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Faculty of Health Sciences

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Systems Epidemiology Approach in Endometrial Cancer. The NOWAC Study

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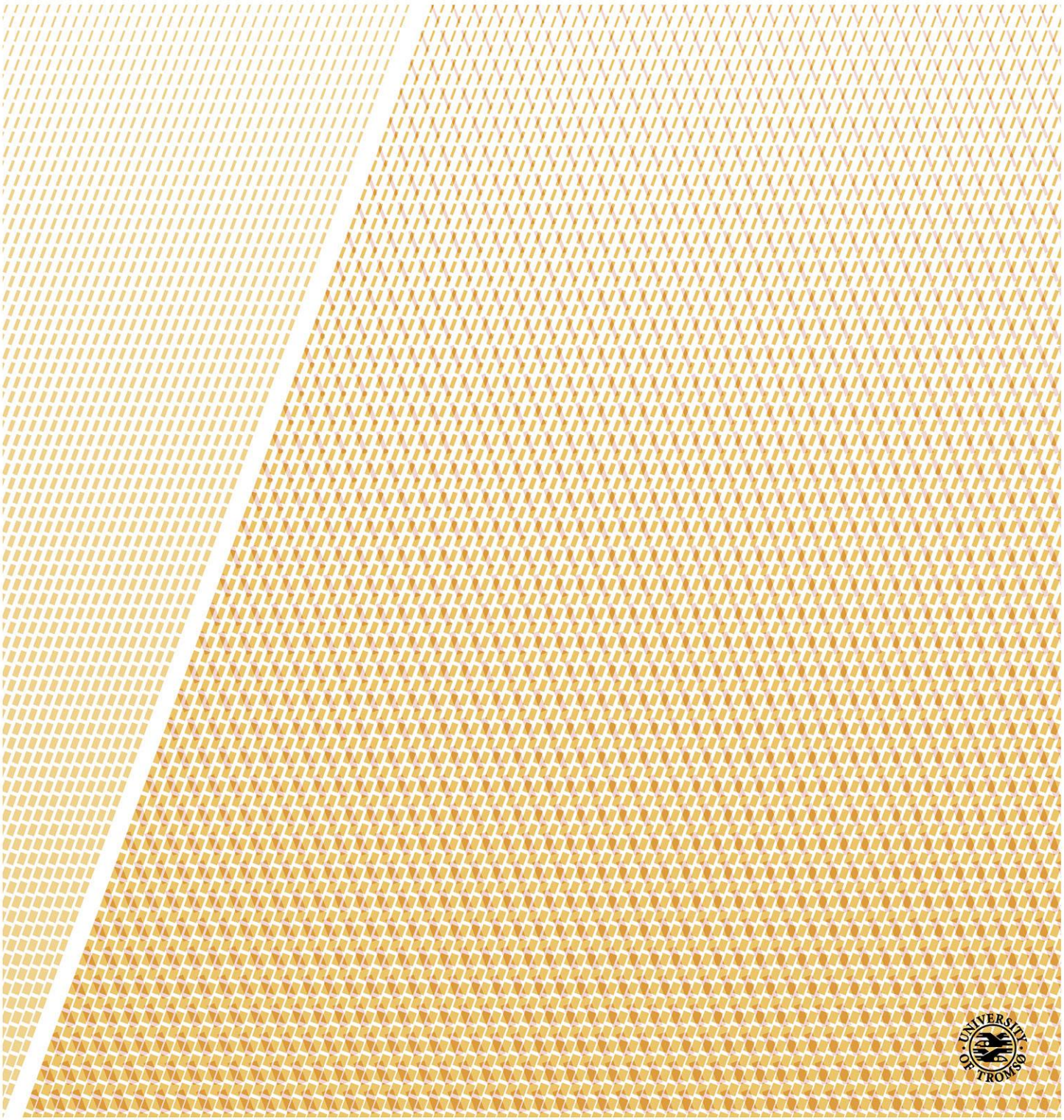

CONTENTS

SUMMARY

Endometrial cancer (EC) is one of the most common gynecological cancers with extensively rising incidence worldwide. Norway is among the countries with the highest rates of EC. Although, most of the established risk factors for EC are well described, there are few studies from Norway investigating them in a cohort design. Moreover, modern clinical medicine, especially oncology, is moving towards personalized and individualized diagnostics and treatment approaches, and therefore there is a great need for studies focusing on detecting of biomarkers and changes in gene expression profiles long before the diagnosis takes place.

The main aim of this PhD project was to evaluate the risk factors that mostly contribute to the development of EC in Norwegian women, and to assess whether these risk factors have any influence on blood gene expression prior diagnosis.

The Norwegian Women and Cancer Study (NOWAC) is a prospective cohort study with approximately 172 000 female participants recruited from the whole Norway since 1991. The participants answered questionnaires regarding lifestyle, diet and health. Further a subset of approximately 50 000 women from NOWAC cohort were randomly recruited to NOWAC Postgenome Cohort and provided blood samples. For paper I, self-reported coffee consumption was evaluated in the light of possible protective effect against EC development in Norwegian population. In paper II, we studied the association between lifetime number of years of menstruation and EC. It was investigated whether this association is attenuated by other well-known modifiable lifestyle risk factors such as high BMI, diabetes, incomplete pregnancies and menopausal hormone therapy (MHT). In Paper III, using the systems epidemiology approach, we evaluated the impact of the major EC risk factors on prediagnostic blood gene expression signatures in a subcohort of 79 EC cases and 79 matching controls.

In line with previous reports, we demonstrated inverse association between coffee consumption and EC, which was especially pronounced in obese women and current smokers. However, in contrast to other studies this was observed only in heavy coffee drinkers (in our study those who drank ≥8 cups/day). In paper II we showed a statistically significant linear relationship between LNYM and EC risk, which remained significant after adjusting for BMI, diabetes, MHT and incomplete pregnancies. Paper III demonstrated that changes in parity status are associated with a number of alterations in immune gene sets in controls compared with EC cases, thus providing a novel view of pregnancy-associated EC protection.

In conclusion, the main findings of this work demonstrate the complexity of endometrial carcinogenesis and emphasize necessity of further investigations on both reproductive and lifestyle

risk factors combined with translational research approaches. The results showing gene expression changes connected to long-term protective effect of parity might serve a solid foundation for further investigations on specific pregnancy-related mechanisms preventing EC development.

LIST OF PUBLICATIONS

This thesis is based on the following papers, hereafter referred to by their Roman numerals.

Paper I

Gavrilyuk O, Braaten T, Skeie G, Weiderpass E, Dumeaux V, Lund E.

High coffee consumption and different brewing methods in relation to postmenopausal endometrial cancer risk in the Norwegian women and cancer study: a population-based prospective study.

BMC Womens Health. 2014 Mar 25;14:48. doi: 10.1186/1472-6874-14-48

Paper II

Gavrilyuk O, Braaten T, Weiderpass E, Licaj I[#], Lund E[#].

Lifetime number of years of menstruation as a risk index for postmenopausal endometrial cancer in the Norwegian Women and Cancer Study.

Acta Obstet Gynecol Scand. 2018 May 21. doi: 10.1111/aogs.13381.

[#]Authors contributed equally

Paper III

Gavrilyuk O, Snapkov I, Thalabard JC, Holden L, Holden M, Bøvelstad HM, Dumeaux V, Lund E.

Gene expression profiling of peripheral blood and endometrial cancer risk factors: systems epidemiology approach in the NOWAC Postgenome Cohort Study.

Manuscript

LIST OF ABBREVIATIONS

ASR	Age-standardised incidence rate
BC	Breast cancer
BMI	Body mass index
CC	Clear cell carcinomas
cDNA	Complementary DNA
CI	Confidence interval
CIR	Cumulative incidence rate
COC	Combined oral contraceptives
CPR	Central Population Register
CT	Computed tomography
D&C	Classic fractional dilatation and curettage
DAVID	Database for annotation, visualization, and integrated discovery
DDD	Defined daily dose
DNA	Deoxyribonucleic acid
E2	Estradiol
EC	Endometrial cancer
EPIC	European prospective investigation into cancer and nutrition
ER/PR	Estrogen/Progesterone
ESMO	The European Society for Medical Oncology
FDR	False discovery rate
FFQ	Food frequency questionnaire
FIGO	International federation of obstetrics and gynecology
FSH	Follicle-stimulating hormone
GE	Gene expression
GOC-28	Name for international randomized trial
GSEA	Gene set enrichment analysis
HR	Hazard ratio
ICD	International statistical classification of diseases and related health problems
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor-binding protein
LNYM	Lifetime number of years of menstruation
MHT	Menopausal hormone therapy
MRI	Magnetic resonance imaging

mRNA	Messenger RNA
NGS	Next generation sequencing
NOWAC	Norwegian women and cancer study
OC	Oral contraceptives
OR	Odds ratio
PA	Physical activity
PAF	Population attributable fraction
PCR	Polymerase chain reaction
PORTEC-3	Name for international randomized trial
RCT	Randomized controlled trial
REK	Regional committees for medical and health research ethics
RNA	Ribonucleic acid
RR	Relative risk
RT	Beam radiotherapy
SE	Systems epidemiology
SHBG	Sex hormone binding globulin
TCGA	The cancer genome atlas research
TMN	Classification of malignant tumors (tumor-nodus-metastasis)
UICC	International union against cancer
WCRF	World cancer research fund

1. INTRODUCTION

1.1 Endometrial cancer

The present PhD thesis and following articles focus on endometrial cancer (EC), malignancy that originates from the inner epithelial lining of the uterus (endometrium) and comprises ca. 90% of all *cancer uteri* tumors (1).

1.1.1 Epidemiology

EC is one of the most common gynecological malignancies worldwide with a strong geographical variation in cancer incidence rates (Figure 1) (2). It is the fourth frequent cancer type in women in developed countries after breast, colon and lung cancer (3). Among gynecological cancers, EC takes the first place in developed countries and the second place world-wide after cervical cancer.

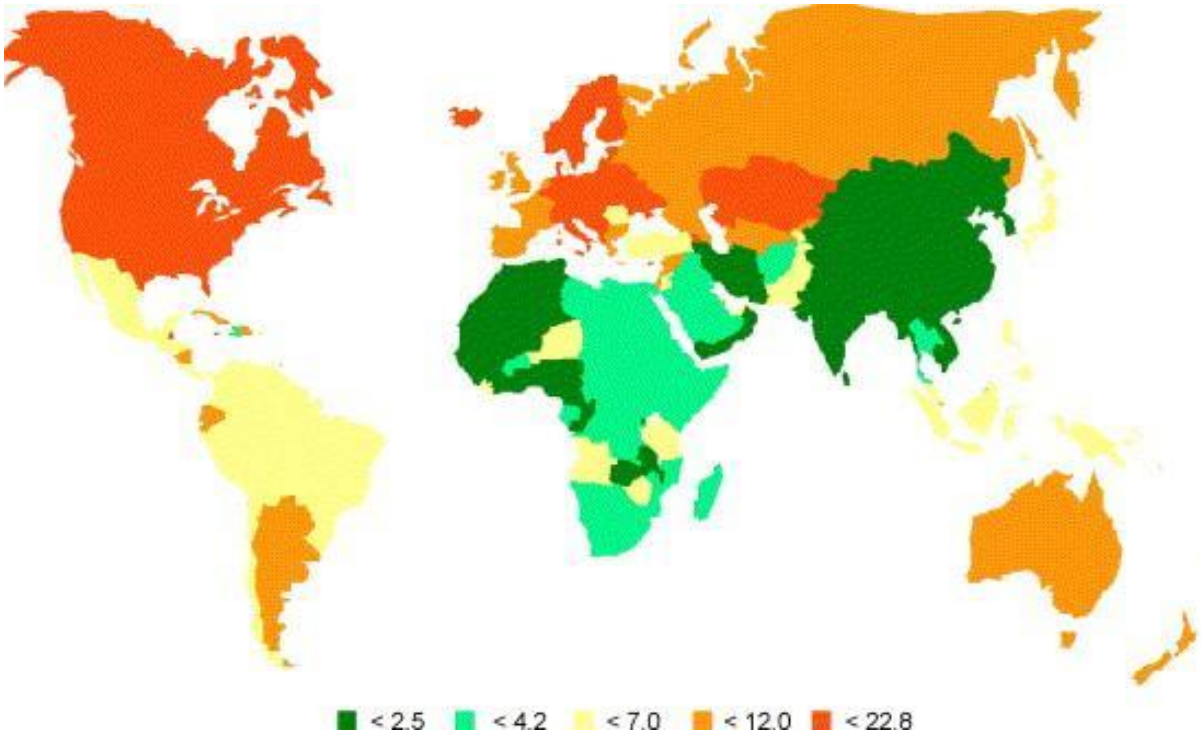

Figure 1. Age-standardised incidence rates of cancer of the uterine corpus per 100 000 person-years (all ages).

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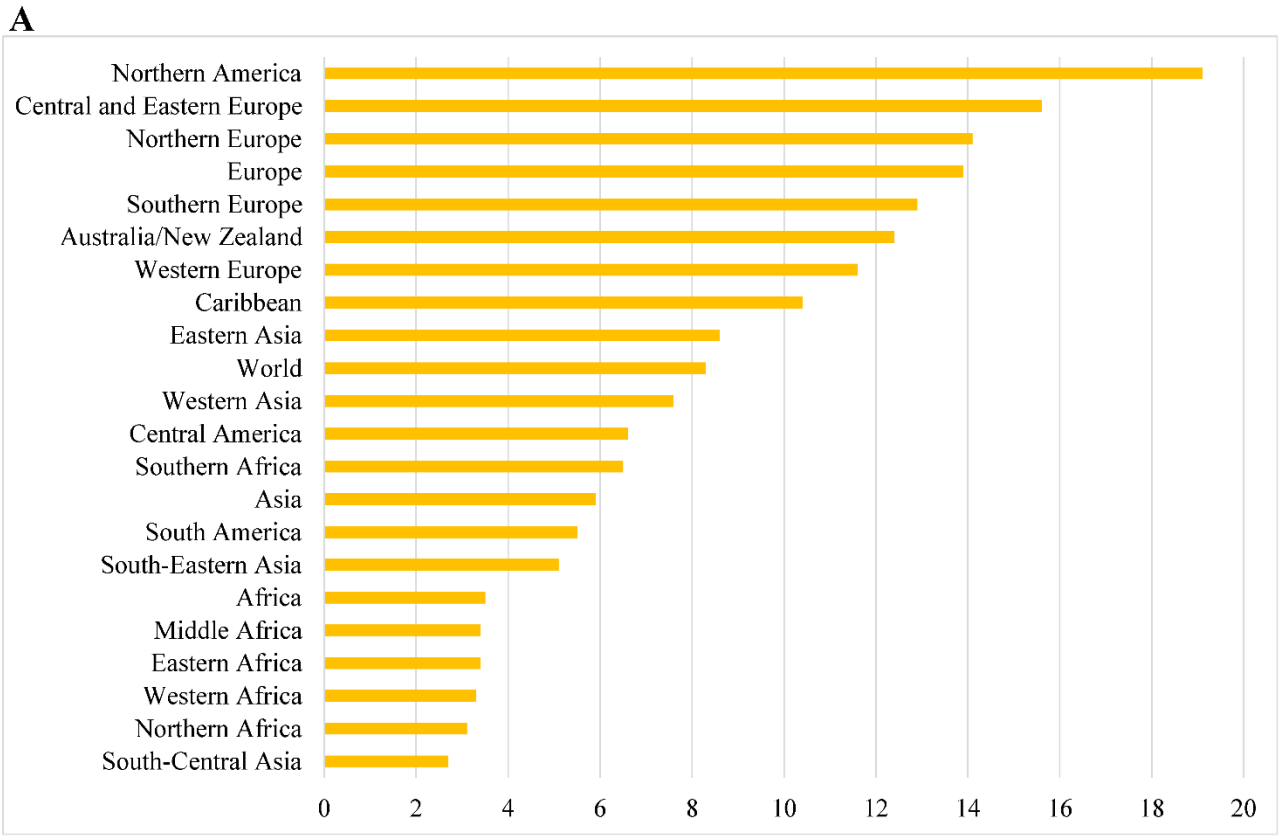
World age-standardised incidence rate (ASR) statistics shows that Northern America, Central/Eastern Europe, Northern Europe, Australia are among the countries with the highest incidence rates in 2012 (4). In contrast, the majority of African countries (except Southern Africa) and countries of South-Central Asia had the lowest incidence rates (Figure 2A). However, such contrast variation in incidence could be partly explained by varying data quality worldwide (5). Among the European countries, the highest world ASR for EC were in Macedonia and Luxembourg

(39.4 and 35.3 per 100 000 respectively) compared to the lowest in Greece and Hungary (10.5 and 10.3 per 100 000 respectively) (Figure 2B) (6).



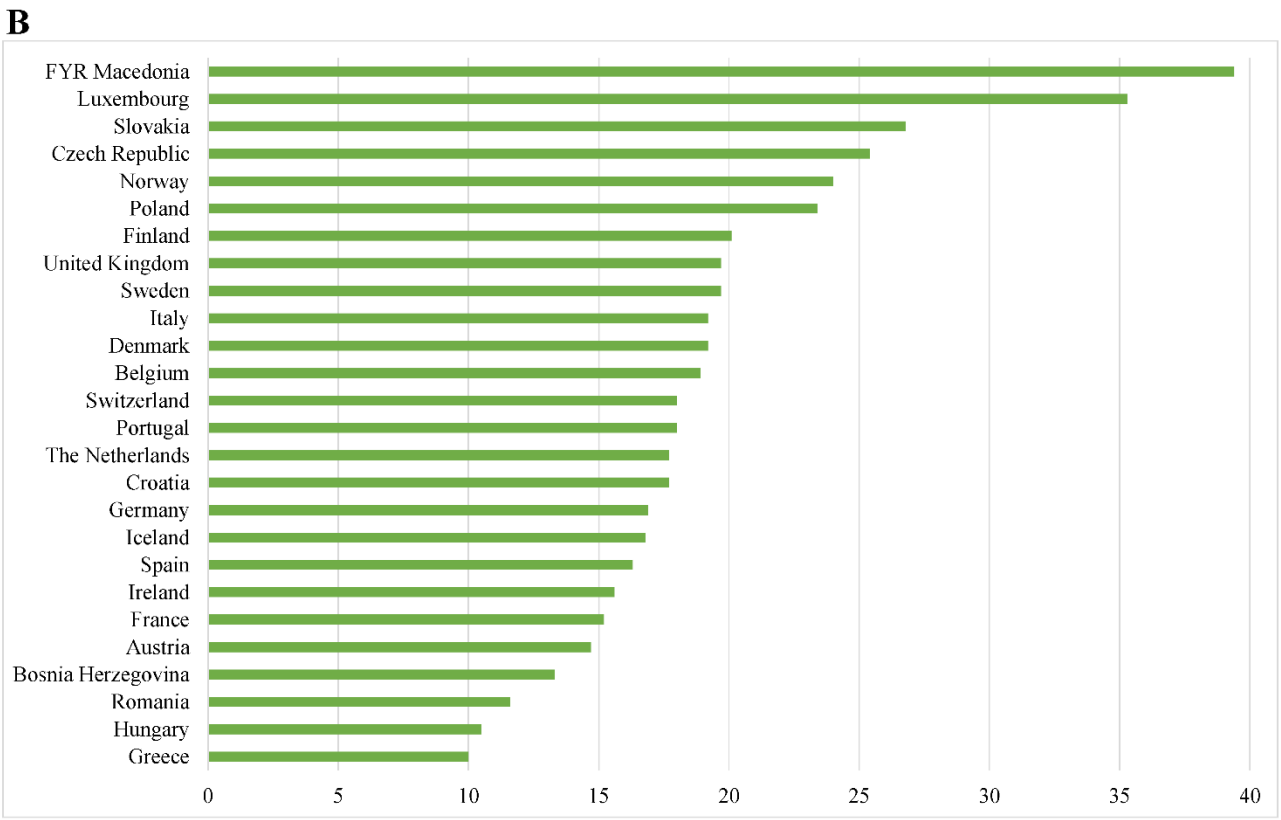


Figure 2. Age-standardised endometrial cancer incidence. (A) Age-standardised incidence per 100 000 population world-wide, 2012 estimates. (B) Age-standardised incidence per 100 000 population in Europe, 2012 estimates.

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EC in Norway occupies one of the leading positions among European incidence rates (the 8th highest in Europe), with World ASR 24.0 per 100 000 in 2012 and Norwegian ASR 27.6 per 100 000 estimated for 2012-2016. The incidence rates has grown dramatically over the last decades in Norway, given that the ASRs were 11.3 per 100 000 and 19.7 per 100 000 in the periods 1957-1961 and 1982-1986 respectively (7).

According to the last updates from Norwegian Cancer of Norway, there were 742 new cases registered in 2012-2016 compared to 181 cases in period 1957-1961 (Figure 3). The incidence rates has grown dramatically over the last decades in Norway and is predicted to rise further by 57% in 2025 compared with the rated observed in 2005 (8).

Registry based data usually provide incidence rates of EC that are recorded within the large general group “uterine cancer” (International Classification of Diseases [ICD] code C54), which consists of epithelial, mesenchymal and mixed tumors. Consequently, the crude number of EC could be lower than reported. However, sarcomas, which comprise 3-9% of all uterine cancers in Norway (9, 10), have had a relatively stable incidence during the last 40 years (0.3-0.4 per 100 000/year)(11). This proves that observed increase of incidence rates of uterine cancer is mainly attributed to increase of EC incidence.

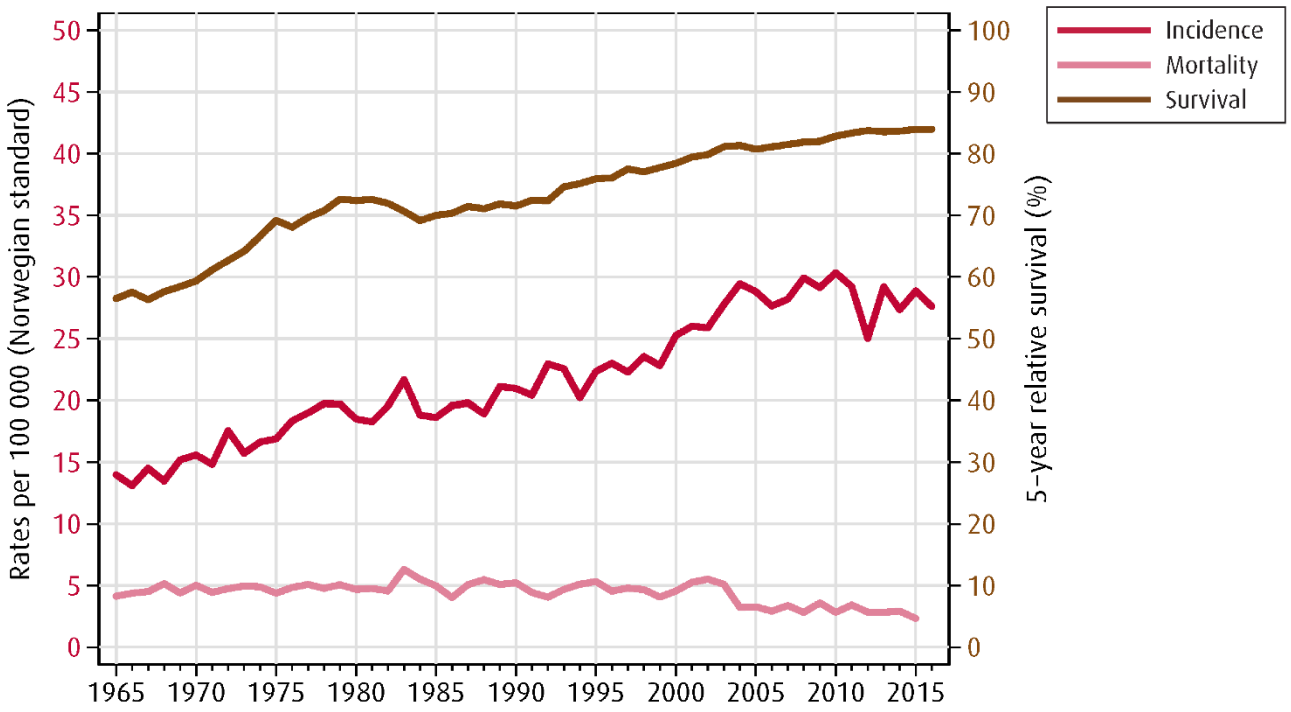

Figure 3. Incidence, mortality and survival rate in cancer corpus uteri per 100 000 person-years in Norway 1965-2016.

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Mortality, five-year survival rate and prognosis

In terms of mortality, the rate for EC in Norway in 2015 was 2.3 per 100 000 accounting for 67 cases (7). The overall prognosis of EC is considered to be good as the symptoms appear at early stage and lead to detection of this malignancy earlier. Ward et al. (12) showed that during 5 years after diagnosis, 42 % women diagnosed with low grade localized EC will most likely die from cardiovascular disease, than from cancer (7.2%). In contrast, those who are diagnosed with high grade advanced EC will most likely die from this malignancy regardless of age (56%) compared to cardiovascular causes (15.1%). The same study showed that, when looking at 5-year interval from diagnosis, EC is the most frequent cause of death during the first 5 years, but then, cardiovascular disease is the leading cause for the next 5-year intervals (Figure 4) (12).

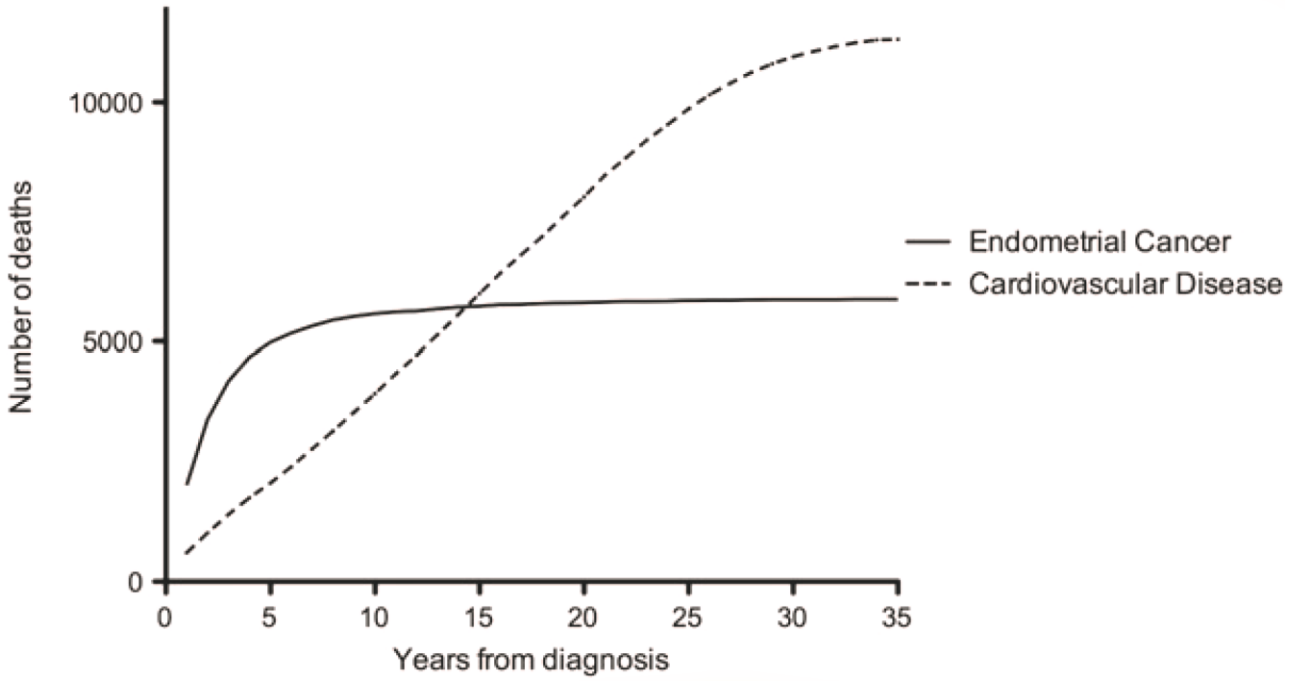

Figure 4. Cumulative mortality rates in patients with endometrial cancer due to cardiovascular causes, other malignancies and other causes.

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The five-year total relative survival (for all EC patients combined) in Norway is considered to be high and accounted for 84% in 2012-2016. The increase in total survival is mostly accounted by improvements in survival of localized disease (Figure 5). This could be partly explained by more successful detection of patients with metastatic lymph nodes and as a result, more frequent performance of staging lymphadenectomies. However, at the same time, favorable survival at early stages of EC could cause onset confounding taking in account that some tumors are diagnosed at early stage would not progress further. For advanced stages with regional and distant spreading of metastasis prognosis is less favorable, where the five-year survival rates decrease to 61% and 38% respectively (Norwegian data, Figure 5).

International Federation of Obstetrics and Gynecology (FIGO) using its own staging system, defines the following distribution of 5-year survival: 85% for stage I, 75% for stage II, 45% for stage III and 25% for stage IV (9). However, age, histological subtype, grade and surgical stage have a huge impact on variation of survival rates (13). Thus, due to heterogeneous pathology 5-year survival vary from 92% to 42% (14) for stage I and from 68% to 17% if there is regional spread or distant disease (15). It is well established, that patients with type II EC has lower survival rates compared to those who have type I EC.

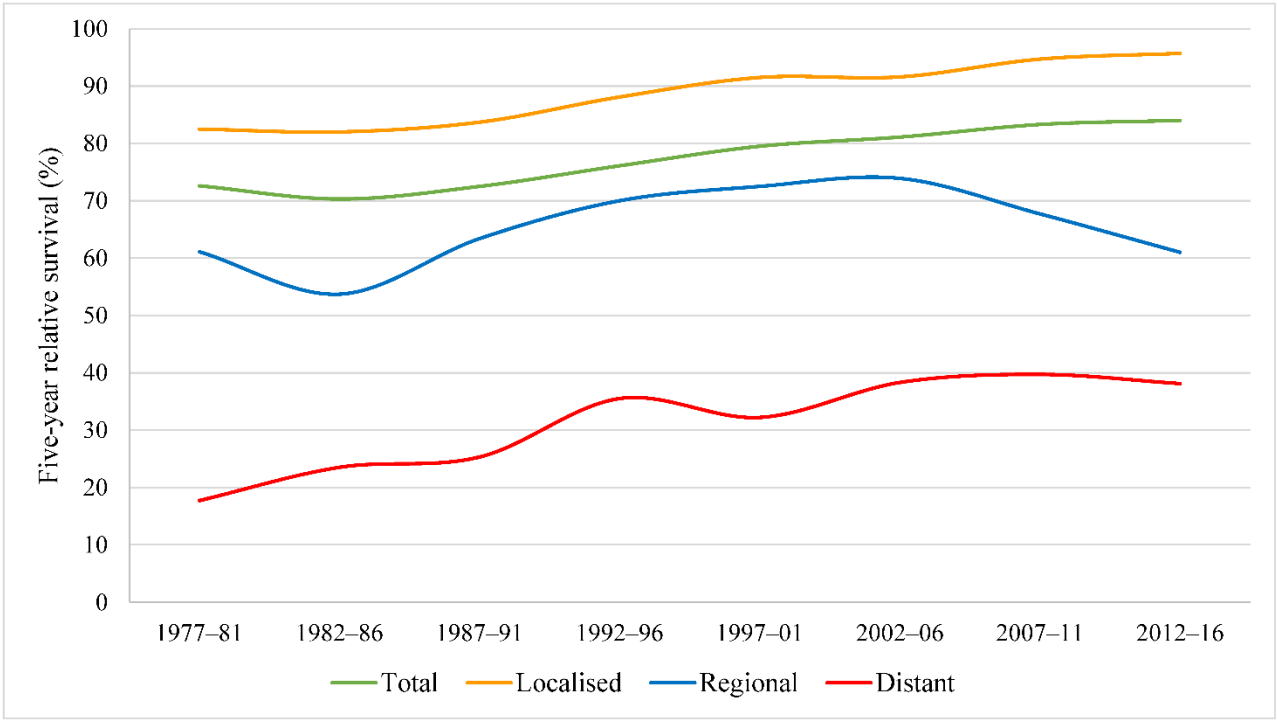

Figure 5. Five-year relative survival (%) for uterine cancer according to primary site and period of diagnosis (1977-2016).

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1.1.2 Clinical features and diagnosis

Clinical presentation and preoperative diagnostics

Around 90% of EC patients have reported abnormal vaginal bleeding, which is considered to be the first classical presenting symptom of cancer uteri in postmenopausal women (16). For premenopausal women intermenstrual bleeding or menorrhagia are the most common first clinical signs of EC. The physicians should be aware of uterine bleeding especially in postmenopausal women until other reasons excluding EC are confirmed. Abnormal vaginal bleeding accounts for 5-10% postmenopausal EC cases (16) and only 0.33% for premenopausal EC cases (17), although the chancing of getting EC are increasing with age. Both pre- and postmenopausal women presented with abdominal bleeding should be particularly examined if they have additional risk factors such as obesity, diabetes, menopausal hormone therapy (MHT) or tamoxifen use. Other warning symptoms can be increased vaginal discharge, abdominal pain and distention.

Preoperative diagnostic is based on evaluation of such parameters as histopathological subtype, estimation of the myometrial infiltration depth and potential infiltration into the cervical stroma and other organs. The first diagnostic steps include gynecological examination, vaginal ultrasound with endometrial thickness >3 mm as a suggested cut-off (18) and investigation of

histological samples obtained either by Pipelle de Cornier curettage device or a classic fractional dilatation and curettage (D&C)(19).

Preoperative histopathological diagnosis could be very challenging due to the difficulties in distinguishing the difference between endometrial hyperplasia (endometrial precancer) and already early stage of endometrial adenocarcinoma. The most challenging samples are those obtained from endometrial polyps and secretory endometrium. Moreover, there is still low reproducibility and inter- and intraobserver variation among pathologists (20, 21). At the present time, several risk scoring classification systems are available now for risk assessment of developing of EC from endometrial hyperplasia. Among them is D-score, method based on morphometry taking into account following prognostic criteria: the volume percentage of stroma, the standard deviation of the shortest nuclear axis and the outer surface density of the glands (22).

The next step in EC diagnostics is pelvic magnetic resonance imaging (MRI) that is used for measuring the tumor size and assessment of myometrial invasion. Finally, computed tomography (CT) or X-ray examination could be used for revealing intra-or extra-abdominal spread.

Treatment guidelines

During the last 20 years essential steps were made in cancer treatment strategies, moving from traditional "killing paradigm" based on eradicating the primary tumor towards more "personalize targeted therapy", which is aimed to select the therapy suitable for each individual patient. In Norway hysterectomy usually in combination with bilateral salpingoophorectomy with or without lympadenectomy has been used as a standard treatment of EC for surgical treatment (23, 24). Debulking surgery is recommended for advanced stages (24). For non-endometrioid subtypes (clear cell and serous endometrial carcinomas) and for carcinosarcomas it is also recommended in addition to perform omentecomy and lymphatic dissection (25, 26).

Lymphadenectomy, both pelvic and para-aortic, are still recommended for complete surgical 2009 staging, however, performing of these procedure in women with low grade and early stage disease, is still controversial and one of the most debated issues. Thus, several randomized controlled trials showed that lymphadectomy could statistically significantly improve surgical staging but did not bring any benefit for disease-free or overall survival both at stage I and in patients with higher-stage disease (27, 28). Moreover, recent review concluded that there is an evidence of increased surgery-related systemic morbidity or lymphoedema/lymphocyst formation in women who received lymphadenectomy (29). In Norway, where the rates of lymphoadenoectomy are higher compared to other European countries, it is recommended to evaluate DNA ploidy from

sampled lymph nodes and then to perform pelvic and para-aortic lymphadenectomy in patients with presumed high-risk tumors (24). Investigation of parameters that might help to select the patients with low risk of lymph-node metastasis takes one of the leading places among studies evaluating preoperative risk of EC. These studies showed that loss of ER/PR expression in curettage specimens is connected to increase risk of lymph node metastasis (30, 31). Another study reported that having endometrioid subtype of tumor with no evidence of deep myometrial infiltration, enlarged lymph nodes or distant metastasis on MRI along with serum CA125 levels < 35 U/mL is connected to 97% negative predictive value for detection of lymph node metastasis (32).

Adjuvant therapy is meant to treat lymph node regions that might contain spread of metastasis in order to avoid the recurrence of EC. Based on the Norwegian guidelines, for FIGO stage I, the risk of recurrence of disease is classified into low, medium and high risk and depends on histological subtype (24). The patients are considered of being at high risk of recurrence if they have FIGO stage II or higher (24). Many of other European centers use a refines risk stratification system suggested by The European Society for Medical Oncology (ESMO). This approach also includes evaluating of various histopathological factors like lymphovascular space invasion (LVSI) for selecting patients for adjuvant therapy (15). Due to the lack of evidence of efficacy the principles for optimal adjuvant therapy for high-risk EC patients are still controversial and on debates. In Norway, adjuvant chemotherapy based on combined regimen of carboplatin and paclitaxel (TC) or paclitaxel, epirubicin and carboplatin (TEC) is commonly used for high-risk patients. For low risk women with FIGO stage 1A and grade 1 and 2 adjuvant radiation can be used. Further, adjuvant radiation in form of brachytherapy or external beam radiation is still used for treatment of intermediate-high risk patients in many countries (15), although in other centers this type of treatments is almost replaced by chemotherapy (33). However, there are ongoing clinical trials PORTEC-3 and GOC-258 that investigate the effect of combination of chemotherapy (CT) and beam radiotherapy (RT) in high-risk patients and recently reported the first results, showing the possible benefit of combined CT/RT in high-risk patients (34).

Hormone therapy is still one of the treatment options for patients with low risk of EC, who wish to preserve fertility and for those with advanced disease, who are not eligible for other types of treatment (35, 36).

After treatment, EC patients have three to five years until recurrence of disease is diagnosed. The recurrence rates for patients with low, intermediate and high risk are reported to be 5-10%, 15-20% and more than 30% respectively (37). For non-endomerioid tumours the recurrence rates are somewhat higher, up to 50% (38).The recurrences are usually treated with surgery, chemotherapy, radiotherapy separately or in combination (39).

1.1.3 Histopathological features

Histopathology

In classification provided by World Health Organization, endometrioid adenocarcinoma represent the most common subtype, which comprises 75-80% of all EC cases (40). This EC type is well-differentiated cancer with preserved glandular architecture, lack of intervening stroma and is known to arise from endometrial hyperplasia (Figure 6A)(40). Other histological subtypes are combined into a of group of non-endometrioid cancers and consist of mucinous carcinoma (9% of cases, Figure 6B), serous carcinomas (3-10% of cases, Figure 6C), clear cell carcinomas (CC) (2-3% of cases, Figure 6D) and undifferentiated carcinomas (41). These less common non-endometrioid subtypes account for 20 % of EC diagnosis and are usually found in atrophic endometrium with no obvious precursor lesion (15, 40). Further, if two histological subtypes are present in tumor, endometrial carcinomas are defined as mixed if among these two subtypes at least one is non-endometrioid tumor, presented in more than 10% of lesion (42). The knowledge and accurate assessment of different histological subtypes is one of the crucial components in assessment of EC risk and patient outcome. In contrast to well-established agreement in histological subtypes of ovarian cancer, EC still has a lot of disagreements and huge heterogeneity in both of diagnostic assessment of endometrial specimens and reproducibility among pathologists (43-46).

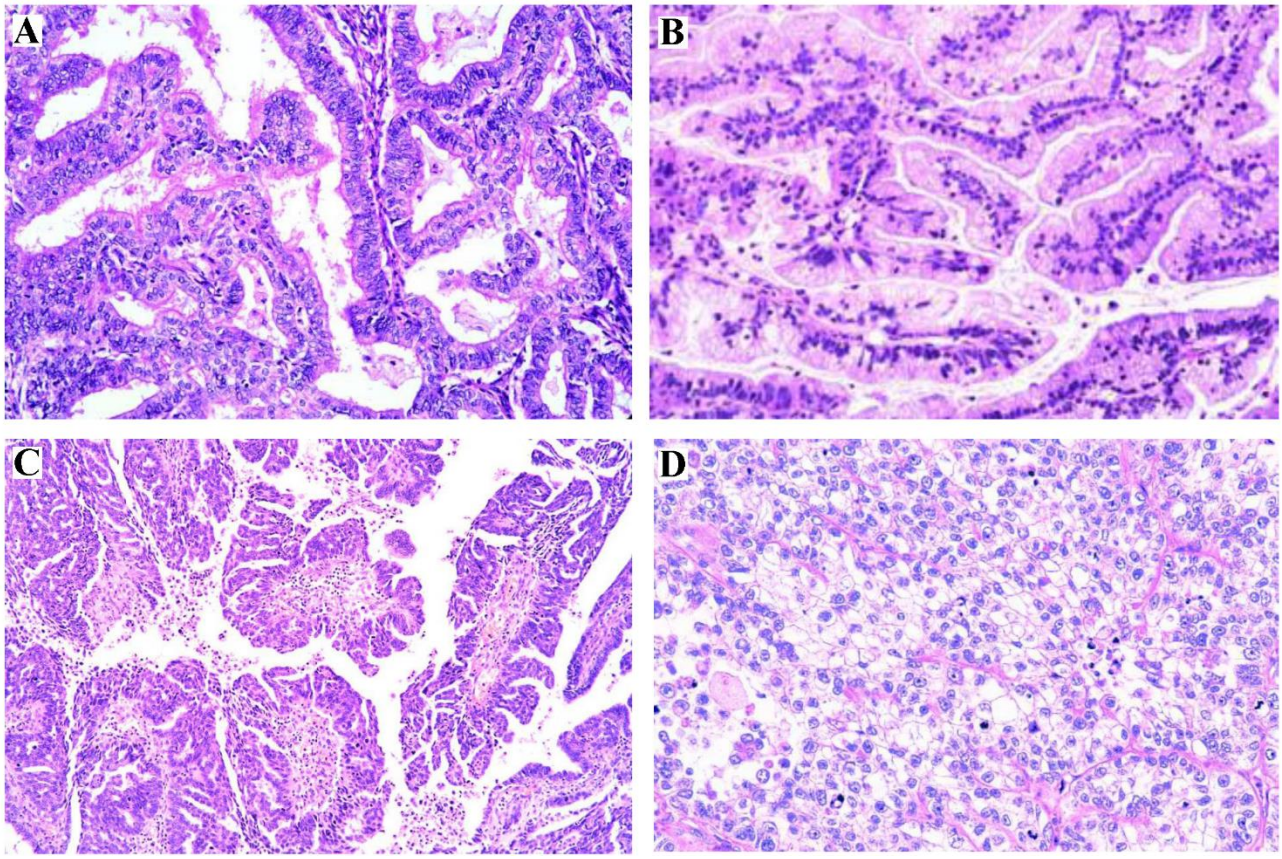

Figure 6. Histological classification of EC. (A) Endometrioid adenocarcinoma. (B) Mucinous adenocarcinoma. (C) Serous adenocarcinoma. (D) Clear cell adenocarcinoma.

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FIGO grade and stage

The grading of EC tumors are performed histologically using either a 3-tiered FIGO system or a 2-tiered (binary) systems. The FIGO grading system is based on architecture, i.e. percentage of solid (non-squamous) growth and cytologic atypia (40). Thus, the grade 1 tumor defines as a well-differentiated tumors with a glandular pattern and ≤5% of solid growth, grade 2 has 6-50% and grade 3 more than 50% of solid growth pattern respectively. Cytologic (nuclear) atypia could change architectural grading through increasing from grade 1 to 2 or from grade 2 to 3. Based on a binary grading system, grade 1-2 and grade 3 are often transformed into low grade and high grade, respectively. Even though, this grading system is currently not used in clinical practice, it showed less interobserver variability and better prognostic power (47-50).

Staging of EC

Two surgical-pathological staging system has been used for dividing the extent of uterine cancer growth into stages. One is classical TNM classification, which is mainted by the UICC (51). In this

classification, T represents the size of the tumor and spread to nearby tissues, N represents the number, size and localization of lymph node metastasis and M tells about distant metastasis. However, for EC historically FIGO staging system has been more frequent applied since 1988 (52). Based on the updated and more available knowledge about risk factors related to tumor behavior and survival, the new version of FIGO staging was introduced in 2009. In this last updated version, the accurate determination of depth myometrial invasion and cervical stromal involvement is crucial for dividing EC into 4 stages (Table 1), although pathological assessment of myometrial invasion can be also challenging (53).

Table 1. FIGO 2009 staging system for endometrial cancer.

Stage I	Tumor within corpus uteri
IA	Minimal myometrial invasion (no or less than half)
IB	Myometrial invasion equal to or more than half of the myometrium
Stage II	Tumor invades further to the cervical stroma, but does not extend beyond the uterus
Stage III	Local and/or regional spread of tumor
IIIA	Tumor invades the serosa of the corpus uteri and/or adnexas
IIIB	Vaginal and/or parametrial involvement
IIIC1	Positive pelvic lymph nodes
IIIC2	Positive para-aortic lymph nodes with or without positive pelvic lymph nodes
Stage IV	Tumor invades bladder and/or bowel mucosa and/or distant metastases
IVA	Tumor invades bladder and/or bowel mucosa
IVB	Distant metastases, including intra-abdominal metastases and/or inguinal lymph nodes

Management of EC in regard to some histopathological factors.

As it was mentioned before, risk estimates and treatment management of EC depends on many factors such as age, stage, grade, lymphovascular invasion and histological subtype. Women younger than 60 with endometrioid type, FIGO I stage, grade 1 or 2, myometrial infiltration less than 50% and without lymph vascular space invasion are associated with low risk getting metastasis, and no adjuvant therapy is recommended. Myometrial cancer infiltration with more than 50% is generally linked to lymph node metastasis and associated with poor survival independently

of FIGO stage and histological type (54). Patients that have EC grade 1 or 2, endometrioid adenocarcinoma, mixed endometrioid and mucinous carcinoma are associated with favorable prognosis and in most of the cases are treated by simple hysterectomy (55). On contrary, grade 3 endometrioid, serous and clear cell carcinomas are associated with disproportionate number of deaths. Non-endometrioid subtypes (clear cell and serous) are considered to be high-grade by definition irrespective of growth pattern and cytologic atypia due to the property for spreading outside of the uterus early in the disease process (13). Serous adenocarcinoma is known for its aggressive behavior due to the fast development of deep myometrial and extensive lymphatic invasion, so that patients have extrauterine spread already at the time of diagnosis (56). Moreover, this cancer type is known for its frequent recurrence and a fatal outcome. Clear-cell carcinoma is considered to have a poor prognosis, because most of the cases are diagnosed in advanced clinical stages (56), however, if clear cell adenocarcinoma limited to the uterus, than the patient has better prognosis than one with serous subtype of the same stage (40). In general, it has been shown by other studies, that within this "group of subtypes with poor prognosis" patients with grade 3 endometrioid or clear cell carcinomas has more favorable prognosis than patients with serous carcinomas (44). When it comes to the histotype-spesific treatment strategies, it has been suggested that for those non-endometrioid subtypes with a tendency to intraperitoneal spread it is better to use chemotherapy in contrast to historical radiation therapy that is used for extensive intrauterine as well as extant disease in EC (56). In addition, non-endometrioid subtypes along with carcinosarcomas usually require omentectomy due to the increased risk of intra-abdominal spread (25, 26).

1.1.4 Molecular alterations

Genetic changes are one of the main driving forces behind malignant transformation of a cell. At present, a wide variety of genetic alterations have been demonstrated to contribute to EC development and progression. Since the publication of Bokhman's work in 1983, where he distinguished two types of EC based on clinicopathological features of tumors (Table 2) (57), many attempts have been made in order to fit various molecular genetic alterations into the model (Figure 7) (58).

Nevertheless, Bokhman's classification has never been used for the staging and risk assessment of endometrial tumors in clinical settings mostly due to its oversimplicity (i.e. existence of significant overlap between Type I and Type II tumors, high heterogeneity of tumors resulting in diagnostic difficulties even for experienced pathologists, etc.). Therefore, there is a need in modern clinically relevant classification of molecular alterations in EC which could be a reliable instrument in the assessment and prognosis of tumor development.

At present, a variety of genes are known to possess altered expression in different components of EC tumorigenesis (Figure 7). Among the most frequently perturbed genes in EC are PTEN (59), PIK3CA (60), KRAS (61), β-catenin (62), p53 (63), p16 (64), HER2/neu (65), ARID1A (66), etc. However, there is no pathognomonicity in a singular genetic change and particular type of EC, hence trends in changes of groups of genes should be considered for the appropriate staging and stratification of tumors.

Table 2. Classification of EC into two types

	Type I	**Type II**
Clinical, endocrinological, and morphological components		
Distribution	60–70%	30–40%
Reproductive function	Decreased	No disturbances
Onset of menopause	After age 50 years	Younger than age 50 years
Background endometrium	Hyperplasia	Atrophy
Oestrogen associated	Yes	No
Associated obesity, hyperlipidaemia, and diabetes mellitus	Yes	No
Tumour grade	Low (grades 1–2)	High (grade 3)
Myometrial invasion	Superficial	Deep
Potential for lymphogenic metastatic spread	Low	High
Prognosis	Favourable	Unfavourable
Sensitivity to progestagens	High	Low
Outcome (5-year survival)	86%	59%
Clinicopathological and molecular correlates		
Prototypical histological type	Endometrioid	Serous
Oestrogen-receptor or progesterone-receptor expression	High	Low
Stage at diagnosis	Early (FIGO stage I–II)	Advanced (FIGO stage III–IV)

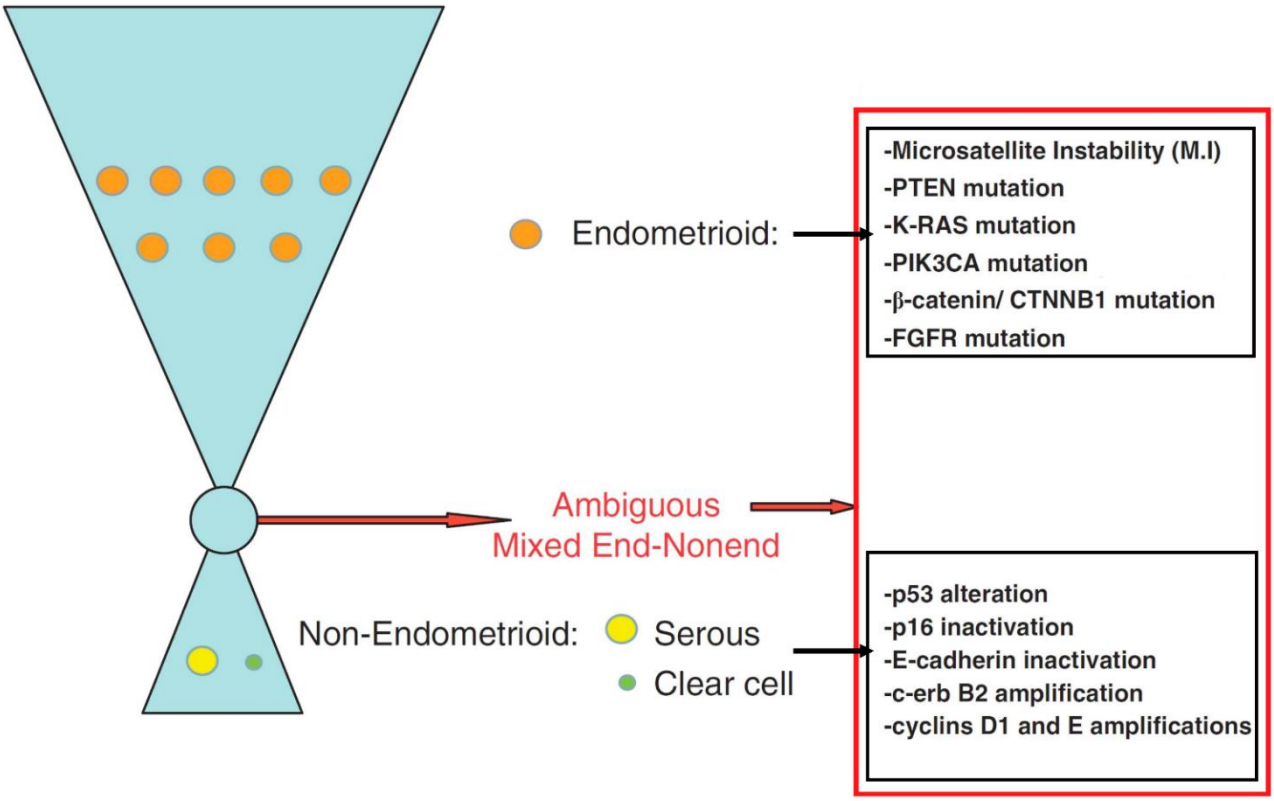

Figure 7. Genetic alterations in EC.

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In 2013, The Cancer Genome Atlas Research (TCGA) Network proposed a novel integrated genomic classification of EC (67). Using multiomics approach, ECs were classified into 4 genomic classes:

1. **POLE ultramutated**. Tumors with very high mutation rates and hotspot mutations in the exonuclease domain of POLE (a subunit of DNA polymerase ε that has a role in DNA replication), few copy-number aberrations, high frequency of C>A transversions, mutations in PIK3CA, PTEN, PIK3R1, FBXW7, and KRAS genes, and favourable outcome.
2. **Microsatellite instability hypermutated.** Tumors characterised by microsatellite instability due to predominantly MLH1 promoter methylation, high mutation rates, few copy-number aberrations, KRAS and PTEN mutations.
3. **Copy-number low.** Microsatellite-stable grade 1 and 2 tumors with low mutation rates, exhibiting increased frequency of CTNNB1 mutations
4. **Copy-number high**. Tumors, demonstrating abundant copy-number aberrations and low mutation rates, increased number of TP53, FBXW7, and PPP2R1A mutations, rare PTEN and KRAS mutations, and poor outcome.

The high clinical potential of this classification has been validated in numerous studies (68). However, the high cost of the laboratory techniques used by TCGA hampers the implementation of

the classification into clinical practice, therefore combination of existing tools (IHC, FISH, etc.) and omics analysis should be further considered.

1.1.5 Established risk factors

Numerous risk factors that account for EC development have been described up to date (23). In this thesis, I will mainly focus on age, age at menopause, age at menarche, cumulative number of years of menstruation, obesity, pregnancy and parity/nulliparity, breastfeeding, oral contraceptive (OC) use, MHT, diabetes mellitus, physical activity and coffee consumption.

Age

EC is still a disease of elderly women with the mean debut age at 50 years. Higher age at diagnosis is considered to be an important prognostic factor in terms of lower survival rates and increased mortality, although it could be partly explained by the fact that elderly patients in general develop more aggressive histological subtypes, and in addition get less aggressive therapy due to more frequent complications. EC is also described in women younger than 35 years (51) and even in teenagers (69). In Norway the increasing of age-spesific incidence rate is observed between in age 45 and 70 with a peak at age period 75-79 (Figure 8).

Exogenous Hormonal Risk Factors in EC

OC

Since its introduction in 1960, combined oral contraceptives (COC) has gained both widest geographic distribution and undergone substantial evolution in hormone formulations and doses. Nowadays, COC represents the most common modern contraceptive method in developed countries and third most common in developing countries (70, 71). Apart of effective protection of unintended pregnancy, COC account for improvement in menstrual bleeding, reduction in risk of iron deficiency anemia and ectopic pregnancy, protection against some cancer types and other beneficial effects (Figure 9) (72). However, some adverse effects such as increased risk for cardio-vascular events (thrombosis, stroke) and risk for cervical and breast cancer (BC) are well-known, especially from the use of previous generations of COC (73).

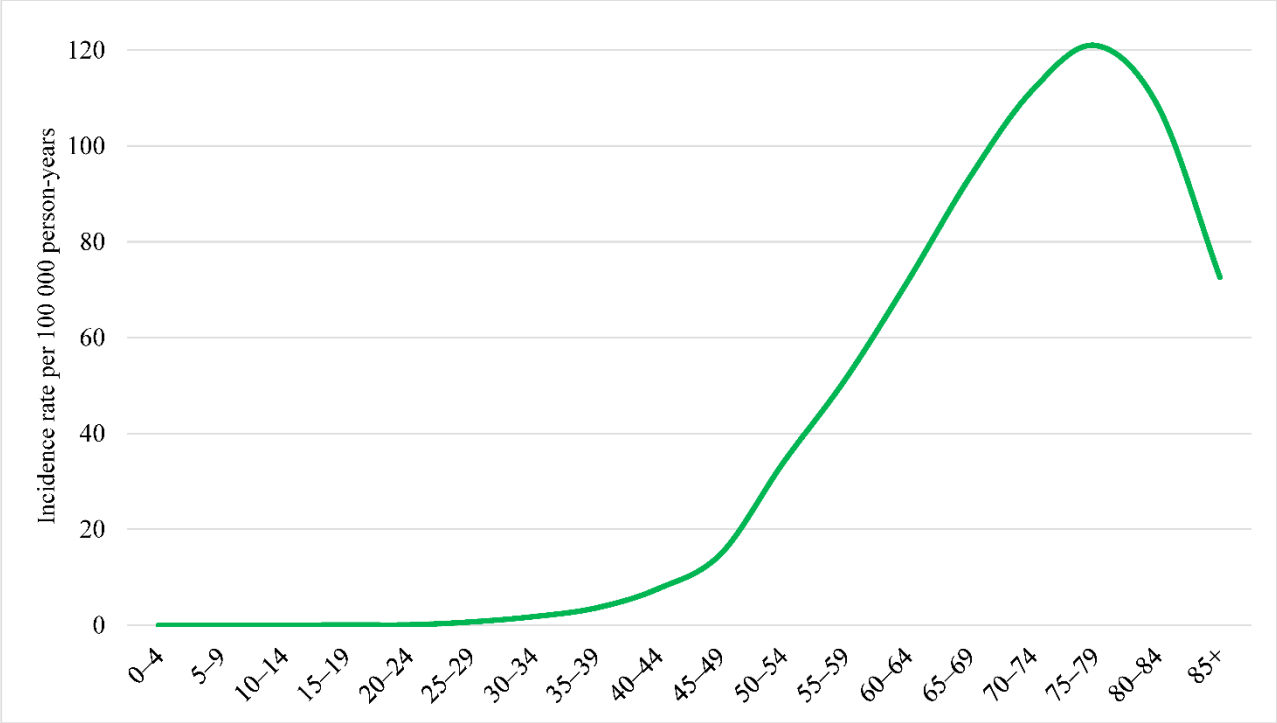

Figure 8. Age-specific incidence rates of uterine cancer per 100 000 person years and five-year age group, in Norway during the period 2012-2016.

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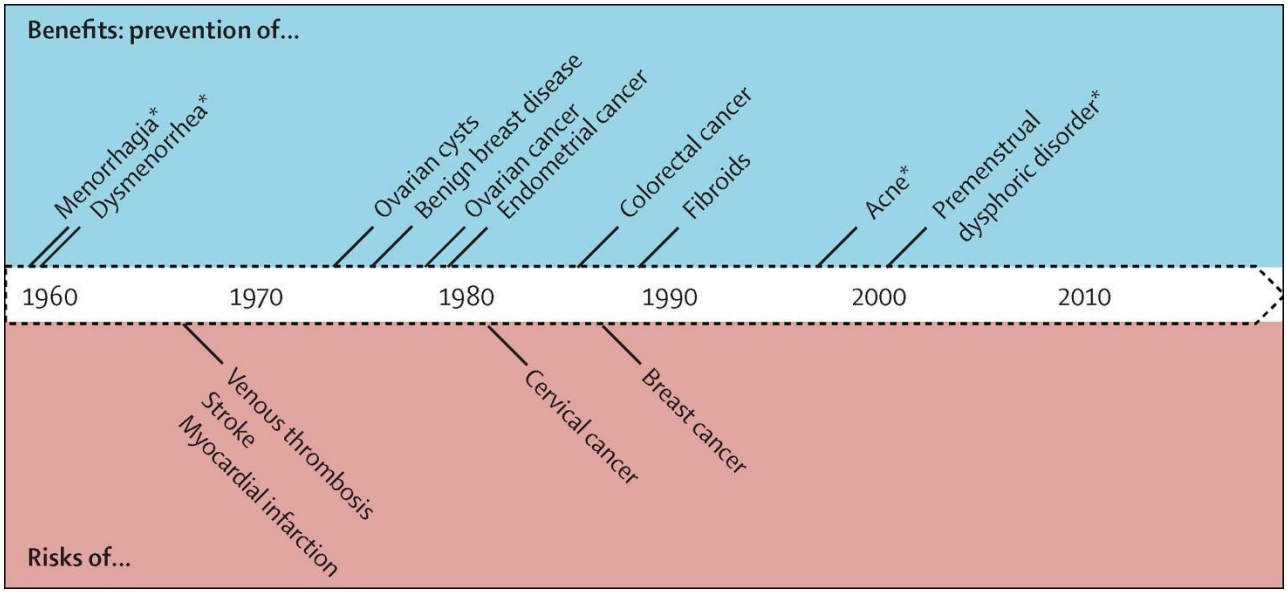

Figure 9. Non-contraceptive benefits and risks of oral contraceptive use

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The beneficial lasting protective effect of OC use in regard to EC is well-established by numerous studies (74). The risk of EC is almost halved with the use of OC and the reduction effect comes first 2-5 years after use. It has been also shown, that the risk reduction is directly related to the duration of OC use and remains minimum 15-20 years after the end of use. Population-based case-control study from Danmark in 2000 showed that OC use in 1-5 years reduce the risk of EC in women under 50 years (OR 0.2; 95% CI 0.1-0.3) (75). Another study from Sweden reported a decreasing trend for EC risk med increasing duration of OC use (76). There were no association with OC use and EC risk if the duration of OC use was under 3 years. While, three and more years of OC use gave the risk reduction with OR0.5 (CI 95% 0.3-0.7). Halving of risk of getting EC during the next 20 years due to OC means from 0.05% to 0.03% risk reduction for 25 years women, and from 0.16% to 0.08% risk reduction for 30 years old women (77). Later on, the collaborative Groups' analysis of 36 epidemiological studies that reported their findings between 1987 and 2004 confirm the evidence that OC prevent EC and has a long-term protection (Figure 10) (78). Every 5 years of use was associated with a risk ratio of 0.76 (95% CI 0.73–0.78; $p<0.0001$) with more risk reduction for carcinomas than sarcomas. The risk reduction persisted for more than 30 years after the last OC pill was used, showing no apparent decrease between the RRs for use during the 1960s, 1970s, and 1980s, despite higher estrogen doses in pills used in the early years (78).This study claims that OC use conferred long-term protection and about 400 000 cases of EC before age 75 years had been prevented during the 50 years from 1965 to 2014.

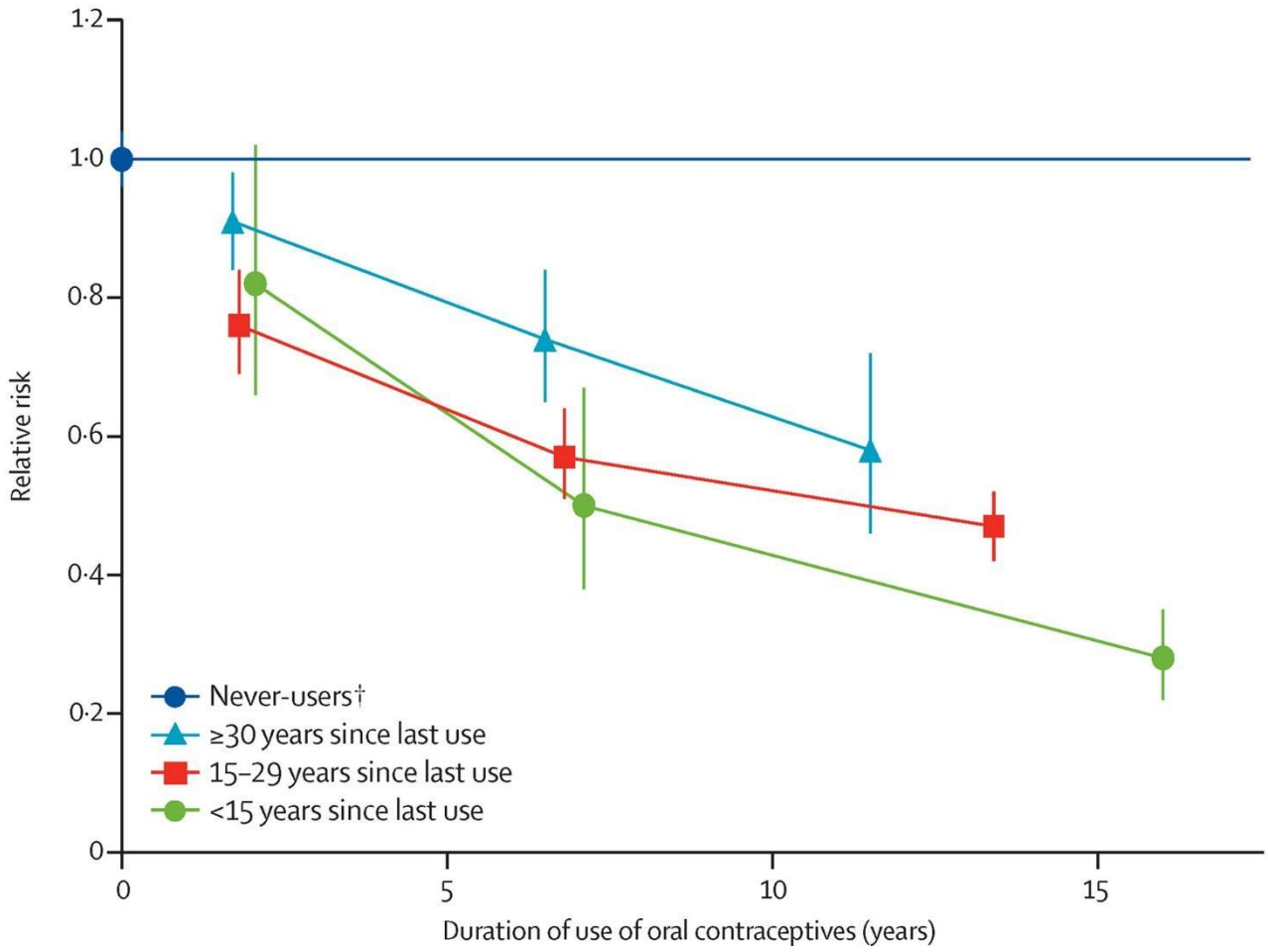

Figure 10. Relative risk of endometrial cancer in users of oral contraceptives by duration of use and time since last use of oral contraceptives.

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The exact mechanisms by which OC reveals protective effect on endometrium especially many years after cessation remain unclear. The most discussed hypothesis proposes that those women who use continuous COC have fewer days of unopposed estrogen exposure period every month (79). It is indeed known, that mitotic activity rates in endometrial cells are lower during first four days of menstrual cycle, then increase rapidly and remain steady up to day 19, and finally, drop to zero for the rest of the cycle period (80). In addition to shorten of period with unopposed estrogen exposure, a synthetic progesterone also is believed to contribute to protective effect on endometrium (81).

Menopause Hormone Therapy (MHT)

Since 1940s when the first MHT preparation, Premarin, came to the market, many changings have been done in the formulation of MHT. The first introduced hormone therapy was based on estrogen

only and has been produced to provide a relief for menopausal symptoms and in addition, prevent many of adverse effects of aging. However, later it was shown that those women who received menopausal unopposed estrogen therapy have a substantial increased risk of EC (82). Several case-control and prospective studies confirmed an increasing risk of EC due to long-term use of unopposed estrogen, and relative risk (RR) varied from 3.1 up to 15 (83, 84). First analogue reports led to decline in use of estrogens preparations (85) and initiated the changings in MHT's formulations in form of adding progestin in order to minimize the proliferative effect on endometrium (86). The results from the Million Women Study showed later that those who currently used estrogen only therapy had a 50% increased risk and users of tibolone preparations had 80% of increased risk (87). The same study showed that risk was lower in women with a body mass index (BMI) < 25 compared to those who had BMI >= 25. Moreover, it has been shown that the risk of endometrial hyperplasia, precursor of EC, is not reduced if unopposed estrogen is given in a cyclic regimen (88). Later coming reports indicate that EC risk could be substantially decreased by MHT with progestin given in either a cyclic or continuous regimen (89), however, it has been also shown monthly users of estrogen-progestin MHT in cyclic regime are at higher risk of developing EC compared to those who use this type of MHT in continuous regime (90).

Endogenous Hormonal Risk Factors in EC

Reproductive Risk Factors

High levels of endogenous estrogens increases the risk of EC via increasing of mitotic activity of endometrial cells (91). On the contrary, progesterone, can slow down this mitotic activity induced by estrogen and promote differentiation of epithelial cells making them less susceptible to malignant change (92). Each pregnancy is a unique health condition associated with addition intense progesterone production, which compensates stimulating effect of estrogen on mitotic activity in endometrium and, therefore, protects against EC development (93). Over several decades, numerous studies have demonstrated that in comparison to nulliparous women, parous women have decreased risk of developing EC. This was showed by both case-controls (94-96) and prospective studies (97, 98). The last updated pooled-analysis from 2015, including 10 prospective, 35 case-control studies and 1 pooled analysis of 10 cohort and 14 case-controls studies, where the final sample size comprised 69 681 patients, revealed a significant inverse association between parity and EC risk with RR 0.69, 95% confidence interval (CI) 0.65–0.74; $I2$=76.9%) (99). Further, dose-response analysis from this study showed a nonlinear relationship between the number of parity and EC risk. Another non-hormonal mechanism that is believed to have a role in association between EC and parity, is connected to mechanical clearing of uterus lining from precancerous cells

that have undergone malignant transformation (100, 101). This theory has raised based on the findings that revealed that later age at last birth is associated with lower EC risks. Indeed, one of the last pooled analyses showed that in comparison to women who had their last child after 25 years, those who gave birth of their last child after 40 years had a 44% lower risk of EC (OR = 0.56, 95% CI: 0.47, 0.66). They also showed a linear decline in EC risk within increasing of age at last birth and 13% decrease in EC per 5-year delay in last birth (102).

The studies investigating the relationship between miscarriages and abortions in relation to EC development have been less conclusive. Some of the studies showed a protective effect (103), however, others could not find any association (104). The possible explanation of mechanisms involved in this association is very poor described in the literature. It was hypothesized that pregnancies that ended before the gestational age of 22 weeks could increase the risk of BC due to increased estrogen level and relatively low progesterone level at this time of pregnancy. This could provoke BC cells to grow in the light of future lactation, and then, in case of early ending of pregnancy, keep these undifferentiated cells. Interestingly, this hypothesis is still up to present time have not been applied to EC (105). The findings regarding provoked abortions and risk of EC are also quite contentious, showing positive (106), negative (107) and null association (98).

Breastfeeding

Breastfeeding is believed to cause protective effect against developing EC through suppression of gonadotrophin-releasing hormone following suppression of ovulation, decreasing circulating estrogen levels and increasing of progesterone levels.

First findings connected to the association between EC and breastfeeding have been for along time inconclusive and inconsistent (108). Most of the previous studies reported inverse association (109), however, there were some reports that could confirm this finding (98). Recent meta-analyses from the Epidemiology and Endometrial cancer consortium showed that ever breastfeeding gives a 11% reduction in EC risk (pooled OR 0.89, 95% CI 0.81–0.98) and longer duration of breastfeeding is associated with lower EC risk (110). Moreover, this study showed that the protective effect of breastfeeding lasts during the first 6-9 months of lactation period. According to some studies, it could be explained by additional effect of suckling stimulus that contributes to lowest levels of estrogens which are found in women that breastfed exclusively (111).

Menstrual Risk Factors

Age at menarche and age at menopause are the two most frequently studied risk factors in hormone dependent conditions including EC. Table 3 gives a brief overview for some of these studies.

Table 3. The risk of EC in relation to early menarche and late menopause

Author, year	Study type	Indicators	Type of measurement	Increase or decrease in risk
Brinton et al, 1992 (104)	Case-control study	Early menarche	Relative Risk	2.4 risk increase for age <12 vs ≥15 y
Reis and Beji, 2009 (112)	Case-control study	Early menarche	Odds ratio	9.43 vs later age of menarche
Zucchetto et al, 2009 (113)	Case-control study	Late menarche Late menopause	Odds ratio	0.7 decreased risk for ≥14 vs <12 y 1.8 decreased risk for age ≥ 55 vs < 50 years
Dossus et al, 2010	Prospective study	Late menarche Early menopause	Relative risk	7%-8% decreased risk 7%-8% decreased risk

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Link between late-age menarche, early-age menopause to decreased EC risk, along with association between early-age menarche, late-age menopause and increased EC risk are based on lifetime exposure to estrogens and number of menstrual cycles/number of menstruations women experience during the life. Older age at menarche is associated with a shortening of menstruation span and decreased risk of EC due to later initiation of ovulatory cycles and start of excessive exposure to estrogens. The recent dose-response meta-analysis has shown a 4% risk reduction for per 2 years delay in age of menarche (115). At the same time, later age at menopause can prolong the lifetime of menstrual activity and exposure to estrogens, and therefore increase EC risk. Relationship between EC risk and these two variables could be also confirmed by reciprocal association of age of menarche and age at menarche: the effect of later menopause can be attenuated by later age of menarche and on contrary, the effect of earlier menarche can be attenuated by earlier menopause (105). Several studies aimed to show the link between the number of menstrual cycles/years of menstruation and EC risk, and most cited ones are described in Table 4.

Table 4. The risk of EC in relation to number of years of menstruation and lifetime number of menstrual cycles

Author, year	Study type	Type of measurement	Main Variable	Risk estimates
Wang et al, 2015 (116)	Case-control study	Odds ratio	TNMC-Total number of menstrual cycles	**≤ 424** 1.00 (ref) **> 424** 1.40 (1.01-1.95)
Salazar-Martinez, E et al, 1999 (95)	Case-control study	Odds ratio	Index of anovulation (years without ovulation)	**≤ 26** 1.00 (ref) **27–59** 0.25(0.12–0.53) **60–104** 0.22(0.11–0.46) **≥ 105** 0.17(0.08-0.35)
Zucchetto et al, 2009 (113)	Case-control study	Odds ratio	Years of menstruation	**<33** 1.00 (ref) **33-36** 1.63(1.15-2.29) **>37** 2.43 (1.72-3.44)
Pettersson et al, 1986 (117)	Case-control study	Odds ratio	Menstruation span (number of years of menstruation)	For women <70 years **<25** 1.00 (ref) **25-29** 1.4 (0.5-4.1) **30-34** 2.61.(1.0-6.9) **35-39** 4.5 (1.7-12.0) **40+** 4.7 (1.4-15.9)
Xu W. et al, 2003 (107)	Case-control study	Odds ratio	Years of menstruation	**< 30** 1.00 (ref) **30+** 1.3(0.95-1.78) **35+** 1.93(1.38-2.7) **40+** 2.7 (1.7-4.4)
Yang et al, 2016 (118)	Case-control study	Odds ratio	Lifetime number of ovulatory cycles	**196.3-402** 1.00 (ref) **403-444.5** 1.3(0.85-2.00) **444.6-479.9** 1.5(0.92-2.42) **480-602.3** 1.9 (1.11-3.44)
Cusimano et al., 1989 (119)	Case-control study	Odds ratio	Years of fertile life	**< 31** 1.00 (ref) **31-35** 1.02 (0.35-2.99) **36-40** 1.21 (0.45-3.23) **> 40** 0.89 (0.24-3,28)
McPherson CP et al, 1996 (106)	Cohort	Relative risk	Years of ovulation	**≤ 33** 1.00 (ref) **33.01-36.25** 1.25 (0.76-2.09) **36.26-38.25** 2.00 (1.21-3.31) **38.26-40.50** 2.84 (1.74-4.62) **>40.50** 3.63 (2.21-5.95)
Dossus et al, 2009 (98)	Cohort	Hazard ratio	Risk per year of total menstrual lifespan	0.93 (0.91-0.95)
Wermli et al, 2006 (120)	Cohort	Hazard ratio	Menstruation span	**<30** 1.00 (ref) **30-34** 1.47 (1.01-2.14) **35-39** 2.69 (1.01-2.14) **40-44** 9.25 (2.88-29.7)

BMI

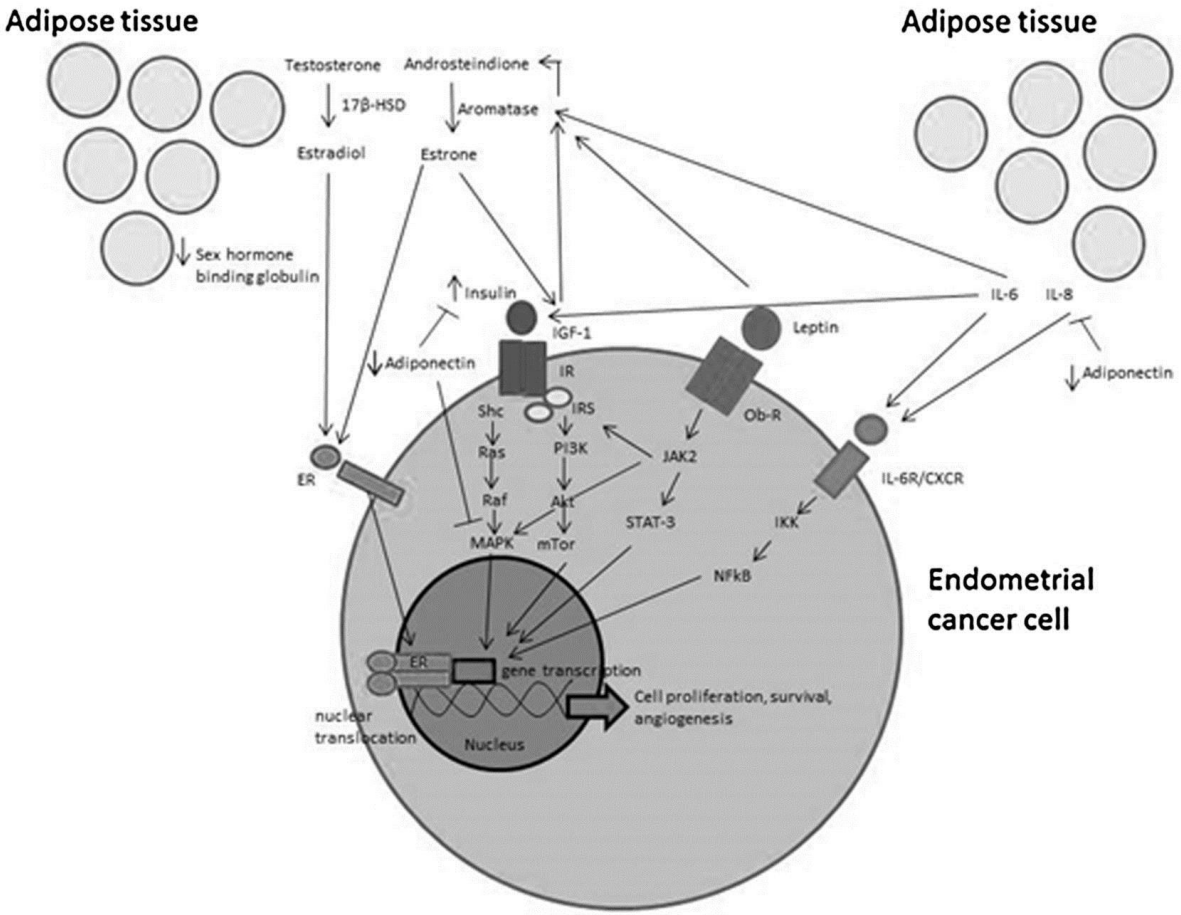
Excess body weight and obesity became a major challenge for public health (121). During the past four decades, the prevalence of obesity among women has more than doubled (122). In Norway the increasing of obesity is also observed which account for 20% of adult population (123). Increasing obesity epidemic contributed to increase of EC incidence rates specially in the Western World, although the lay public awareness and knowledge to this problem is shown to be limited (124). It has been shown, that obese women may have up to 6-fold higher EC risk compared to lean woman (125), and that association between BMI and EC in Europe is significantly stronger than in regard to most other cancer types (126). Crosbie and colleagues in their meta-analysis (127) reported that

effect of BMI is non-linear and those women who had BMI higher than 42 kg/m2 had a 9.11 greater risk of developing EC compared to women with BMI 22 kg/m2. Million Women Study (128) found a significant positive trend in the RR of incidence with BMI for EC (RR per 10 unit increase in BMI=2.89, 95% confidence interval 2.62 to 3.18). Studies investigation association between obesity and EC risk separately for pre-and postmenopausal women found higher risk for older women. Bjørge et al in their study of Norwegian women found the most pronounced effect of BMI (especially high BMI) in older age group (129).

The mechanisms lying behind the association between obesity and EC are linked to the following processes (130):

- excess estrogen production due to aromatization of androgens into proproliferative estrogens;
- direct mitogenic effect of estrogens produced from adipose tissue, which is not counterbalanced by progesterone due to reduced progesterone production in the light of chronic anovulation; this is considered to be the predominant determinant in pathogenesis of EC in obese premenopausal women (125);
- increase in local production of the mitogens insulin and IGF-1 (both are endometrial growth factors) through a reduction in insulin sensitivity;
- inhibited production of sex-hormone binding globulin (due to increased insulin level) that causes increase the levels of active estrogen;
- chronic release of high levels of inflammatory mediators;
- production of cytokines (leptin and adiponectin) in fat tissue that take part in endometrial carcinogenesis (115, 131);
- effect of transcription factors that regulate both tumorigenesis and cellular lipid metabolism (132);

However, several studies suggested that the mechanisms linked to obesity and endometrial canser risk development are different in pre- and postmenopausal women. In premenopausal women obesity is associated with anovulatory cycles and through this mechanism is associated with increased EC risk (133). In contrast, postmenopausal women with generally a lower oestrogen levels compared to premenopausal women, have adipose tissue as a primary source of endogenous E2. Thus, it is suggested that in these women the rate of production of circulating oestrogen is related to the size of the adipose depots (125). Summary of pathways involved in association between obesity with EC development are illustrated in Figure 11.



Abbreviations: 17β-hydroxysteroid dehydrogenase (17β-HSD), interleukin-6 (IL-6), IL-6 receptor (IL-6R), chemokine receptor (CXCR), leptin receptor (Ob-R), Janus Kinase 2 (JAK2), signal transducer and activator of transcription 3 (STAT-3), IkB kinase (IKK), insulin receptor (IR), oestrogen receptor (ER)

Figure 11. Pathways involved in association between obesity with endometrial cancer development.

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Other acquired or life-style factors

Smoking

Numerous epidemiological studies have been evaluated the association between cigarette smoking and risk of EC, showing an inverse association among ever smokers and somewhat stronger protective effect in current smokers compared to former smokers (134). Moreover, it has been shown that protective effect remains after cessation, if it occurs 1-4 years prior to EC diagnosis (134).

There are several anti-estrogenic mechanisms through which smoking can protect against EC:

- cigarette smokers are as usual leaner compared to non-smokers and thus potentially has less adipose tissue that is known to be an additional source of estrogens;
- smoking can decrease estrogen-derived cellular proliferation of endometrial cells through increasing of 2-hydroxylation of estradiol, increasing androgen levels (135) and by slowing down the decay of progesterone (136);
- direct destructive toxic effect of smoking on the oocytes (137), reducing number of ovarian follicles causing earlier menopause (138);

Remarkable, smoking has a unique ability to attenuate the effect of endogenous and exogenous hormones on endometrial carcinogenesis. Several studies reported that menopausal status plays an important role in association of EC and smoking, revealing reduction in EC risk in postmenopausal women and no association or even increased risk in premenopausal women (139). Further, among current smokers, in comparison to premenopausal women, postmenopausal women have about 20% lower estriol excretion rates (140). It has been also demonstrated that smoking has an impact on level of circulating estrogens and can attenuate the effect of oral estrogens on for example bone density and serum lipids (141, 142). Moreover, EC risk reduction by smoking is known to be stronger among MPT users versus nonusers (139).

Physical activity (PA)

The known link between PA and EC is mostly based on weight control and following improvements in hormone metabolisms. Most of the studies investigating this association showed an inverse relationship with up to 22% of risk reduction associated with recreational PA (143). Further, numerous other studies also reported inverse association (144-147). Thus, recent findings from NOWAC Study showed dose-response trend in decreasing the EC risk within increasing of PA levels from lowest PA level giving HR=1.6 (95% CI 1.16-2.2) to highest PA level with HR =0.73 (95% CI 0.45-1.16) compared to the median level (148). This study showed that 21.9% of EC could be avoided, if women with PA level ≤ 4 in 1-10 degree scale could have instead increased their level of PA up to 5-10. The main area for discussion in analyzing the data based on association between EC risk and PA is linked to BMI, which is believed to be an important confounder affecting hormone profiles. However, several studies, including recently mentioned NOWAC Study, were able to report no significant effect modification for BMI, confirming independent effect of PA (144, 145, 149-151). Modifying other hormonal risk factors involved in endometrial carcinogenesis is another hypothesis lying behind the association between PA and EC. Thus, it was hypothesized that increased physical activity could contribute to later menarche and amenorrhea, two conditions that are linked to reduced EC risk (152). Moreover, alternative mechanism could be

based on enhanced absorption of steroids due to increased bowel motility in physically active women (153).

Diabetes

Along with the well-known effect of unopposed estrogens, insulin resistance and enhanced metabolism of related growth-factors are associated with increased risk of EC. Studies investigating this association have reported up to 80% increased risk of EC in women with type 1 diabetes and a 2-fold increased EC risk in individuals with type 2 diabetes (154-156). In addition, some of the studies pointed the importance of having diabetes in younger ages, showing a higher RR of EC that had diabetes at age less than 40 and 50 years old (157, 158).

The most described changes involved in the association between diabetes and EC development are:

- growth-enhanced properties of insulin, increased activity and levels of IGF-I receptor in tumor cells, caused by suppressed gene expression of endometrial IGFBP-1 (159-161).
- insulin resistance, compensatory hyperinsulinemia and elevated levels of insulin growth factor cause inhibition of hepatic synthesis of sex hormone binding (SHBG) and stimulate ovarian synthesis of sex steroid hormones (162);
- deregulation of fatty acid synthase activity, chronic inflammation and oxidative stress (163);

The overview over steps of pathogenesis in relationship between cancer and diabetes is illustrated in Figure 12.

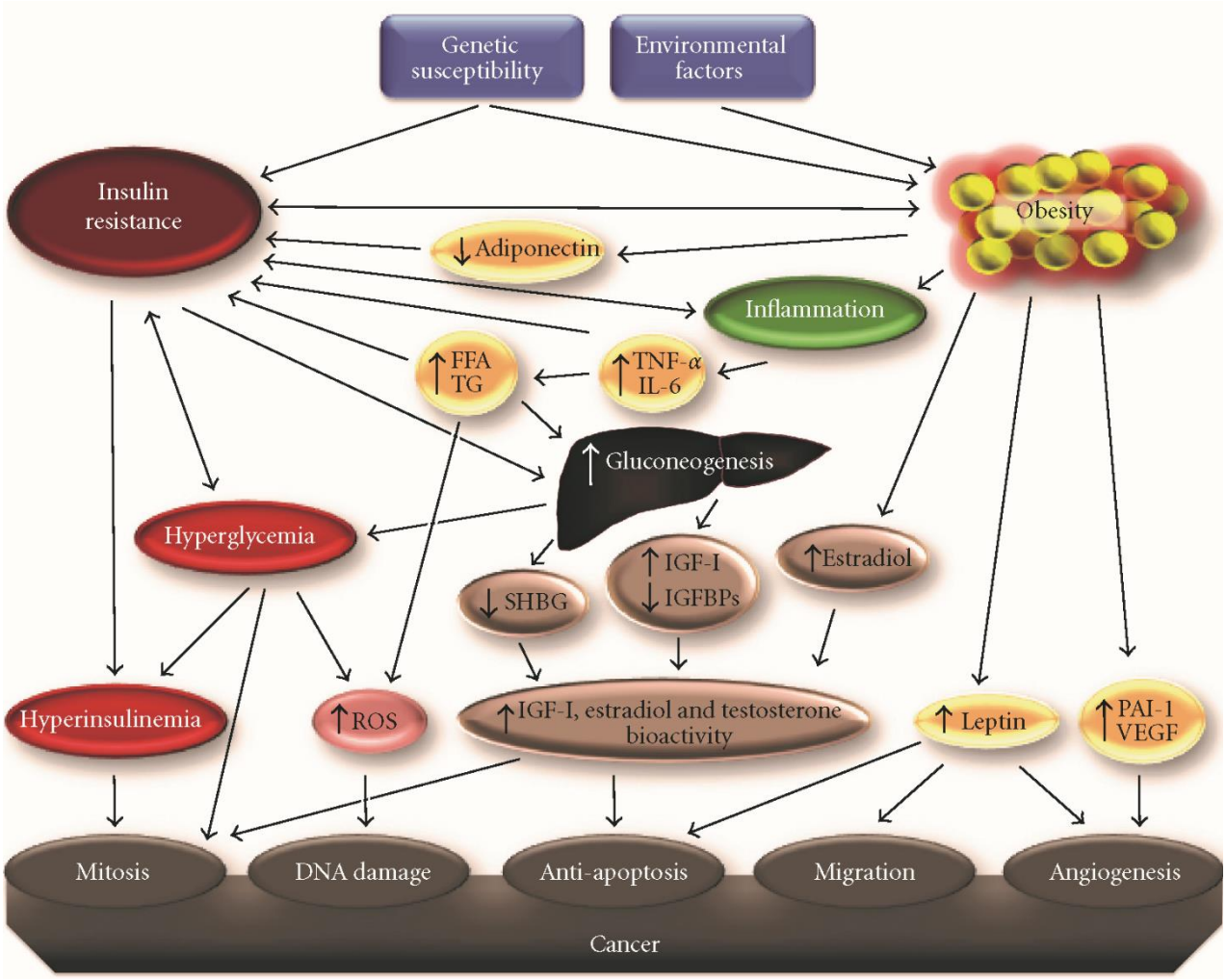

Figure 12. A multi-step model of cancer development associated with insulin resistance. TG: triglycerides; FFA: free fatty acids; TNF-α: tumor necrosis factor α; IL-6: interleukin-6; ROS: reactive oxygen species; SHBG: sex-hormone-binding globulin; IGF-I

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Nutritional risk factors and EC

Intensive rise in EC incidence catalyzed a cascade of studies related to prevention strategies including investigating effect of diet. It has been hypothesized that diet independently of obesity may play a role of modulating of chronic inflammation which is known to be an important risk factor and one of the possible reasons for EC development (164). During the last decades there have been a numerous studies investigating different aspects of diet related to EC risk such as saturated fat intake (increased EC risk) (165, 166), soy/fiber products (decreased EC risk) (167) and vitamin supplementation (decreased risk)(168). However, according to the report from World Cancer Research Fund 2013 (WCRF), there is a limited evidence of association between EC risk and specific dietary components with exceptions on coffee consumption (protective affect) and possible negative association with glycemic load (169). However, recent case-control study from Italy reported a statistically significantly lower EC risk in women with high vegetable intake, high

adherence to the Mediterranean diet and low dietary inflammatory index (170). This could be explained that Mediterranean diet is phytoestrogens and several antioxidants that have a protective effect on EC development.

Coffee consumption

Coffee is one of the most frequently consumed hot beverage in the world, which is in spite of known adverse effects, is more associated as a potential source of antioxidants and anti-mutagenic compounds. The latter attractive features of coffee have raised the interest of investigating association between coffee consumption and different cancer types, including EC. Since 1986, when the first study investigating effect of coffee on EC cancer was conducted (171), variety of studies with different design have address this epidemiological question and found in most of the reports a decreased risk of EC (171-176) (Figure 13). The RR of total consumption in two recent meta-analyses from 2015 and 2017 were almost identical: 0.80 (95% CI: 0.74-0.86) (177) and 0.79 (95% CI 0.73-0.87) (178), respectively. A meta-analysis from 2015 found in addition stronger effect in never hormone users (RR 0.60 95% CI 0.50-0.72) and in women with BMI ≥ 25 (RR 0.57 95% CI 0.63-0.94) along with dose-dependent relationship in caffeinated coffee, decaffeinated coffee and caffeine intake. Meta-analysis from 2017 has also reported a 24% EC risk reduction in postmenopausal women (178).

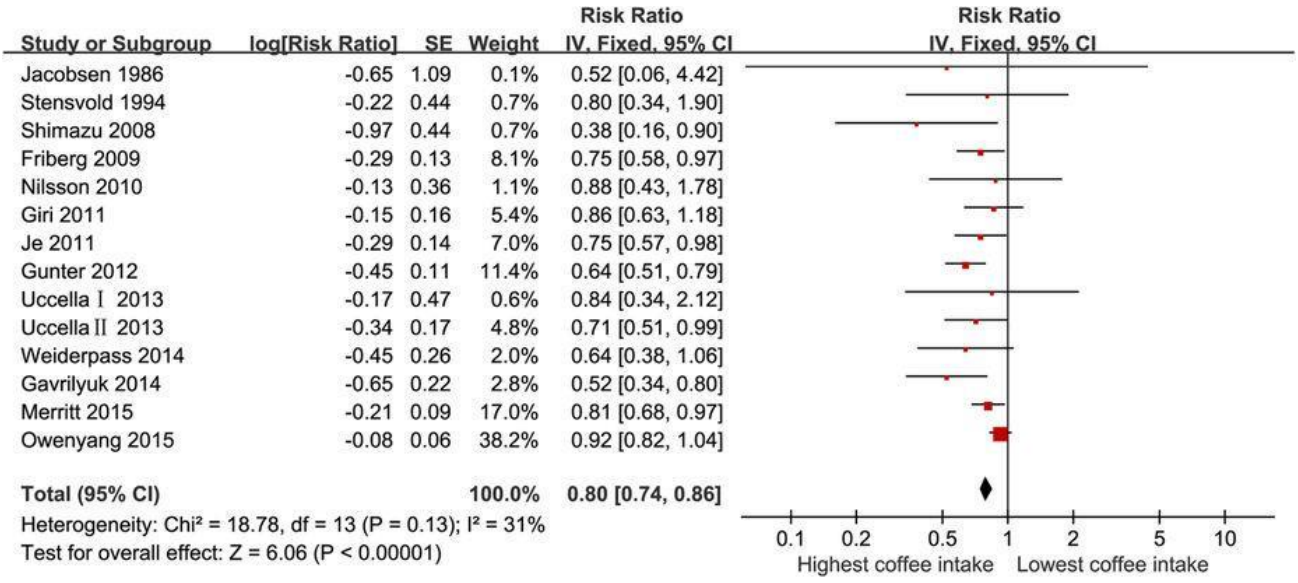

Figure 13. Overview of prospective cohort studies used in meta-analysis 2015.

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1.2 Gene expression

Gene expression in the light of central dogma of molecular biology

Nucleus of eukaryotic cells are the unique carriers of individual set of protein-coding genes that are stored in DNA and determine the cell function. The central dogma of molecular biology, which was first introduced to the scientific world by Francis Crick, describes how a gene is ultimately expressed (179). Basically said, when the cell receives a command about expression of a certain gene RNA polymerase sticks to this actual region of DNA where this gene is located and makes a RNA copy of it (transcription) (Figure 14). Then, this RNA copy goes out of the cell's nucleus and transfer biological information further into protein through translation process in ribosomes. This simply explained process of how the biological information can be transferred from DNA to RNA and further to protein product is actually gene expression.

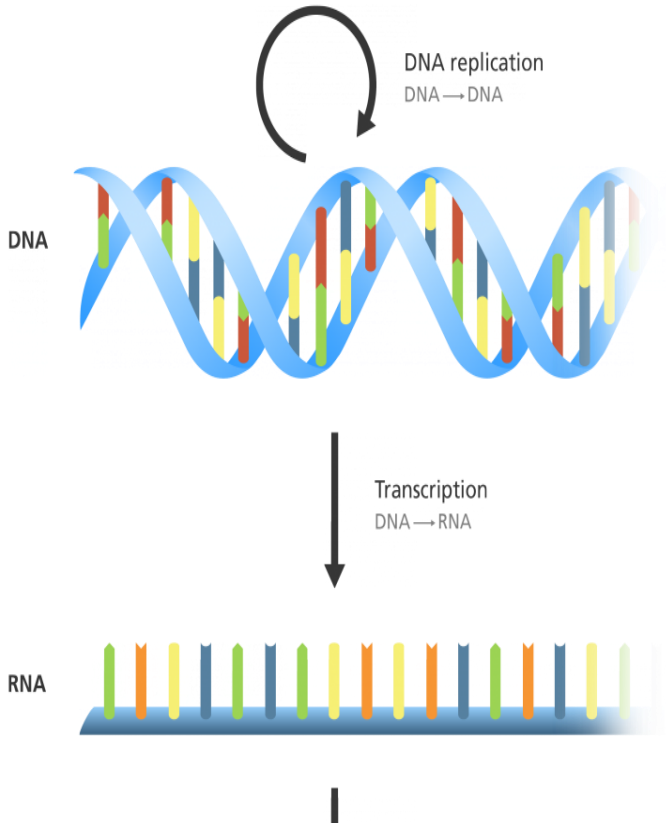

Figure 14. The central dogma of molecular biology.

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Remarkably, that even though each cell nucleus contains thousands and thousands of genes, only a part of those genes transforms into messenger RNA (mRNA) transcripts at any given time and thus, produce of certain amount of a particular protein. In this context, measurement of gene expression level is linked to the level of abundance of mRNA produced during transcription (180).

Controlled and regulated production of a certain number of proteins is the basement of balance between synthetic (transcription, translation) and degradative (enzymatic breakdown of RNA transcripts and existing protein molecules) biochemical mechanisms. Control of synthetic mechanisms is crucial in regulating what proteins and in what amounts should be present in the cells. This ability allows cells to be able to adapt to changes caused by different agents in their environment and as a result, to change gene expression in response to exposure (for example, particular risk factor).

Microarray technology

The use of microarray technology allows to measure the expression of a big number of genes. In this thesis, the analysis of gene expression data is based on the measurements obtained from whole blood RNA samples, which were stored in PAXgene tubes. Generally, microarray analysis consists of the following basic steps (Figure 15):

I. Construction of Microarrays
   - Preparation of probes (cDNA fragments or oligonucleotides) complimentary to a set of both coding and non-coding human genes (expression of approx. 20 000 genes might be tested by a microarray platform);
   - Spotting probes onto a solid substrate (for example, glass slides or membrane);

II. Preparation of samples
   - Blood or tissue sample collection;
   - mRNA isolation, purification;
   - Synthesis of cDNA or cRNA from mRNA;
   - Fluorescent (in our project) or radioactive labelling of cDNA/cRNA;

III. Hybridization
   - Hybridization (selective complementary base pairing between the study samples and probes on the array);
   - Washing away unbound material;

IV. Analysis
   - Scanning by quantifying of signal intensities (mRNA abundance) using a chemiluminescence detector;
   - The level of expression of a certain gene correlates with intensity of the signal: the stronger the signal, the more expressed the gene;
   - Data analysis;

In this thesis, studying of blood gene expression allowed us to reveal which genes were activated or deactivated at the time of blood donation, and how these changes in gene expression were related to the association between different exposures and EC.

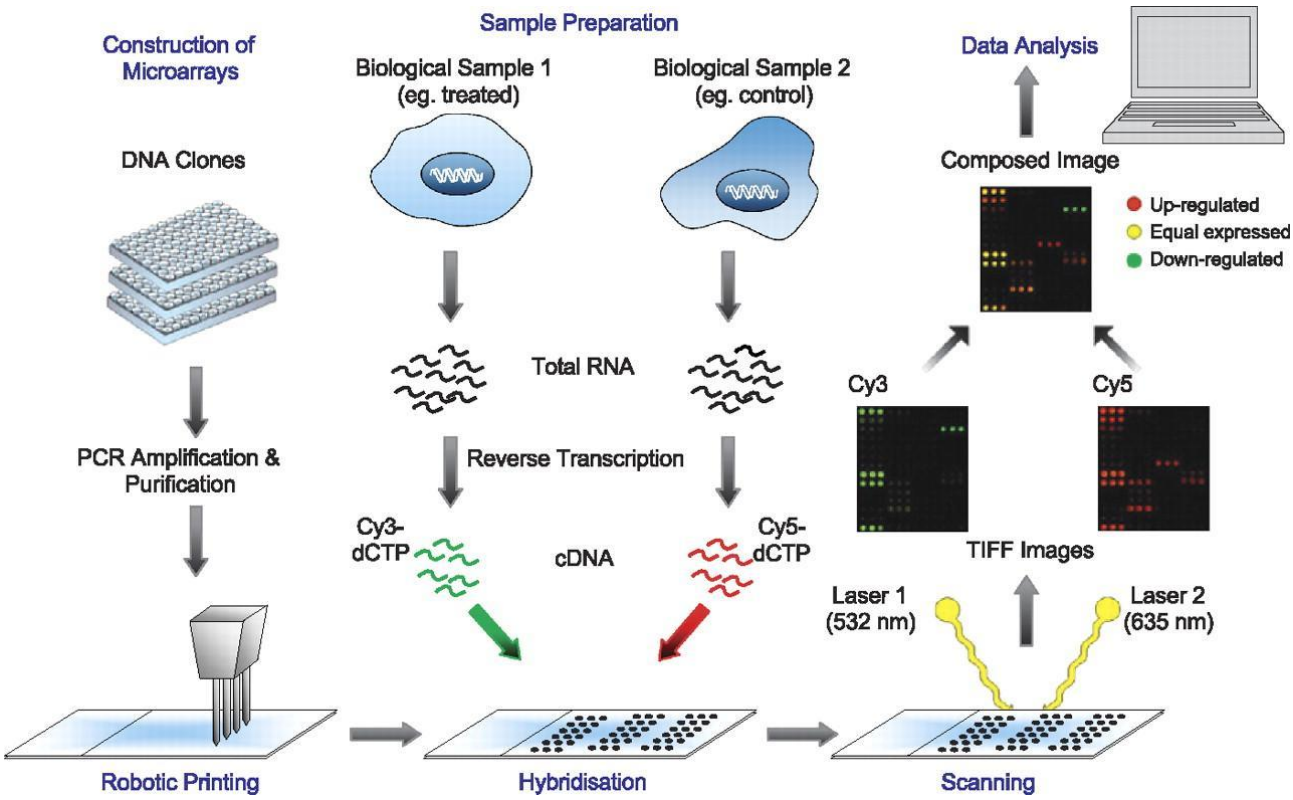

Figure 15. Example of basic steps of microarray technology.

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Blood as a target tissue: potential benefits and limitations

Rapidly developing high-throughput genomic technologies expanded the opportunities for genotyping of large number of samples and our better understanding of different steps of carcinogenesis. However, using of such technologies have several limitations, included necessity to obtain in most of the cases a sample from a certain specific tissue. Such way of sample collecting, like for example, biopsy from a visceral organ could be possible in case of planned surgery or as a part of diagnostics in already manifested disease, otherwise, in many other cases and especially, in case of obtaining tissue-sample in a healthy control is difficult to perform (181). In this context, collecting of peripheral blood is relatively non-invasive procedure that does not necessarily require hospital admittance or even attendance and could have been performed at the first level of health care institutions (general practitioner's office). Moreover, being a "surrogate transport tissue" (182), that interacts with all other tissues in the body, peripheral blood mirrors all the physiological

processes connecting to both normal functioning and pathological changes in our body. Altogether, non-invasiveness of the sample collection process, feasibility of their use in human population studies and unique opportunity of blood to reflect all the physiological processes, make blood sampling a valuable tool for integrating the principles of basic science in modern epidemiology.

"If you have cancer and you are a mouse, we can take good care of you…"

Judah Folkman

Performing biomedical research using murine models and cell lines, and further translation of the obtained results on human trials gets an increasing number of critical discussions. Although non-human models have contributed a lot to our general understanding of pathogenesis of cancer and other diseases, there is still a huge significant divergence in humans and mice, for example, in terms of physiology, immune systems functioning and carcinogenesis (183). According to some studies the average rate of successful implementation of animal models in human clinical cancer trials is quite low and comprises less than 8% (184). Thus, developing of biobank research turned a biomedical research towards an alternative approach, when it became possible to develop preventive, diagnostic and treatment strategies based on compatible human samples.

1.3 Systems epidemiology approach

The traditional epidemiology has been built up to determine the occurrence of the disease in a population, aiming detecting the higher risk symptoms by investigating the association of the certain exposures and diseases, and further on, developing a health improving messages to the public with further primary and secondary prevention strategies. The described approach, however, has a minimal focus on the mechanisms and sometimes almost ignores the biological background lying behind these associations. This phenomenon is well described as a "black box of epidemiology" (Figure 16) (185).

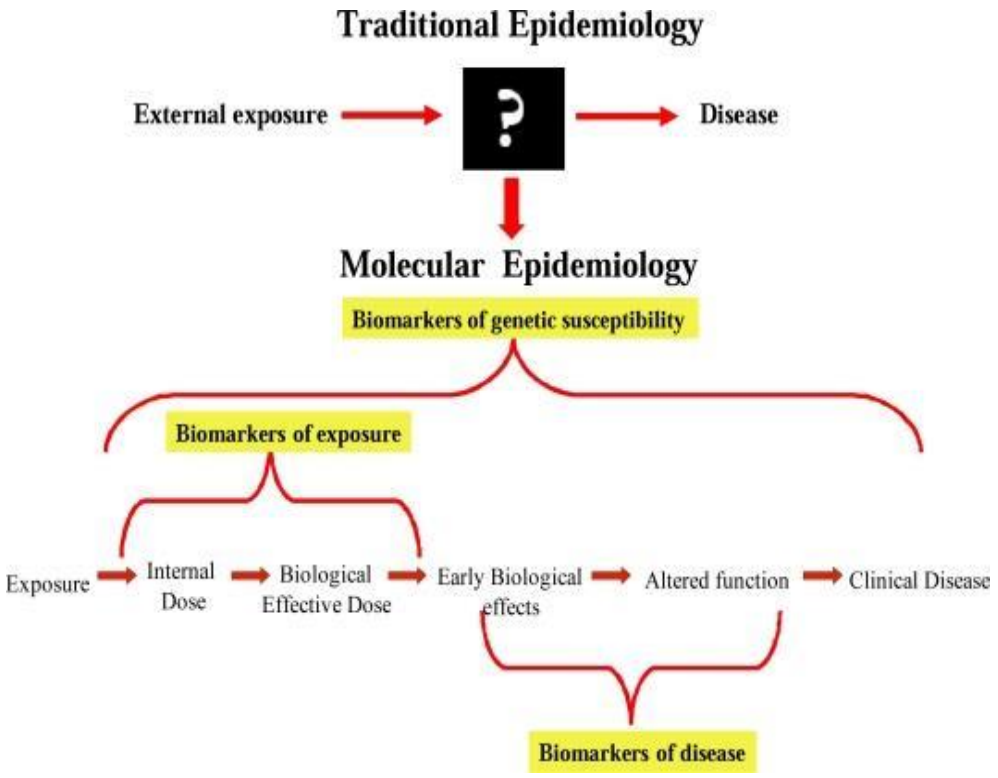

Figure 16. "Black box epidemiology" phenomenon.

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On the contrary, classic molecular science has for a long time investigated how a specific single gene or a protein solely can influence a biological phenotype. Further advances in high-throughput technologies opened a new revolutionary era of "multi-level omics approaches" and created new definitions for novel integrated "systems disciplines" like for example, systems biology (SB) (186) or recently presented systems immunology (SI) (187). Thus, SB discipline moved traditional molecular biology to the next step of basic science development, focusing on cellular signaling networks between the cells, stroma, organs, and finally, on how all these changes work in the entire organism. This expands our understanding, how intracellular environment of the normal cells is affected by carcinogenesis, and how these findings can be further implemented in developing of new cancer-therapy strategies and predictive models. However, this approach has also several limitations and among others-a minimal focus on individual life's exposures and on possible normal variation of the gene expression in human based on a big large-scale population-based data. In this context, Norwegian Women and Cancer (NOWAC) Study is a big cohort population-based study, that on the one hand contains a big sample size for valid estimations of relationship between different lifetime exposures and diseases, and on the other hand has biological samples that were taken during different life periods of cohort participants. Biological material could be further analyzed on the high dimensional gene expression level according to the available

exposure information and could tell us, how different risk factors affect the gene signatures in both those with disease (cases) and without it (controls) in doe example, nested case-control study. This integrated multi-level approach, already known as systems epidemiology (SE) (188) could serve as an important additional gap in further investigating of multifactorial nature of carcinogenesis.

2. OBJECTIVES

The overall objective of this doctoral thesis was to elucidate the effect of some of the risk factors for EC and to implement systems epidemiology approach to the large-scale population-based Norwegian Women and Cancer Study.

More specific objectives are highlighted as following:

- To gain further insight into association between coffee consumption and EC risk in postmenopausal women within NOWAC cohort and to examine how this association interact with BMI (Paper I)
- To assess the combined effect of reproductive and menstrual factors using a composite variable LNYM (Lifetime number of years of menstruations) and to calculate population attributable fraction of LNYM within NOWAC cohort (Paper II)
- To investigate gene expression signatures in blood by direct testing of hypotheses obtained in papers I/II (Paper III);

3. MATERIALS AND METHODS

3.1 Study populations

This thesis is based on data from prospective cohort study, the Norwegian Women and Cancer study (NOWAC) (Paper I and Paper II) and its subcohort – The NOWAC Postgenome Study (Paper III).

3.1.1 The Norwegian Women and Cancer Study (NOWAC) (Paper I and Paper II)

The Norwegian Women and Cancer (NOWAC) Study (https://site.uit.no/nowac/) was initiated in 1991 as a study that was created initially to build up a national-based cohort that would be representative for the entire Norwegian female population. Such design would allow calculation of both RRs and attributable risks in regard to certain cancer types and exposures with a primary aim of investigating the relationship between OC use and BC. All the NOWAC participants were selected based on the random sampling of women from the Central Population Register (CPR). This selection was possible due CPR's information about all women living in Norway, including those temporary residents, refugees etc. The availability of this information is based on the unique national 11-digit identity number, which is assigned to each inhabitant and is used in all official registers in Norway. The personal number then is transformed into a serial number by Statistics Norway, department that was responsible for sending the reminders to non-responders in form of a postcard.

The process of enrollment of NOWAC participants is complex and consists of several waves, where the first recruitment period was distributed into 24 different mailings over seven years. Such arrangement was constructed due to logistics, financial reasons and need in methodological sub-studies. These women who answered the first questionnaires received also invitation to fill in a follow-up questionnaire. In addition, in order to increase response rate, two reminders per serie were sent to both first and follow-up questionnaires. The last updated version of NOWAC recruitment scheme representing all mailings that were sent during different periods is shown in Figure 17 and Figure 18. This scheme has been constructed according to sending of first, second, third and fourth questionnaires:

- The red boxes represent the **first** or "enrollment"/"baseline " **questionnaires** that have been sent in three main time points: 1991, 1995-1997, where of 179 388 women aged 30-70, 102 443 agreed to participate (all together series 1-24 with a response rate 57.1 %) and

2003-2007 (series 35-36, 40, 41, 43-45) with a response rate 48%. Response rates were calculated with correction for emigration, death and unknown addresses;

- The green boxes and yellow boxes represent the distribution of second and third questionnaires respectively. These "follow-up questionnaires" have been sent to the women that had already received questionnaires before. Depending on the planned study design, they had different content and were sent in different periods. Dispensation of a **second questionnaire** was started during the period 1998-2002 and were sent to those recruited in the first 24 mailing series (series 25-29 with response rate 81%) (189). The second wave of sending of these "green" follow-up questionnaires was in 2011 and 2014 and was meant for those women who received first "red" questionnaires in 2003 and 2004. Distribution of a **third questionnaire** started in 2001 (2001, 2003, 2004, 2005 and 2010) and completed some additional information for women who received both "red" and "green" questionnaires. Women who responded to both second and third questionnaire comprised 80.7% (unpublished data);
- Finally, the next round of sending of fourth questionnaires for the new participants has been recently started in 2017;

As it was mentioned above, the questionnaires were designed according to the different purposes (research hypothesis). Moreover, they have been sent in different time points. As a consequence, different subgroups of NOWAC participants had a various set of information. During all sending rounds women received a standard letter of invitation with a short description of study aims (see Appendix 1 as an example of invitation letter and first enrollment "red" questionnaire), prove of approval from the ethical committee, approval from the legal right to keep data computerized (by Norwegian data inspection board) and information on right to be withdrawn from the study at any time. The core information presented in the first questionnaires was devoted to questions related to OC use (ever/never use, use before first birth, total duration and etc). Among general information women were also asked about age at menarche, menopausal status, number of children, lactation, smoking, medical history, physical activity and dietary intake. The later series on questionnaires varied in content and had in addition questions on other lifestyle factors such as sun bathing habits, HRT and medication use. In addition, a more detailed food frequency questionnaire (FFQ) was added in 1996.

3.1.2 The NOWAC Postgenome study (Paper III)

The NOWAC Postgenome study has been built up within the original NOWAC study (Figure 17 and Figure 18) based on the prospective collecting of blood samples from women that already

participated in study and gave an informed consent for blood sampling. These women were randomly selected in series of 500 from the whole NOWAC Study. All potential participants received a folder consisting of a consent form, special tubes for blood collecting, and two-page questionnaire that women answered at the time of blood sampling. Those who agreed to participate, have been offered to perform the blood sampling in their local GP's office. Obtained blood samples then were sent by ordinary mail to the NOWAC centers, where they were further prepared to be stored in -80 °C. Initially, as a part of collaboration with EPIC Study (190), all the blood samples were processed in order to preserve frozen buffy-coat samples. However, a pilot study in 2001 that was later conducted in NOWAC, showed that the quality of the obtained samples was insufficient in terms of RNA isolation and gene-expression analyses. Therefore, during the next waves of sample collection, which took place in period 2003-2006, all the blood samples were split in 2 aliquotes: 1 – for plasma and buffy coat isolation and 1 – for RNA isolation using a PaxGene Blood RNA System (191). The latter tube type contained a special RNA stabilizing agent, which was meant to improve the quality of RNA preservation. By 2006, the blood sample collection comprises 48 943 women born between 1943 and 1957, and became a baseline for core postgenome cohort (blood drops in Figure 17 and Figure 18).

3.2 Ethical approval

All the studies included in this thesis are based on informed written consent from each participant in NOWAC Study and performed in compliance with the Declaration of Helsinki. The information on cancer diagnosis is obtained by Statistic of Norway via linkage to the Cancer Registry of Norway, which delivers further the information to researchers using the serial number instead of original identity number. Women participated in NOWAC Study were informed about these linkages. In addition, those women who donated blood samples were informed that the blood samples would be used for gene expression analyses. In accordance with the Norwegian Biobank Act, the studies based on gene expression analysis were approved by the Regional Committee for Medical and Health Research Ethics (REK Nord) and the Norwegian Data Inspectorate (REK number for the biobank in NOWAC 141/2008, REK number for the study described in paper III-2012/413).

Some general issues concerning study sample used in the papers

Due to a variety of information used in the current thesis from different types of questionnaires, it was decided to show the examples of mailing series/invitation letter, which can be found in appendix:

- Appendix 1: Example of first red questionnaire. Serie 11 (Paper I and Paper II);
- Appendix 2: Example second green questionnaire. Series 28-29 (Paper I and Paper II);
- Appendix 3: Example for pamphlets on OC and hormone replacement therapy. (Paper I, Paper II and Paper III);
- Appendix 4: Example third yellow questionnaire. Serie 39 (Paper I and Paper II);
- Appendix 5: Letter of invitation and information to the NOWAC study;
- Appendix 6: Example of English version of blood questionnaires;

Paper I and Paper II are restricted to postmenopausal women due to limited number of premenopausal women who had information required for subgroup analyses available. At the same time, in paper III we included both pre- and postmenopausal women. This approach was chosen to keep as much cases as it possible due to complexity of the study design. The other inclusion criteria along with exclusion criteria are otherwise specifically described in the next sections devoted to each paper.

3.3 Study sample for Paper I

For different subgroup analysis in Paper I we used the data from NOWAC questionnaires of different series and years (Figure 17), but initial sample size was based on women who filled in the baseline questionnaires 1991-1997 and 2003-2007 (all-together 129 854 women).

We then selected those who was postmenopausal from the start-point of inclusion to the study or became postmenopausal by the end of the follow-up for this paper, which was set to 31st of December 2010. After all exclusions highlighted in Paper I, we ended up with 97 926 postmenopausal women at start of follow-up, of which 462 women developed incident EC.

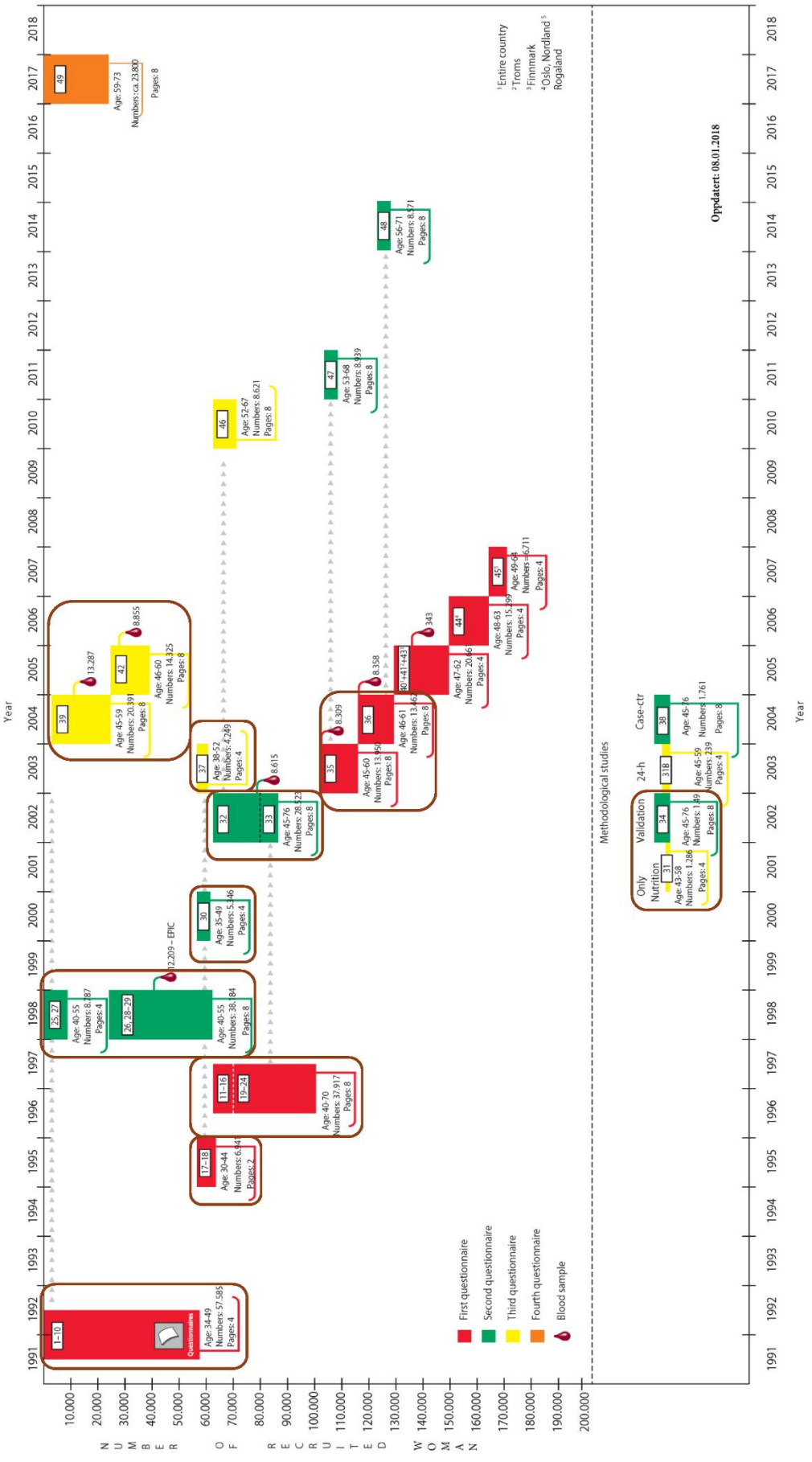

Figure 17. Data used in paper I (brown rounded rectangles). Based on enrollment to Norwegian Woman and Cancer Study.

3.4 Study sample for Paper II

With the only exception on age at menopause, the information about other variables used in analysis for paper II was obtained from the baseline questionnaires (first "red questionnaires"), which were received by women in the period 1996-2006 (Figure 18). Women's age at entrance to the current study varied from 27 to 65 years with the largest group among those who were between 42 and 58 years old (87%). For those women, who became postmenopausal later during the follow-up (from the start of the current study until 31 December 2014, we used updated information on postmenopausal status from the follow-up questionnaires (second "green questionnaires"). After all exclusions, which are in details described in corresponding paper II, the final study cohort included 117 589 postmenopausal women, of which 720 women developed incident EC.

3.5 Study sample for Paper III

Paper III is nested case-control study based on information obtained from 8-pages questionnaires and blood samples collected in 2002-2005 (Figure 18). The main concept of this paper represents a systems epidemiology approach by testing the epidemiological hypotheses obtained from a large-scale data on a gene expression level data within the same cohort. We therefore chosen the factors/variables, which were evaluated in paper I/paper II and among them selected those, that had strongest effect on EC risk in the whole NOWAC cohort. The main steps of selecting the NOWAC participants and exclusions based on quality control procedures are in details described in corresponding paper.

After all exclusions based on quality control described in details in paper III, the final dataset available for analysis consisted of 158 individuals (79 case-control pairs) with 47 248 microarray probes for each.

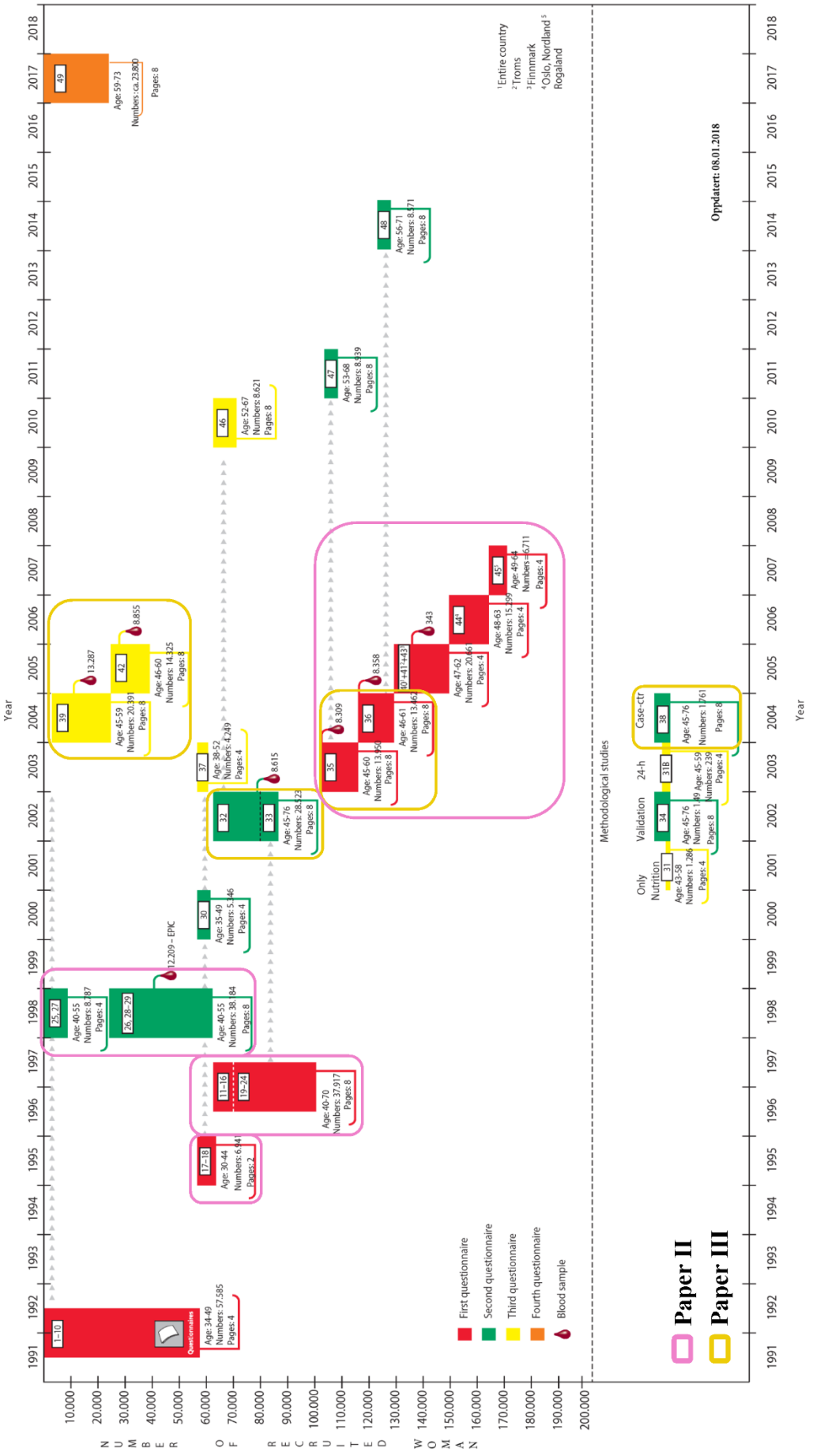

Figure 18. Data used in paper II and III. Based on enrollment to Norwegian Woman and Cancer Study.

3.6 Central variables

Coffee variable

Information on coffee consumption was derived from NOWAC questionnaires (example in Appendix 1), where the women were asked to report how often they consumed the coffee during the preceding year by ticking suggested fixed frequencies. Of note, this information was very differently presented in various series of first, second and third questionnaires. For example, questionnaires from 1991 to 1995 in general had a very limited number of dietary questions compared to 1996 and onwards. In addition, the formulation of coffee questions differed along the whole way of recruitment and follow-up. As a result, one group of women got the questions just on total consumption while another group answered just on their preferences in different brewing types. The distribution of number of participants and cases that had information on a certain type of coffee is illustrated in Appendix 7.

Formulation of "number of cups categories" were also differently presented. In order to increase the statistical power and sample size, we have pooled the data together and got a common version of frequencies for both the total coffee version and the brewing method version of the questionnaires (Appendix 8).

LNYM variable

Lifetime number of years of menstruation (LNYM) is a central variable of paper II and one of the investigated risk factors in paper III. This is a composite variable, which was calculated in a following way:

LNYM = age at menopause *minus* age at menarche *minus* cumulative duration of full term pregnancies (calculated as the number of full-term pregnancies, including live and stillbirths, times 0.75 years) *minus* duration of breastfeeding (calculated as the cumulative number of months of breastfeeding in all pregnancies) and *minus* duration of OC use, *minus* 12 weeks for each incomplete pregnancy (for those women who had this information available). All the mentioned variables were added on a continuous scale in years. LNYM was further classified into 5 categories: <25, 25-29, 30-25 34, 35-39, ≥40. Additional analysis related to including incomplete pregnancies into LNYM calculation was performed just for paper II.

Parity

Parity was calculated as categorical variable for showing distribution in all three papers. As a continuous variable, parity was used for adjustment in multivariate analysis in paper I and as a part of LNYM in paper II. In paper III, parity is a central variable that was also calculated on a continuous scale, showing the changes in gene expression within having each additional child.

Body mass index (BMI)

BMI was calculated as weight divided by height squared (kg/m2). For all three papers we used information on height and weight that were measured at baseline (first time the participants filled the questionnaires containing these questions). For paper I and paper II, BMI was categorized as <20, 20-24.9, 25-29.9, and ≥30 to show the detailed distribution. In the subgroup analysis of these papers BMI was classified into 2 categories: <25 and ≥25. For paper III, BMI was calculated as a continuous variable.

Menopausal status/age at menopause

Both for paper I and paper II menopausal status was derived from the questions on menstrual regularity. Women were classified as premenopausal if they answered that they still had regular menstruation. If women reported that their menstruation had stopped at the time of enrollment or during the follow-up they were classified as postmenopausal. However, we have differently treated the missing information on this variable in paper I and paper II. In paper I, in case of uncertain information (irregular menstruations, MHT use or otherwise insufficient information) we set 53 years old as a cut-off for age at menopause as it was previously used in earlier NOWAC reports based on the definition used in The Million Women Study (192). In paper II, missing information on age at menopause was treated according to smoking status as the recent publications showed that women who smoke have earlier menopause (193).

In paper III, due to a limited sample size we have included both pre- and postmenopausal women, but have used "age at menopause" as a continuous variable, showing how gene expression changes with increase of age at menopause.

OC use

OC use was defined as ever or never users. Duration and type of OC were not evaluated.

Smoking status

Smoking status in paper I and II was coded as never, former, current or missing. Women who reported either being current or former smokers were also categorized as "ever smokers". Duration of smoking and number of cigarettes were not considered in the papers included in this thesis.

Data from Cancer registry of Norway

The Cancer Registry of Norway is one of the oldest national cancer registries in the world (established in 1951). It is obligatory for all medical practitioners in Norway to notify about the new cancer cases and for pathology departments to send the copies of their reports to the Cancer Registry. By 2001-2005 the completeness of recording uterine cancer cases was high (99%) (194).

Topography codes were converted first to ICD 7th version in 1970 and then to ICD-10 in 1993. At the present time, The Cancer Registry of Norway provides information for both ICD-7 and ICD-10. For all three papers, we have used ICD Revision 7 and 10 with corresponding code 172 for corpus uteri in ICD-7 and analogue code C54 from ICD-10. Using different ICD-coding did not affect the main findings of our papers.

3.7 Statistical methods

The main focus of this thesis is not connected to statistical analysis, therefore only brief description of the used methods will be mentioned here. Otherwise, more detailed information on statistical steps could be found in respective papers.

Statistical analysis was performed using SAS version 9.2 and STATA version 14.0 for paper I and paper II respectively. Cox proportional hazard regression models (195) were used to examine the association between the relevant exposure variables and postmenopausal EC risk. Multivariate analysis in both papers was carried out to control for the potential confounding effect of other variables (for details see corresponding papers). The analyses of Schoenfeld residuals were used to test the proportional hazard assumptions and there was no evidence of deviation from proportionality. We also used Wald test to assess the heterogeneity in effects between different brewing methods (paper I) and to check for any non-linear relationship between LNYM-variable and postmenopausal EC risk. In paper II we have also used Royston-Parmar flexible parametric proportional hazard models (196) to estimate the baseline HRs according to different LNYM categories and cubic splines (197) to show the dose-response associations between LNYM and EC risk.

Gene expression analysis for paper III was performed at the Norwegian Computing Center. The analysis was done by using R with Bioconductor packages. First, potential confounders were evaluated by comparing cases and controls using independent sample t-test, Mann Whitney U-tests and Chi square tests. Then, using Limma packages (198) analysis with gene-wise linear models was conducted in order to evaluate the difference in single gene expression between cases and controls. The same method was used to identify the differentially expressed gene sets and to evaluate whether these genes and gene sets were influenced by one of the variables (parity, LNYM, coffee consumption, BMI or age at menopause). Description of steps in statistical analyses with equations are in details presented in paper III.

4. MAIN RESULTS

Paper I

High coffee consumption and different brewing methods in relation to postmenopausal endometrial cancer risk in the Norwegian women and cancer study: a population-based prospective study.

For the present analysis we included 97 926 postmenopausal Norwegian women from the population-based prospective Norwegian Women and Cancer (NOWAC) Study. Among them, 462 developed incident EC during an average of 10.9 years of follow-up. After multivariate adjustment, we found a significant risk reduction among participants who drank ≥8 cups/day of coffee with a hazard ratio of 0.52 (95% confidence interval, CI 0.34-0.79). We did not observe a significant dose-response relationship. We also did not observe significant heterogeneity in risk when comparing filtered and boiled coffee brewing methods. A reduction in EC risk was observed in subgroup analyses among participants who drank ≥8 cups/day and had a BMI ≥25 kg/m2, and in current smokers. The results of this paper suggest that in Norway, in population with historically high coffee consumption rates, EC risk decreases in women consuming ≥8 cups/day, independent of brewing method. According to our results the protective effect of coffee consumption is more pronounced in obese women and in current smokers.

Paper II

Lifetime number of years of menstruation as a risk index for postmenopausal endometrial cancer in the Norwegian Women and Cancer Study.

Lifetime number of years of menstruation (LNYM) is a measure the effect of all reproductive factors combined, reflecting the cumulative endogenous estrogenic exposure during lifetime. Based on the data from a prospective population-based cohort study of 117 589 postmenopausal women, including 720 EC cases, we studied association between the number of years of menstruation and EC risk. Lifetime number of years of menstruation (LNYM) were computed taking into account age at menarche, age at menopause, cumulative duration of full-term and incomplete pregnancies, breastfeeding duration and duration of OC use. Using Cox proportional-hazards model, we found a statistically significant linear relationship between LNYM and EC, with a 9.1 % increase in risk per

year (p for trend < 0.001). The risk of EC increased gradually along with increasing duration of menstrual span. Using the group ≥ 40 years of menstruation as a reference, the hazard ratio for group <25, 25-29, 30-34, 35-39 were 0.17 (95% CI 0.22-0.27), 0.25(95% CI 0.17-0.36), 0.43 (95% CI 0.32-0.58) and 0.68 (95% CI 0.51-0.92), respectively. The linear relationship remained significant after stratification for BMI, adjustment for diabetes, hormone therapy, and incomplete pregnancies. We found similar associations among all strata of BMI and among non-users of OC. In addition, due to a strong dose-dependent association between LNYM and EC risk we were able to calculate PAF in 5 years-interval. PAF calculations showed that if women with LNYM ≥35 decreased LNYM to less than 35 years, 48% of EC could be avoided. The proportion of avoided cases increase to 64% and 67%, if the cut-off for LNYM category changed to 20 and 25 years respectively.

In line with previous reports, our study support that increasing lifetime number of years of menstruation is an important risk predictor for EC, which is independent of other proposed risk factors.

Paper III

Gene expression profiling of peripheral blood according to endometrial cancer risk factors: systems epidemiology approach in NOWAC Postgenome Cohort Study.

Increasing worldwide incidence of EC, the most common gynecologic cancer in the world, requires extensive search for novel preventive tools and early intervention approaches. Several factors, including parity status, breastfeeding duration, use of OC, coffee consumption, BMI, use of hormone replacement therapy, and lifetime number of years of menstruation have previously been reported to modify EC risk. However, establishment of reliable predictive models is impossible without knowledge on genetic changes prior to diagnosis. In this work, we aimed to establish if known EC risk factors influence peripheral blood gene expression in a prospective design. First, we selected variables that were shown to have an impact on EC risk in the whole Norwegian Women and Cancer (NOWAC) cohort (165 000 women). Then, we tested the association between these variables and changes in gene expression profiles in blood in a nested case-control study (79 case-control pairs) of women from the NOWAC postgenome cohort. Lastly, we undertook a gene set enrichment analysis (GSEA). When we looked at overall gene expression, we found no difference between EC cases and controls. Introduction of parity status into the statistical model, revealed changes in expression of 1379 genes (false discovery rate (FDR) 20%) in controls, while we did not

observe any expression changes in cases. 27 genes (FDR 20%) were associated with BMI increase in controls, whereas there was no association between changes in BMI and gene expression in women with EC. In GSEA, the major part of significantly enriched gene sets (2407, FDR 20%) were attributed to parity increase among cancer-free women. We found that increased number of parities has a major impact on changes in peripheral blood gene expression in women diagnosed with EC later in life. The descriptive study design does not allow us to provide accurate explanation of our findings in biologic terms but this work brings solid background for further research on the development of predictive EC risk models.

5. GENERAL DISCUSSION

This PhD project is one of the examples of the developing field of systems epidemiology, where unique combination of both lifestyle exposure and information on functional genomics will hopefully give more understanding in the processes involved in endometrial carcinogenesis. Such multidisciplinary projects, however, also have many aspects for quality control and a lot of challenges when it comes to methodology. Therefore, the first part of the discussion is devoted to methodological issues and the second part describes the interpretation of the obtained results in the light of existing literature.

5.1 Methodological challenges

In the world of competitive research inaccurate reporting of data is not a seldom event. This hampers the generalizability and correct interpretation of results both for the whole research community and for future patients especially when it comes to diagnostics or treatment of such diseases like cancer. Thus, quality control of data should be an integral and essential part in research at various stages and first of all before data gathering starts.

5.1.1 General issues related to NOWAC study

Study design

The present project will focus on a study with observed data based on prospective design, although to date many researchers investigating cancer have also used cross-sectional and other types of case-control studies as a model. It is known that cross-sectional design can provide information about possible association between exposure and outcome (199), but since the information is obtained at a given point of time it is difficult to make any conclusions about the causality of this association. In this context, using a prospective design like in NOWAC Study is more safe and reliable as the exposure is measured before the outcome and therefore the time-effect relationship is known (200). Another advantage of using a prospective design is an excess to follow-up, which is in case of NOWAC is complete due to unique opportunity to use the linkage to national registries such as mortality registry, migration registry and cancer registry (201).

When it comes to integrated systems epidemiology analysis, the initially correct planning of the study design is particular essential. In order to succeed in catching of any significant associations between exposures and related changes in gene expression, the studied cohort should be first of all, large enough to reach the sufficient calculation power. NOWAC study has a large

sample size and random sampling, which reduce sampling errors and therefore increases the precision of estimates. Prospective design and involving of many participants gives enough statistical power to detect small differences in smaller subgroups like NOWAC Postgenome Cohort using a nested case-control design. Moreover, using a representative smaller subcohort is more practical in terms of high costs of all kind of functional genomic analyses. Secondly, NOWAC Postgenome Cohort is constructed in a such way that in a matched case-control design all the cases ad controls were kept together through the all steps of laboratory work. This approach aims to avoid batch effects and systematic bias. Finally, this unique design allows testing the hypothesis in functional genomic obtained earlier from the same cohort like it was demonstrated in paper III. This approach minimizes many types of bias and measurements errors, which are known to occur if for example if the testing hypothesis is derived from the study from another country, which could differ in sampling procedures and simply different patterns of lifestyle characteristics.

Validity

Validity represents the level of confidence that we can put to the studied cause-effect relationship and investigates whether the obtained findings represent the real situation (202). Internal validity evaluates whether the results are correct for the studied group of participants, e.g. if the current study gives unbiased results (203, 204). Implying this definition to the current thesis, internal validity assesses if the observed difference between the studied groups related to our dependent variable (*EC risk*) is attributed to the studied exposure (*coffee consumption, LNYM, parity, age at menopause, OC use or BMI*). External validity (representativeness or generalizability) shows if the chosen population in a given study (in our case, NOWAC Study) differs from the general population, and whether participants differ from non-participants. This type of validity is generally good secured in NOWAC as this study has a random selection of participants though the Central Population Registry. However, as participants anyway "select themselves" and decide to participate or not, the possibility of invalidity of study arise and thus, methodological studies could be of great help. Such evaluation of validity has been done within NOWAC as well. Evaluation of data from Cancer registry of Norway showed that cumulative incidence rates (CIR) in NOWAC for all cancer sites included EC in women of the same age were almost identical with the corresponding CIR for the entire population (205). External validity is also particular important in terms of possibility of estimating the public health effects of a given association by calculating absolute or attributable risks. The analysis of validity study from NOWAC did not reveal major source of bias that could make calculations of population attributable risks invalid.

However, still the number of different types of errors can be rather overwhelming due to so many sources of possible bias that are identified in modern research, and in this part of discussion I will mainly focus on the 2 main groups of possible bias: selection bias and information bias. The role of confounding will be later mentioned in the discussion of the main results.

Selection bias

Selection bias results from skewed selection to participation or follow-up. In spite of rather high participation rate in NOWAC Study (57%), there is still a chance of getting selection bias if the non-participants had a systematically different risk profile than the participants. Of course, we cannot be certain whether the participants have higher or lower frequencies of risk factors than non-participants. And as we do not have any relevant information about the non-participants, it is difficult to assess the direction of the possible selection bias.

Validity evaluations from NOWAC showed that the highest response rate was among the women from Northern Norway, that response rate was higher for short questionnaires and decreased with the increasing of age of those who received questionnaires (189). In case of EC, it could lead however to selection bias as EC is strongly associated with age. Thus, if among non-participants there was a high rate of elderly women, we could have underestimated the effect of age on EC risk. In addition, if for example obese women or ex-active users of MHT are in particular among the elderly women, the impact of these variables may be also underestimated. Using the data from Norwegian fertility registry and registry of education it was shown that women who agreed to participate had higher age at first birth and more than 12 years of education in comparison to source population. However, for example, proportion of women with three or more children was approximately the same among responders and non-responders. Validity studies within NOWAC in general showed no significant differences while comparing the distribution of exposure variables in samples with response rates from 55 to 70% (206). Another NOWAC study (189) investigated possible selection bias comparing women responding to the NOWAC follow-up questionnaire and women responding to the NOWAC baseline questionnaire in relation to the information given at enrolment. Almost no differences were found, except the fact that those women who completed the follow-up questionnaires were slightly younger and better educated. In accordance to this, a certain percent of selection bias must be expected among the participants of the Postgenome cohort as they participated and filled inn questionnaires several times due to a specific recruitment process to this sub-cohort. However, expect educational level and MHT use, there were no major differences among NOWAC participants who donated blood and responded only once compared to those, who responded 2-3 times (207).

Information bias

Information or measurement bias occurs when the study subjects or personnel/instruments give systematically inaccurate measurements or there is a systematical difference in the way data is obtained (208). This may affect both independent and dependent variables. Recall bias (differential bias) refers to disease/outcome status and can arise when for example, cases and non-cases remember the exposure information differently. However, in case of NOWAC study, recall bias will be generally prevented due to prospective design, meaning the assessment of exposure information before the occurrence of cancer. Non-differential misclassification on contrary results when misclassification of either exposure or outcome is not linked to exposure or outcome status. And, since NOWAC study represents a big cohort and all participants are equally measured using the same questionnaire or blood collection kits the possible misclassification bias will be mostly non-differential.

The role of confounding

Confounding occurs when there is the confusion of two supposedly causal variables, and the effect we observe in a studied association by one variable is actually due to the effect of another variable (209). It is well-known, that most proposed risk factors for EC interact with each other and therefore can alter the studied association. For example, obesity in many cases leads to chronic anovulation and infertility, as a result we could observe many nulliparous women with high BMI. However, nulliparity by itself is a risk factor for EC, and lack of controlling of this factor can also lead to an overestimation of the impact of obesity. On the other hand, it is also well known, that BMI increases with increasing parity, which on contrary is linked to inverse association with EC. This example shows, how complicated is relationship between these hormone-associated risk factors in EC development. In order to avoid the effect of potential confounding, in Paper I and Paper II depending on model, we have used multivariate analysis adjusted for age, parity, smoking, BMI, MHT use and OC use. However, it is always important to keep in mind, that obtained results may also be influenced other unmeasured variables, not yet known to be related to EC.

5.1.2 Validity of variables used in the present thesis

Age at menopause

Menopausal status is one of the central variables in all three papers. Due to its nature of being a prolonged biological process, start of menopause could be difficult to identify and this could logically lead to inconsistent recall of a precise menopausal age. This could also explain the higher

difference between self-reported age at menopause and accurate age in natural menopause compared to menopause caused by other reasons like operation or hormone intake. However, in the majority of validity studies, self-reported age at menopause is considered to have a good reliability (210-212).

Self-reported age at menopause was also validated by NOWAC in a study provided by Waaseth et al (213). In this study the measurement of plasma levels of E2 (cut-off for postmenopausal women < 0.2) and FSH (cut-off for postmenopausal women> 0.26) were used to validate menopausal status/classification used in both blood (two-pages) and standard questionnaires (eight-pages) (213). The study revealed, that NOWAC data provide a valid information on menopausal status, showing 92% sensitivity (95% CI 89–96%) and 73% specificity (95% CI 64–82%) for this variable in blood questionnaire, and 88% sensitivity (95% CI 84–92%) and 87% specificity (95% CI 80–94%) in standard eight-pages questionnaire respectively.

However, such validation of menopausal status using plasma concentration of E2 and FSH is not suitable for all analogue studies, as it requires the presence of blood samples for all women. Moreover, there is still no independent established serum biomarker for accurate identifying of menopause, so for many studies self-reported retrospective information is still the only one option (214).

Age at menarche

Age at menarche was included in paper II as the start point of calculating LNYM. As it was obtained as a retrospectively self-reported information with a long-term perspective back in time, recall bias could be particularly present here. Moreover, along with information on age at menarche, other factors like weight, height, level of physical activity in adulthood and medical records on presence of anorexia nervosa or any other hormone-associated diseases should be also taken into account as they are shown to modify the start of menstrual function (215). It is known, that on average the first menstrual bleeding occurs between 10 and 15 years (216), but according to some reports (217) age at menarche decreased dramatically since 19th century worldwide, including Northern Europe. However, studies obtained from Norway showed that menarcheal age was close to stable and between 13.1-13.3 years since 1950's (218), which in accordance to the mean age at menarche in all three papers in this thesis. Moreover, studies that validated self-reported age at menarche revealed relatively high correlation between self-reported information and correct data obtained in adulthood with quite Pearson's correlation ranged from 0.52 to 0.83 (219, 220). In recently published analogue study based on Tromsø Study Cohort (216), Pearson's correlation was

0,84 and was not attenuated by increasing age of women when they had to recall this information. All in all, these studies give a solid background to conclude that self-reported age at menarche is relatively accurate to be used in epidemiological studies.

Parity and pregnancy-related variables

Parity was among the variables that received the highest validation in a validity study provided by NOWAC (205). Moreover, parity has been stable since the end of the 1970's, although there was a decreasing in parity from 1970 (Figure 19).

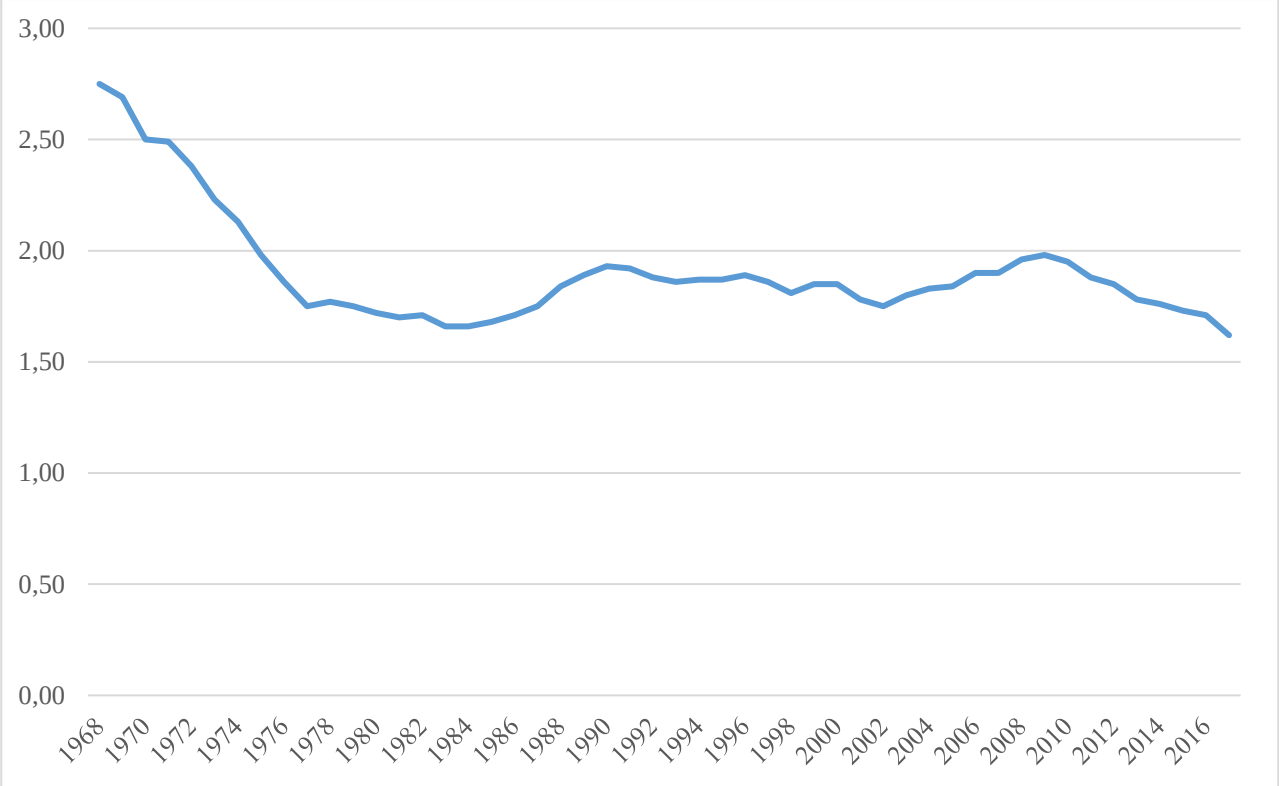

Figure 19. Average number of parities in Norwegian population. Raw data obtained from Statistics Norway.

Breastfeeding rates varied a lot over the years. In 1960s only 20% of Norwegian women breastfed until the baby was 3 months old. Revolutionary introduction of "Breastfeeding-help" in Norway in 1968 led to raise of breastfeeding rates and already from 1980's the introduction of new public breastfeeding policy at obstetrics departments all over the country and extending of maternity leaves in Norway led to increased public focus on breastfeeding (221). Women that were included in paper II had their fertile years (with potential for breastfeeding) during these 70s-90s when there was an increased attention to changings in breastfeeding policy, thus it could give us more evidence to believe that they correctly recall their breastfeeding duration.

Validation of questions related to incomplete pregnancies and abortions is always more challenging due ethical specificity of these issues. In general, response-surveys on abortion show that response rate differs a lot depending on several factors. Thus, in some surveys, unmarried women compared to married were less likely to report abortions, women with fewer children were also less likely to report abortions compared to those who gave birth to more than 3 children (222). Moreover, the response rate could be affected by the way the question was asked. For example, in the first versions of NOWAC questionnaires question on abortion were divided into spontaneous and induced. This subdividing was replaced by general questions on all abortions in general because of social stigmatization of having induced abortion. In Norway, the women's right for induced abortion was legalized in 1978. Until 2005 Statistical central register was responsible for registration all induced abortions in Norway. In 2005-2006 National Health Institute of Norway took over this responsibility and, and finally in 2006 Abort Registry was developed. Thus, there is available statistics giving the overview of the number of induced abortions since 1978. Moreover, it has been reported that he number of induced abortions in Norway was stable over the years in all fertile groups and in general the prevalence is not high compared to other countries (223).

Oral contraceptive and MHT use

Since OC and MHT use are not constant characteristics, misclassification and misreporting for these variables can arise. For both OC and MHT users, NOWAC performed validation by reproducibility tests.

Data from Drug Consumption in Norway based on Defined Daily Doses (DDDs/day) indicate that the total use of oral and transdermal hormonal contraceptives increased from 20 000 in 1967 to 200 000 in 2000 and 270 000 in 2016 (Figure 20) (224). In Norway, combined OC's had a dominant frequency of use in the period 1967 with the first peak of use in 1981 and 5-years following fall, stable sale rates in 1991-1995 along paralleled with sequential OC's (Figure 20 upper panel) (77). An interesting trend is then observed in 2 periods. The first one, from 1995 to 2000 when combined OC's undergone of dramatic fall of sales but sequential OC's on contrary, had a parallel rise of sales. The second 5 years (2000-2005) the opposite scenario has been observed. Finally, since 2006 combine OC's got a rising and dominant frequency of use up to date (Figure 20 lower panel) (224).

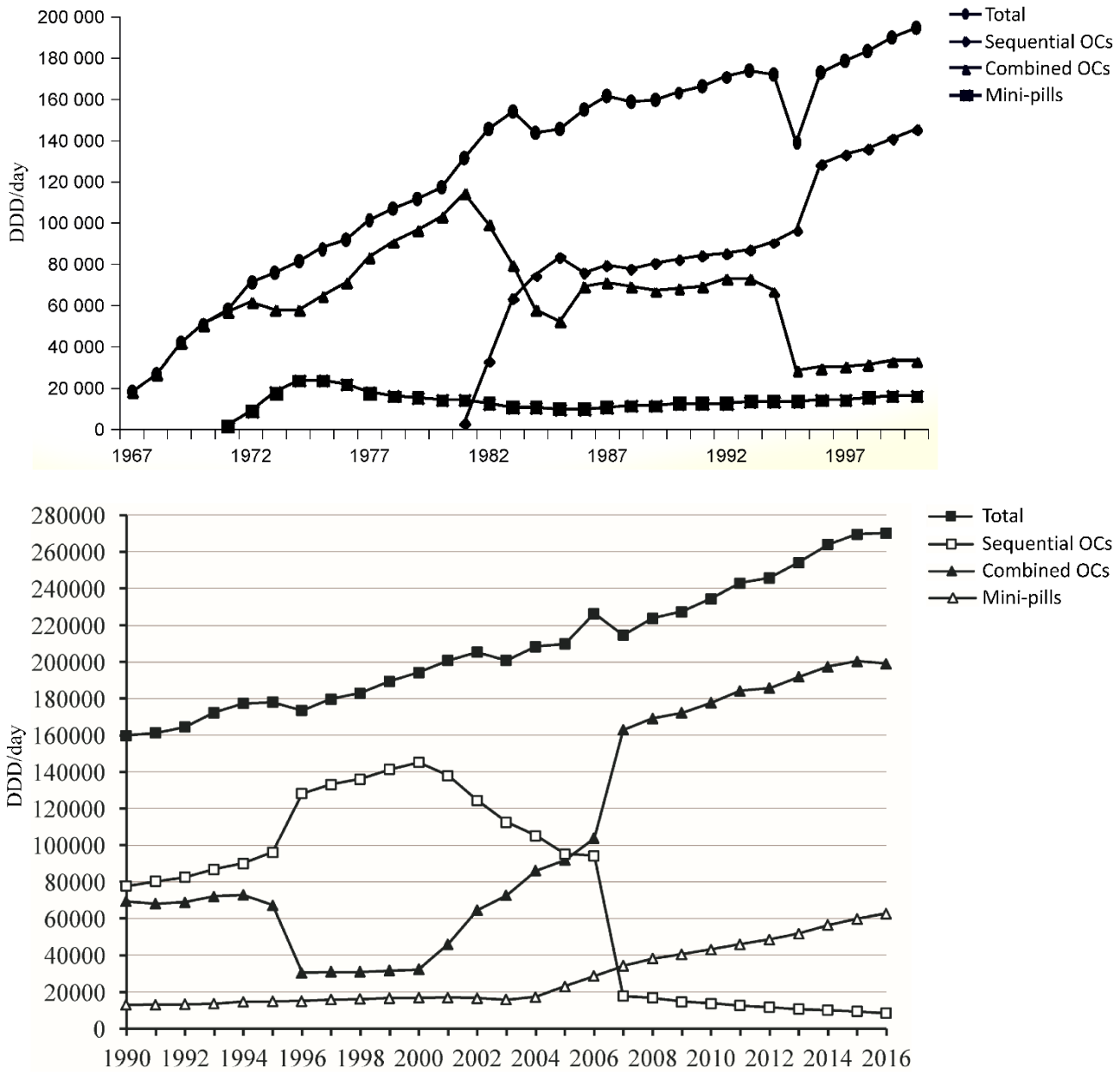

Figure 20. Sales of oral and transdermal hormonal contraceptives in Norway between 1967 and 1997 (upper panel) and between 1990 and 2016 (lower panel).

Adapted with permission, copyright 2006, 2017 by Norwegian Institute of Public Health

In order to receive more precise information about OC use, in 1991 all women invited to participate in NOWAC Study also received a booklet with photographs of 33 of the 36 known OC brand sold in Norway. Three months after returning the first questionnaire, women received a second identical questionnaire, which were answered by 61.1% of earlier responders. Information on the new OC types was updated in each new version of questionnaires. In order to acquire the extent and level for agreement between these two responses in accordance to OC ever use, current use, use before the first full time pregnancy kappa was used, showing satisfactory agreement for all the categories mentioned above (k=0.97, k=0.86, k=0.87 respectively). As one of the limitations for

our LNYM study (paper II) one can mention absence of updated information about any changes in exposure status in OC during as this information was obtained only from baseline questionnaire.

When it comes to MHT use, so together with validation of menopausal status, study of Marit et al (207, 213) evaluated the validation of current users, showing 100% specificity. Former users of hormonal preparation are always less reliable in their reporting compared to current users. As for OC use, to recall former MHT use in NOWAC, women received a prospect with photos for all known brands of menopausal hormonal preparations that were available on Norwegian pharmacological market since 1953. However, we could expect some underreporting because of general awareness and controversial information about potential harmful or beneficial effects of MHT during the periods, when the participants of our studies answered these questions. In Norway the first MHT preparation based on ethinylestradiol and the first patch (Estraderm) containing estradiol were introduced in 1953 and 1989 respectively (225). Preparations contained progestins were first available in Norway since 1960. Later, during 1990s other MHT preparations as tibolone, vaginal ring and other progestins joined the Norwegian drug market. Later, norethisterone and levonorgestrel became the most preferable progestins that have been used in Norway (225). Generally, Norwegian women were somewhat restrictive in use of MHT comprising less than 6% of users among postmenopausal women in the late 1980s (226). Further then, the pattern of use has changed in the 90s the use of MHT preparations increased and accounted for 35% of postmenopausal users. In this period, the users of estrogen-progestagen preparations comprised 70% of all MHT users in Norway. In accordance to analogue reports from other countries, in Norway this type of MHT has not been shown to increase EC risk in contrast to estrogen-only regimens which gave RR 3.2 (CI 95% 1.2-8.0) (192). Unfortunately, we do not know for sure, if there is or not misclassification in category "former MHT users". For some of the analogue studies in Norway, linkage to the Norwegian Prescription Database, would have been one of the best alternative ways to validate the information on former MHT use. However, it was founded just in 2004, thus this type of validation is not suitable for our study as information on MHT use was collected long before this date.

LNYM as a chosen version of total estrogen exposure measurement. Challenges with comparing with analogue studies.

As it was illustrated in Introduction section (Table 4), there is a big heterogeneity between analogue reports and up to date there is no standard or common method to calculate the lifetime number of menstruations or number of cycles. Indeed, the methodological study provided by Yang et al (118) that investigated the correlation between different algorithms for computing lifetime number of

cycles, showed an elevated EC risk in the highest quartile of cycles (408.0-602.3, corresponding to 34-50.2 years), but this was statistically significant (odds ratio 1.95, 95% CI: 1.11,3.44) only in one of 5 tested algorithms. Moreover, it has been shown, that the choice of mathematical algorithm used in the calculation of a core composite variable might by itself independently effect the studied association (118).

In our study, we have chosen to use years of menstruations instead of number of cycles due to fewer number of uncertain factors that is difficult to validate compared to calculating of number of cycles, which is considered to be more imprecise due to more factors that should be taken into account in calculation but difficult to measure such as for example, individual validity of cycle length, and regularity of the cycles. However, it was reported by several studies that cycle length and regularity were unrelated to EC risk (107). Moreover, when it comes to using cycles as an indicator for cumulative estrogen exposure and EC risk, it is essential to distinguish, whether the cycle was ovulatory or not. Further, it is shown, by some studies that ovulatory cycles and LNYM might be two different independent risk factors (227). Therefore, as a baseline for our study we used the formula for calculating LNYM from EPIC study (98) as the authors used quite a standard approach with using minimum of required components, such as age at menarche, age at menopause, number of pregnancies, duration of breastfeeding and OC use. However, EPIC has a limitation of using information from different centers, where questions on required variables could be formulated and collected in a different way. There is a need for such "LNYM" studies in general and within each of participating cohorts in EPIC in order to have a better comparison. In this context, one of the strengths of our study is that we provide the results for comparison with future studies, showing both a "standard" approach of calculating LNYM (age at menopause, age at menarche, number of full-term pregnancies, duration of breastfeeding and OC use) (see different models in paper 3, Table 3) and in addition, other alternatives of calculating the LNYM for those studies, that like ours, had for example supplementary data on different types of incomplete pregnancies.

BMI

Challenges in validation of self-reported information on BMI is a well-described problem for epidemiological surveys using questionnaire data. Validation of this information was also performed in NOWAC (228). This study showed that although there was in general a substational agreement between self-reported and values measured by medical staff, there was an underreporting of weight in overweight and especially in obese women. Such underreporting of BMI could have effect the results of subgroup analysis for BMI categories (indeed, we had few women in "obese category"), however, in case of paper II and paper III, this variable was included as a continuous

variable and did not affect the main results. In paper I, we have reported that protective effect among those who consumed 8 or more cups of coffee was stronger in obese women. These findings are indeed in line with the results from recently published meta-analyses, showing more pronounced protective effect of coffee consumption in overweight and obese women (178).

As other acquired life-style factors, body weight can also change during follow-up. Previous reports (229) pointed the importance of weight change over time. Most of them showed that an increased EC risk may be attributed to increase in weight at age 18-25, even after adjusting for current BMI. Interestingly, replicating of these results in non-Western populations also showed a positive association between obesity at age 20 and EC development (230). Body fatness in childhood is showed to be less significant, however, still remains important as it correlates with adult obesity, and thus, many adverse health outcomes, including cancer (231). In one of the NOWAC Studies, it has been recently shown (unpublished data by Rylander et al, article under review) that both moderate weight gain (5-10 kg) and high weight gain were associated with increased risk of EC, independently of high BMI at baseline. However, the association was weaker for weight gain than for high BMI itself (personal communication with Rylander).

It is shown, that in contrast to BMI, measurement assessing the extent of central versus peripheral obesity is better predictor for other hormone-dependent cancers like for example BC (232). Thus, measurement is based on a ratio of waist to hip circumference, where value 0.8 and higher is associated with central adiposity and following metabolic phenotype, independent of weight and thus, BMI (233). In contrast to other cancer types, studies investigating relationship between waist/hip ratio and risk of EC have been sparse and inconsistent. A population based case-control study from Shanghai showed a particular role of upper-body obesity, which was associated with increased EC in spite of low BMI (234). A meta-analysis of prospective observational studies reported a non-significant increase in EC risk within each 0.1 unit increase in waist and hip circumference. However, when waist and hip were taken into the risk assessment separately, they had also shown independent risk increase with RR 2.16 and 1.30 per 10 cm increase in waist and hip circumference respectively (229). The importance of waist to hip measurement becoming more and more relevant taking into account that central obesity is strongly associated with other EC risk factors, such as hyperinsulinemia and diabetes type II (235). Finally, as an additional validation for BMI, measurements of levels of adiponectin, which is secreted by adipose tissue, could be used as a serum biomarker for obesity. The levels of adiponectin are inversely correlated with BMI (236) and EC risk, showing that each 5 μg/mL increase of adiponectin level reduces the risk of EC by 18%, this effect is consistent after adjustment for BMI, menopausal status and MHT (237).

Smoking

Although smoking is not the central exposure in all three papers, it is indeed an important modulator of hormonal metabolism in women especially in postmenopausal period, and therefore, is important confounder that should be included in risk assessment models. Smoking information in NOWAC was obtained from self-reported questionnaires at baseline, and by present time no validity studies have been performed. Therefore, several methodological challenges in interpretation of our results from subanalysis in paper I and paper II can occur. First of all, we cannot exclude the possibility of selection bias due to "healthy volunteers effect" (238). Further, in our studies we have focused just on smoking status as former, current, never without including details like age at smoking initiation, smoking duration, number of cigarettes smoked per day and pack-years. In addition, no data was available on passive and occasional smoking, so these categories are included in the group "never smokers". Then, we do not used information about any changings in smoking behavior during the follow-up. Results from Million Women Study showed that in their study of those who were current smokers at baseline, 20% and 44 % quit to smoke after 3 years and 8 years respectively (239). However, in case of postmenopausal EC, which is our focus for paper I and paper II, it has been shown that both current smokers at baseline and those who quit ≥5 years before baseline had significantly reduced risk of EC compared to never smokers (134).

Coffee consumption

All the studies investigation the components of diet as a potential exposure are always at certain risk of rising various types of bias, and studies focusing on coffee consumption are not the exceptions. Among the problems that arise, might be first of all inaccurate measuring of caffeine consumption along with other bioactive compounds from other sources in diet, like tea, cola, chocolate and energy drinks as this type of information was not available from NOWAC questionnaires. Further, it is also challenging to investigate real changes in the amount of coffee consumed during the follow-up or differences in the cup size and coffee strength. Coffee habits vary much between the individuals - some prefer small amounts of strong coffee like espresso, others drink large amounts of instant coffee. So even though these coffee types contain different amount of caffeine, sometimes it could be equalized by the size of the cup. Hence, in human studies it is difficult to get the final conclusion regarding the effect of one or another type of coffee. Although some other studies showed the satisfactory reproducibility and validity of information on coffee (240) still various bias could arise with coffee assessment extracted from self-reporting food-frequency questionnaires.

In order to update the exposure information (e.g. dietary intake) NOWAC FFQs have been investigated in several validity studies. To increase reproducibility and validity these questionnaires

were tested in terms of biomarkers and 24-hour dietary recalls and have been shown to be in the same range as similar studies have (241). Precisely to coffee consumption, the validity of estimates of this beverage was fairly good as well.

5.1.3 Technical considerations in gene expression analysis (Paper III)

Validation of the methods and findings demonstrated in paper III was not the key focus for this study due to the exploratory and "testing" concept. However, many of the challenges related to validation and interpretation of results are generally common for many analogous gene expression studies. Implementation of microarray technique into the large-scale epidemiological studies along with limited standardization of methods used on different steps of analyses, introduced a variety of factors that we should be aware of. Both pre-analytical issues and analytical errors as well as within and between subject variations might significantly influence results.

All pre-analytical steps of microarray including mRNA isolation, cDNA synthesis, labeling, hybridization, washing, and scanning could produce random or systematic errors. In addition, contamination of samples and technically inaccurate work performed by the lab personnel can take place. Finally, blood samples are always prone to RNA degradation due to the presence of RNase. A study by Dumeaux et al (242) revealed that 46,5% of the overall variation in gene expression is attributed to three technical variables in the pre-analytical step: transportation time (time between blood sample collection and freezing of the sample) and RNA extraction time (time between blood sampling and RNA isolation). In paper III, we have checked the difference between the time of blood sampling and RNA extraction, which showed that the difference between cases and controls regarding time between these two events does not exceed 10 days. All blood samples used for paper III, were collected by the PAXgene blood RNA collecting system, which was shown to work well in terms of effects of pre-storage handling, storage over time and RNA isolation output (243).

Validation studies of data obtained in microarray demonstrate, in general, good agreement with the results obtained by more sensitive techniques including quantitative reverse transcription polymerase chain reaction (qRT-PCR) and next generation sequencing (NGS) (244-247). Although, it has been demonstrated that genes with largest fluctuations in expression levels (both positive and negative) have better concordance than genes exhibiting modest changes (248). Thus, in paper III, we assessed trends in differential expression and, therefore, maximized the significance of obtained results as it has been shown in other studies that interpretation of microarray data should be rather based on relative expression levels than on absolute numbers (248).

The role of microarray data preprocessing has been emphasized by various sources (249, 250). During the initial steps of Paper III preparation, we experienced, how different preprocessing approaches might influence the final outcome. The results of this trial are presented in Table 5.

Preprocessing for paper III in 2017 and the main differences from preprocessing in 2013-2015
In 2017, the dataset was revised again with regard to the initial preprocessing steps of removing individuals considered as technical outliers. There were in total 168 individuals (84 case-control pairs) analyzed at the laboratory. In the data preprocessing step performed in 2013/2015, we marked 11 individuals as technical outliers and removed them along with their matching case/control. We were thus left with 146 individuals for analysis (2013 analysis). We further removed 4 pairs in the 2015 analysis due to unknown metastasis for the case. As our experience with the data and methods for outlier removal increased, we developed a standardized package for outlier removal (unpublished manuscript "*nowaclean package*") and based on this package we decided to only remove four individuals as technical outliers (along with their match). The 2017 data is also based on a more recent update from the Cancer Registry with cancer information through 31.12.2015. For these data, one case with two diagnoses was identified and removed along with its matching control making a data set for analysis consisting of 158 individuals (79 case-control pairs). The other main difference between the data from 2013/2015 and 2017 is that for the former data the chips are scanned using hiScanner, whereas the latter data are scanned using the Bead Reader scanner.

Table 5. "Behind the scenes" of the gene expression analysis. First alternative analysis approaches performed 2013-2015

"*All models are wrong, but some are useful*" *(George Box, 1979)*

Preprocessing, year, main steps	**Analysis approach of GE Methods**	**Main results**
2013-2014 Corrected for background noise using the normal-exponential model Variance stabilized using log2-transformation Filtered probes Mapped probes to genes Data ready for analysis contained 146 individuals (73 case-control pairs) and 9327 gene expression values	Explorative approach without preliminary hypothesis Methods: ● Unsupervised analysis to identify whether there is difference between cases and controls in regard to expression of most variable genes ● Paired analysis using global test to identify which variables are associated with blood GE globally;	GSEA revealed 1 pathway, which was borderline statistically significant: *Sphingolipid pathway* This finding led to development of additional direct analysis of sphingolipids in blood samples. This is an ongoing project.
2015 Corrected for background noise using the normal-exponential model Normalized using quantile normalization on original scale level Variance stabilized using log2-transformtion Removed 4 case-control pairs where the information on metastasis stage from the Cancer Registry are marked as unknown/other Filtered probes Probes were then translated to genes using function "nsFilter" Saved data of size 138 x 9327 The main difference from preprocessing in 2013: 1)data is normalized using quantile normalization 2)We have removed 4 cases with unknown metastasis stage, along with matching controls	● Paired linear supervised analysis (top 50 and top 100 genes) to identify single genes differently expressed between cases and controls; ● Gene set enrichment analysis (GSEA) (among top 50 and top 100 genes). Functional annotation analysis by DAVID.	GSEA revealed 2 pathways, which were borderline statistically significant and just in subgroup analysis (in women with BMI < 25): *Pathways related to altered cholesterol and insulin metabolism.* These findings required additional confirmation and direct measuring of cholesterol and insulin metabolites in blood samples samples.

6. DISCUSSION OF THE MAIN RESULTS

Paper I

"More coffee, please…"

Investigating association between coffee consumption and EC risk has received an increased interest in epidemiology during the last decades. The possible favorable effect of this popular beverage on potential protection against EC lead to increased number of conducted studies and multiple discussions of any further opportunities for public health implications. There are several proposed biologicals pathways lying behind the inverse association between coffee consumption and EC risk. Coffee is a source of many antioxidants and compounds that have anti-mutagenic properties. Among them are caffeine, phenol compounds, isoflavones, chlorogenic acids, diterpenes and various additional substances like melanoids, ferulic and coumaric acids that are further produced during the steps of coffee preparation (177). As it was highlighted in paper I, the level of exposure to different active substances in coffee highly depends on several conditions such as brewing method, choice of coffee beans and phase of administration (251, 252). All these compounds are proposed to take part in regulation of hormonal metabolism through increasing the level of circulating sex-hormone-binding globulin (SHBG) and prevention of hyperinsulinemia by increasing the level of adiponectin (177). Moreover, many of the bioactive compounds in coffee are known for their antioxidant effects and prevention of DNA damage (253).

In Norway, coffee is the second most consumed drink after the water (254). Despite representing only the 0.7% of the world's population, Norway cover 5.5% of the world's coffee import (255). The fact that Norway takes one of the leading places in daily consumption of high amounts of coffee initiated the interest of investigating the effect of coffee on different cancer types in NOWAC Study. Our main findings pointing towards overall inverse association between coffee consumption and EC risk, and in addition, stronger effect in current smokers and women with higher BMI are in accordance with both previous reports (175, 176, 256) and recently published studies (177, 178, 257). It is of note, that the analysis for paper I was conducted during the period with high publication rates of analogues reports that found a significant decreased risk in EC within consumption of already 3-4 cups of coffee. In this context, reporting a protective effect within 8 or more cups of coffee was one of the challenging issues. Our study provides the results based on a population that historically had a high coffee consumption. Thus, proposing the hypothesis that in such populations, heavy drinking of this beverage might epigenetically, generation by generation alter the metabolic mechanisms involved in the association of coffee consumption and cancer. Moreover, one of the main focuses of this paper was to investigate whether the studied association

is different according to brewing method, although we were not able to show significant results in these subgroup analyses. Moreover, in our study we have not done any repeated measurements and therefore, can base our conclusions just on the consumption reported at baseline. Later report from NOWAC by Lukic et al (258) investigating lung, ovarian, colorectal and breast cancer, showed that proportion of high moderate consumers (3-7 cups per day) and heavy consumers (> 7 cups per day) decreased during the follow-up. In addition, as it was mentioned earlier we did not have information on other sources of bioactive compounds that are found in coffee and that are proposed to have a protective effect against EC. However, in spite of the mentioned limitations, we have a solid evidence to consider our findings to be reliable as several meta-analysis that were published later on confirmed our main findings (178, 257) showing a consistent protective effect of coffee consumption, which is especially beneficial for women with BMI more than 25 kg/m2.

Paper II

"To menstruate or not, that is the question…"

The women's natural menstrual lifespan starts from menarche, interrupts by pregnancies and breastfeeding periods, and end ups with menopause. In addition, during the whole life, woman's body goes through myriad changes influenced by numerous exposures that alter hormonal environment. All these factors contribute in different extent to changings in lifetime exposure to natural estrogen and progesterone in hormonal imbalance, and therefore might then contribute to endometrial carcinogenesis. Possible long-term consequences of each single reproductive factor differs substantially between each other and varies between individuals, making investigating of several factors together especially challenging.

In paper II we observed a significant increase in risk with more years of menstruation during the lifetime on a population level, suggesting that LNYM might be used as a measure of collective effect of hormone-related factors during the reproductive period. Several epidemiologic studies have investigated the impact of total number of years of menstruation or number of menstrual cycles on hormone-depended conditions, including cancer (93, 259-261). However, few studies have previously looked at these associations in relation to cancer uteri. Among them, two case-control studies examined this association using a number of natural cycles as a core variable combining the key reproductive and menstrual features (116, 118). One of them, showed a 56% greater risk in EC in women who had a median number of cycles during the lifespan compared to those with less than median (6), however, this study was limited by moderate sample size and 2-groups analysis without showing a trend. To the best of my knowledge, only 2 prospective studies has previously investigated association between number of years of ovulation and duration of

menstruation span in relation to cancer uteri (98, 106). Although they both showed increased risk, they also had several methodological limitations. Regardless the huge heterogeneity between existing studies, the findings related to association between cumulative effect of reproductive factors and EC risk largely overlap with each other and with our findings in paper II as well, regardless whether years of menstruation or numbers of cycles has been used as a composite variable.

The mechanisms lying behind this association are indeed very complex and not well understood. First and the most logic mechanism related special to EC and LNYM is connected to monthly mechanic shedding and removal of endometrial cells that are potentially malignant. Another mechanism, which is proposed to be common for many estrogen-dependent diseases, including cancer, is linked to so-called "estrogen window hypothesis" (262). The proliferation of endometrial cells is increased during the longest phase of the cycle-follicle phase. And as more cycles/menstruations woman has, the longer cumulative period of estrogen stimulation with inadequate opposed action of progesterone she gets. This could explain why earlier menarche and later menopause are so strong risk factors for EC. They are indeed both also linked to anovulatory cycles. Findings supporting "hormonal hypothesis" are linked to association between number of cycles/menstruations and the level of androstendione, an estrogen precursor of estrogen, at menopause (107) and with sex-hormone binding globulin levels (113). In addition, many studies have also pointed out that total menstrual lifespan is crucial and fundamental not only for the reproductive function but for development of many diseases that woman gets later in life. More and more reports are devoted to effects of so-called "ovarian aging", proposing a hypothesis that predisposition to some of the health conditions in women like for example, obesity, cardio-vascular diseases, hormone-dependent cancers are attributed to timing of start or end of both menarche and menopause (Figure 21) (263).

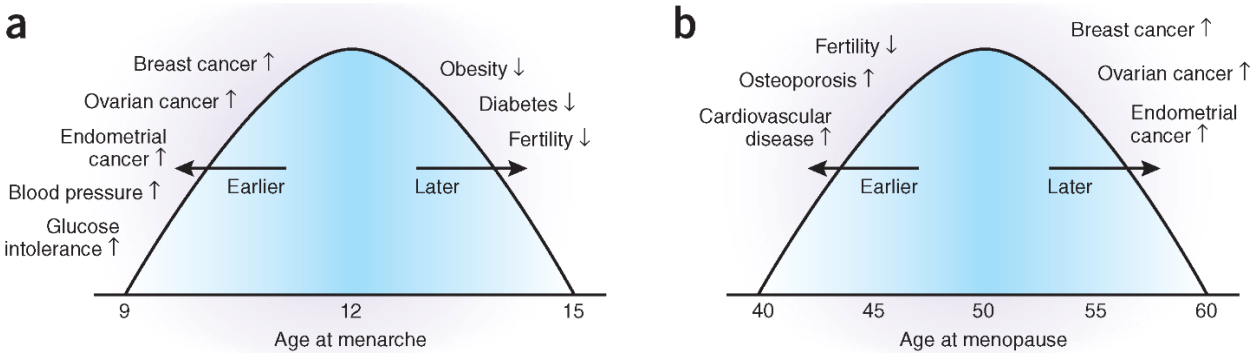

Figure 21. The influence of age at menarche (a) and age at menopause (b) timing on health.

Paper III (testing hypotheses obtained in paper I and paper II)

"The truth is out there…"

This paper attempted to build up the disciplinary bridge between classic epidemiology and molecular biology with a future potential approaches towards further implication into clinical studies. As it was highlighted in paper III, we were limited by a sample size and therefore most probably due to this reason were not able to catch up many statistically significant gene signatures related to coffee consumption, OC use and comparably not many significant findings related to BMI. In addition, in spite of so obvious strong association between LNYM and EC showed in paper II, we have not got significant results neither related to LNYM nor to age at menopause. Although, among of the genes that were significant, when LNYM variable has been tested, was for example, gene 13q34, which is known to be proposed as one of the "genetic markers" of age at menopause (264). Further then, our observation of significant enrichment of "REACTOME_HYALURONAN_METABOLISM" gene set (FDR 20%) in OC users among cases is in line other studies, demonstrating the relevance the hyaluronan metabolism in EC progression (265, 266). Therefore, monitoring of hyaluronan acid in the blood of women using OC might be a valuable tool in EC screening. Of course, we cannot draw any conclusions based on the single genes or gene set with low level of significance, but in my opinion, such a coincidence, could give us a hope that, indeed, these signatures can be relevant, but statistical significance was hampered by sample size. At the same time, few observations related to OC use might be also explained by short-term effect of OC on gene signatures after its discontinuation. Another interesting aspect related to LNYM and age at menopause is our initial expectations to get any gene signatures related to the age at menopause in order to reveal what is the central component in LNYM that may explain the whole association. Few studies, that used analogue composite variables like we did in paper II, attempted to speculate, if among factors summarized there, there are any leading ones. Some studies, proposed that the age at menopause might be a decisive component of lifetime menstruation span (118). Age at menarche and age at menopause, are two factors that affect the length of woman's lifetime menstruation span. At the same time, both of them separately of each other, are strongly related to EC risk. Indeed, older age at menarche is associated with a shortening of menstruation span and decreased risk of EC due to later initiation of ovulatory cycles and start of excessive exposure to estrogens. At the same time, later age at menopause can also prolong the lifetime of menstrual activity and exposure to estrogens, and therefore increase EC risk. We also attempted to check how these two variables might probably affect the association between LNYM and EC risk (unpublished results related to paper II). We found significant correlation coefficients both between LNYM and age at menarche or age at menopause (-0.21 and 0.41 accordingly). However, further analysis using Fisher r- to z-transformation test showed that correlation between age at menopause and LNYM

stronger than correlation between LNYM and age at menarche, supporting the hypothesis that it is more hazardous to get additional menstruations/cycles closer to the end of menstruation span rather than at the beginning. Indeed, it has been already shown the closer to menopause, the cycles are more often anovulatory and the qualitative characteristics of menstruations are substantially changed due to huge hormonal changes (267). Nevertheless, this hypothesis should be interpreted with caution due to existed inconsistence and controversy regarding independent impact of each of the factors on the cumulative risk of LNYM. Moreover, in paper III, the gene signatures related to both LNYM and age at menopause were unexpectedly weak in comparison to parity. At the same time, even though we had so limited sample size we found significant association between increased number of pregnancies and expressional profile in cancer-free controls. So, what is really causation of what? Is it then parity and pregnancies that shift the whole trajectory of association with LNYM? It is obvious that there is indeed interplay of many factors. However, such findings related to parity together with previously mentioned hypotheses proposing predisposition of many diseases by increased number of cycles/menstruations, prove the evolutionary hypotheses that a long menstrual history can have logic dangerous consequences for women. Indeed, many "evolution-orientated" studies propose that menstrual cycles and parity are two conflicting events in women's life. They believe that human endometrium is "designed" first of all to receive and nourish blastocyst, and menstruation is a just a result of unsuccessful reproductive cycle. Therefore, they postulate that excessive number of menstrual periods is not a normal event, calling a menstrual cycle "a culprit", "derivative", "by-product", "a side effect" as neither the brain, breast, the ovary nor the uterus were developed by nature to undergo each month powerful hormone fluctuations for so many years. They also state that as evolutionary consequences of not using uterus for its main purpose, childbearing, modern women get increasing number of menstruation- and bleeding-related diseases like endometriosis, myoma uteri, endometrial polyps and fibroids. Indeed, inverse association between endometriosis and parity along with inverse association between fibroids and parity are extensively studied and confirmed by numerous studies (268).

The importance and pure natural origin of relationship between the lifetime number of periods/parity and estrogen-dependent cancers has been already shown by studies investigating the incidence of hormonal malignancies among women in indigenous populations (269). More industrial style of life through the years affect the women's menstrual pattern as well. Indeed, already in contrast to their foremothers, the modern women experience earlier age at menopause, later age at first birth, fewer pregnancies, fewer months of breastfeeding and later age at menopause. As a result, the number of periods over the life increases from about 160 to more than

400 (270), indicating that lifestyle and reproductive factors interplay and could attenuate the effect of each other.

Our findings related to parity in controls are in accordance to the recently accepted paper from NOWAC where it has been demonstrated a linear decrease in BC risk after each full-term pregnancy independent on other risk factors and marked differences in gene expression between BC cases and cancer-free controls (paper in press). Gene set enrichment analysis revealed significant enrichment of immunologic gene sets among controls. The authors outlined a novel theory about pregnancy-associated long-lasting protective properties of the immune system hampering BC development later in life, which was recently confirmed by an experimental study (271). Moreover, in another preliminary analysis (work in progress) we have tested significant genes and gene sets from BC on endometrial and ovarian datasets and found great overlap between BC and EC and no overlap between BC and ovarian cancer.

7. MAIN CONCLUSIONS

The thesis presents the results linked to the best described and known factors of EC risk. However, given the fact that endometrial carcinogenesis is a very complex process, we cannot rule out the possibility of influence of other confounders that we have not taken into account. Moreover, when comparing our findings to other studies especially in the future, it is important to consider that the obtained results are based on the exposures and life-style patterns women had 30-50 years ago. On the contrary, we believe that findings related to reproductive and menstrual factors should be more close to the real situation as during these periods women were not that much exposed to the huge variety of "artificial hormone modification factors" as modern women.

The main conclusion based on the papers are the following:

1. High coffee intake independently on brewing method might be beneficial for EC prevention, especially for women with high BMI.

2. Elevated LNYM increases EC risk among postmenopausal women and can be used as an important tool that represents the cumulative effect of several risk factors and predict EC risk at a population level.

3. Parity status has a major impact on immune gene expression in the blood of healthy women compared with EC patients, thus potentially explaining pregnancy-associated EC protection.

8. FUTURE PERSPECTIVES

Coffee consumption and EC risk

Although epidemiological findings provide evidence of beneficial health effects of coffee on EC risk, before any clinical recommendations could be proposed, further large population-based studies need to confirm these findings. In spite of our negative results in possible risk difference linked to the brewing methods, analogue large population-based studies are very sparse. Hence, it is important to try to investigate the separate effect of proposed bioactive compounds, taking into account other sources of diet that could have contained these components.

Self-reported information on important confounders like BMI, MHT and smoking should be better validated

Thus, other measurements of adiposity are required in identifying the women who at risk of developing of EC. Taking into account that women with the highest BMI were not the biggest subgroup in all three presented papers, it will be essential to reproduce our findings using BMI-values measured by more accurate methods. This is also relevant for validation of group "former smokers" and "former MHT users". In addition, more precise information about type, duration of smoking along with type of MHT and duration of use are preferable.

LNYM is an index of measuring the total estrogen exposure

As it was mentioned earlier, the main challenge in analogue studies is comparison as there is still a big methodological heterogeneity in both how the main multiple variable was constructed, and in number of covariates available for. Thus, additional studies, using the uniform algorithm for calculation of core variable, which in addition have a greater variety of variables available for adjustment analysis, are required in order to make an adequate comparison between the studies.

Gene expression

Our epidemiologic and preliminary findings from gene expression analysis should be verified on deeper functional genomic level to find underlying mechanisms that further can be exploited in the clinical setting. As for paper III we used only mRNA, for the next step we will use several "omics" approaches:

1. Targeted gene expression analysis and gene set enrichment analysis for immune-related changes

2. DNA methylation profile (necessary for studying the cellular content of the samples stored in NOWAC biobank since the preservation technique used doesn't allow to quantify cells by direct methods)
3. Quantitative, qualitative and functional characterization of blood cell composition and serum antibodies by direct methods (for newly collected samples)

In addition, the following improvements in gene expression analysis are generally needed:

- Improvement and higher degree of automation of laboratory procedures will reduce the variance in gene expression data;
- Standardizing of pre-analytical procedures will make it easier to compare and reproduce the results from analogue studies;

The present PhD project had the main focus on preclinical investigation of potential epidemiological and genetic predictive factors that will hopefully contribute to earlier detecting the patients at high risk, development of the new preclinical screening models and novel targeted therapies of EC.

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Paper I

BMC Women's Health

RESEARCH ARTICLE Open Access

High coffee consumption and different brewing methods in relation to postmenopausal endometrial cancer risk in the Norwegian Women and Cancer Study: a population-based prospective study

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Abstract

Background: Coffee and its compounds have been proposed to inhibit endometrial carcinogenesis. Studies in the Norwegian population can be especially interesting due to the high coffee consumption and increasing incidence of endometrial cancer in the country.

Methods: A total of 97 926 postmenopausal Norwegian women from the population-based prospective Norwegian Women and Cancer (NOWAC) Study, were included in the present analysis. We evaluated the general association between total coffee consumption and endometrial cancer risk as well as the possible impact of brewing method. Multivariate Cox regression analysis was used to estimate risks, and heterogeneity tests were performed to compare brewing methods.

Results: During an average of 10.9 years of follow-up, 462 incident endometrial cancer cases were identified. After multivariate adjustment, significant risk reduction was found among participants who drank ≥8 cups/day of coffee with a hazard ratio of 0.52 (95% confidence interval, CI 0.34-0.79). However, we did not observe a significant dose-response relationship. No significant heterogeneity in risk was found when comparing filtered and boiled coffee brewing methods. A reduction in endometrial cancer risk was observed in subgroup analyses among participants who drank ≥8 cups/day and had a body mass index ≥25 kg/m^2, and in current smokers.

Conclusions: These data suggest that in this population with high coffee consumption, endometrial cancer risk decreases in women consuming ≥8 cups/day, independent of brewing method.

Keywords: Coffee, Brewing method, Endometrial cancer risk, Norway, High coffee consumption, Prospective cohort study

Background

Endometrial cancer comprises about 4% of all cancers in women globally and occurs predominantly after menopause [1]. Some of the highest incidence rates worldwide are found in European populations [2], and a consistent increase in incidence has been observed in the Nordic countries [3]. Although the exact cause of this increase is unknown, use of postmenopausal estrogen therapy and exposure to high levels of obesity-related hormones (i.e. estrogen and insulin) may be contributing factors [4,5]. Recently, a growing number of studies have highlighted that coffee, one of the most consumed beverages in the world, may favorably alter hormone levels [6-9] and consequently lower endometrial cancer risk [10,11]. Coffee might also decrease endometrial cancer risk due to its antioxidant properties [12,13] and capability to prevent DNA damage [14]. Interestingly, coffee contains numerous active substances, such as caffeine, diterpenes, chlorogenic acids

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and phytoestrogens. Concentrations of caffeine and other coffee compounds vary by coffee type (e.g. caffeinated or decaffeinated) and might also be modified by different preparations/brewing methods (e.g. boiled or filtered) [15,16]. Although traditions concerning coffee types and brewing methods, and levels of coffee consumption may vary across populations, relatively few countries have investigated the association between specific coffee consumption and endometrial cancer risk.

Two recent prospective studies showed inconsistent results concerning the association between coffee type and endometrial cancer risk. While one study [17] found a significant decrease in risk associated with only caffeinated coffee in obese women, another [18] concluded that both caffeinated and decaffeinated coffee may reduce endometrial cancer risk. Overall, it is unclear whether caffeine is the compound causally associated with endometrial cancer risk reduction. Moreover, to the best of our knowledge, only one Swedish prospective study has investigated the effect of boiled and filtered coffee on endometrial cancer risk [19]. Although chemical analyses in several reports showed that filtration leads to an almost complete removal of diterpenes, the most promising chemopreventive compounds in coffee [16], the comparison of filtered and boiled coffee in the Swedish study did not reveal any differences between these brewing methods and endometrial cancer risk. Of note, results from the Swedish study were inconsistent with previous studies and found no significant effect of coffee consumption on endometrial cancer risk. Thus, it remains unclear what role, if any, different brewing methods or coffee types play when it comes to endometrial cancer risk.

Norway has the second largest coffee consumption per capita after Finland, including a long tradition of using different brewing methods [20,21]. Therefore our large prospective study based on a representative sample of the general Norwegian female population offers a unique opportunity to investigate the effect of high coffee consumption covering different brewing methods, including boiled and filtered, on the incidence of postmenopausal endometrial cancer, which is steadily increasing in Norway (about 700 new cases registered annually) [22].

Methods

The NOWAC cohort

Using the unique 11-digit personal identity number assigned to all people legally residing in Norway (citizens, those with temporary work permission, refugees, etc.), a random sample of women aged 30–70 years was chosen from the Central Population Registry of Norway and sent an invitation to participate in the Norwegian Women and Cancer (NOWAC) Study [23,24]. Linkage was then performed with the Central Population Registry to obtain postal addresses and vital status (alive, emigrated or deceased) of women. Linkage with the Cancer Registry of Norway was also performed to determine cancer incidence until December 31, 2009. The NOWAC Study is a large population-based cohort study, aim of which is to prospectively examine the associations between different lifestyle factors and the risk of various diseases in a representative sample of the general Norwegian female population [24]. Between 1991 and 2010, a total of 172 000 women were enrolled in the NOWAC Study. As part of their participation they completed a self-administered questionnaire on lifestyle, health and diet.

Study sample

The present analysis used information taken from baseline questionnaires collected from NOWAC participants enrolled in 1991–1997 and 2003–2007 (129 854 women out of 239 388 invited, response rate 54.2%). Of these 129 854 women, we selected those who were either postmenopausal at start of follow-up, or who became postmenopausal during the course of follow-up. We excluded 2938 women with prevalent cancer at enrollment, 543 women who died before reaching menopause, 38 women with missing follow-up information on vital status and migration status, 6544 women who had hysterectomy at enrollment, 17 women who developed incident uterine sarcoma during follow-up, 7085 women with missing information on covariates, and 3090 premenopausal women (premenopausal at enrollment and throughout follow-up). We also excluded 11 673 women with missing information on coffee consumption. The final study sample included in the analyses consisted of 97 926 postmenopausal women at start of follow-up. All women provided informed consent, and the study was approved by The Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate.

Dietary assessment

Year of enrollment determined the version of the questionnaire that participants completed, therefore women were asked about either their total coffee consumption (total coffee version of the questionnaire), or their consumption of filtered, boiled, and instant coffee separately (brewing method version of the questionnaire). The categories of coffee consumption were also different in two versions of the questionnaire. In the total coffee consumption version, women were asked to choose from one of the following responses: 6–10 cups/day, 4–5 cups/day, 2–3 cups/day, 1 cup/day, 5–6 cups/week, 2–4 cups/week, 1 cup/week, 1–3 cups/month, and almost never. However, in the brewing method version, women could also choose from the following responses: ≥8 cups/day, 6–7 cups/day, 4–5 cups/day, 2–3 cups/day, 1 cup/day, 1–6 cups/week,

and almost never. But each woman had only one set of alternatives. Therefore, in our analysis we had to create a common version of frequencies for both the total coffee version and the brewing method version of the questionnaire. Therefore we collapsed the categories of lowest consumption from both versions of the questionnaire (1 cup/day, 5–6 cups/week, 2–4 cups/week, 1–6 cups/week, 1 cup/week, 1–3 cups /month and almost never) into a single category (≤1 cup/day), which was used as the reference in all analyses. The final categories used in the analysis were: ≤1 cup/day (reference category), 2–3 cups/day, 4–7 cups/day and ≥8 cups/day (heavy consumers). Participants who responded 6–10 cups/day in the total coffee questionnaire were categorized as ≥8 cups/day in the final categories. About 13% of the study population had missing values on brewing methods.

The size of a cup of coffee was not determined in the questionnaire, but we chose to use 2.1 dl (7.1 oz) as the standard cup size, based on a 24-hour recall investigation in the NOWAC cohort (data not shown). The NOWAC Study Food Frequency Questionnaire (FFQ) has been thoroughly validated by 24-h recalls [25] and a test-retest study [26], which showed good reproducibility and validity of information on coffee consumption (Spearman's rank correlation coefficient r = 0.82, 95% confidence interval, CI 0.77-0.86; calibration coefficient 1.17, 95% CI 1.06-1.28).

Assessment of lifestyle factors

Information on age at inclusion, age at menarche, age at first birth, education level, duration of oral contraceptive use, hormone replacement therapy use (current, former, never), smoking status (current, former, never), physical activity, and current weight and height were collected from questionnaire at enrollment. Body mass index (BMI) was calculated as weight in kg divided by height in meters squared. Menopausal status was updated when information was available. Women who were premenopausal at enrollment, but whose menopausal status changed to postmenopausal during follow-up contributed to the study as from the date they became postmenopausal. Menopausal status was derived from the answers to questions on menstruation regularity in the questionnaires. If women reported that their menstruation was regular, they were considered premenopausal. Women were classified as postmenopausal if they reported that their menstruation had stopped at the time of enrollment or during the follow-up. In case of uncertain menstruation regularity (irregular, hysterectomy, hormone replacement therapy use or otherwise insufficient information), women were defined as postmenopausal if they were at least 53 years old at the time of enrollment, or once they turned 53 during follow-up. This cut-off point has been used in previous NOWAC reports [23], based on the definition employed in The Million Women Study [27].

Ascertainment of endometrial cancer cases

Information on cancer incidence among NOWAC Study participants was obtained by yearly linkage to the Norwegian Cancer Registry based on the unique 11-digit person identification number assigned to each Norwegian legal resident and citizen. By the end of the follow-up period, 462 incident endometrial cancer cases were recorded in our study sample.

Statistical analysis

Follow-up of participants started at age of enrollment in the NOWAC Study (1991-1997, 2003–2007) or at age at menopause (for those women who were premenopausal at enrollment). Women were censored at the date of diagnosis of endometrial cancer, date of death or immigration, date of report of any other cancer, or the end of follow-up (31 December 2010). Cox proportional hazard regression models were used to estimate the hazard ratios (HRs) of developing endometrial cancer with 95% CIs in each category of coffee consumption compared with consumers in the lowest category of consumption (≤1 cup/day). We were unable to use coffee abstainers (non-consumers) as a reference group as we had too few endometrial cancer cases in the groups of only boiled and only instant coffee drinkers (Table 1). The variable total coffee consumption included responses to total coffee consumption (from total coffee version of the questionnaire) or a combination of responses to boiled, filtered and instant coffee consumption (from the brewing method version of the questionnaire). As there were very few heavy consumers of instant coffee (n = 2), we did not investigate the effect of instant coffee consumption when estimating risk stratified by brewing method. However, instant coffee consumption was included in total coffee consumption. A test of the proportional hazard assumption was checked graphically and by assessment of Schoenfeld residuals for all relevant variables.

To test for trends, we looked at the percentage points of risk reduction conferred by each additional cup of coffee consumed when consumption was coded as a continuous variable or we assigned a median value to each category of coffee consumption when coded as a categorical variable. Multivariate analyses were carried out to control for the potential confounding effects of age at first birth, parity, age at menopause, oral contraceptive use, hormone replacement therapy use, smoking status, and BMI. We assessed the heterogeneity in effects between different brewing methods by Wald tests. Because of the proposed modifying role of BMI and smoking status, we performed subgroup analyses estimating the risk conferred by total coffee consumption on endometrial cancer stratified by these factors although we did not find any significant interaction between total coffee consumption and smoking (p = 0.72)

Table 1 Distribution of NOWAC postmenopausal participants according to consumption of total, filtered, boiled and instant coffee

	Total coffee, %[a]	Boiled coffee		Filtered coffee		Instant coffee	
		Combined with other brewing methods, %[b]	Only boiled coffee, %	Combined with other brewing methods, %	Only filtered coffee, %	Combined with other brewing methods, %	Only instant coffee, %
No of participants	n = 97 926	n = 93 858	n = 32 049	n = 93 858	n = 60 025	n = 93 858	n = 22 501
≤ 1 cups per day	16.1	80.0	54.4	40.1	25.6	91.7	77.5
2-3 cups per day	32.9	7.9	16.1	25.9	30.4	5.4	13.3
4-7 cups per day	37.8	8.4	20.2	25.9	33.4	2.4	7.5
≥ 8 cups per day	13.2	3.7	9.3	8.1	10.6	0.5	1.7
No of cases	n = 462	n = 452	n = 174	n = 452	n = 322	n = 452	n = 112
≤ 1 cups per day	17.7	77.9	51.2	44.2	27.6	92.7	79.5
2-3 cups per day	37.1	10.4	21.8	27.0	34.2	4.6	10.7
4-7 cups per day	38.3	10.2	23.0	25.0	32.9	2.3	8.0
≥ 8 cups per day	6.9	1.5	4.0	3.8	5.3	0.4	1.8

[a]Total coffee is combination of responses on total coffee consumption in the total coffee version of the questionnaire and filtered, boiled and instant coffee consumption in the brewing methods version of the questionnaire. [b]Combined with other types: those women who positively responded to question about "total coffee consumption" or/and other brewing method (filtered, boiled, instant).

and total coffee consumption and BMI (p = 0.66). We also performed analyses in which boiled and filtered coffee were adjusted for each other, using coffee consumption as both a categorical and a continuous variable. All analyses were conducted using SAS for Windows (version 9.2; SAS Institute Inc., Cary, North Carolina, USA). All tests were two-sided with statistical significance set at $p < 0.05$.

Results

Distribution of NOWAC participants and baseline characteristics

Participants who answered on brewing method version of the questionnaire were divided into two subgroups: those who consumed coffee prepared by only one brewing method (boiled, filtered, or instant) and those who used a mixture of brewing methods.

Among all coffee consumers, women with total coffee consumption of 2–3 cups/day and 4–7 cups/day represented the largest groups (32.9% and 37.8%, respectively). Although women drinking only filtered coffee also mostly reported to drink between 2–7 cups/day women drinking only boiled coffee appear to consume less with 54.4% drinking ≤1 cup/day (Table 1). In the group that we classified as non-consumers, there was a substantial proportion of women who were actually instant coffee drinkers in very small quantities (91.7% of participants in the group of ≤1 cup/day).

Table 2 shows the distribution of known risk factors and main baseline characteristics of our study cohort according to total coffee consumption. Women with a higher total coffee consumption had lower age at enrollment, at menarche, at first birth and at menopause, were less often nulliparous, were more often current smokers, and were leaner than women with lower coffee consumption. This tendency was observed within all brewing methods. During an average of 10.9 years of follow-up, 462 cases of incident endometrial cancer were diagnosed in 97 926 women, contributing 1 071 943 person-years of observation. The mean age at diagnosis of endometrial cancer was 61.5 years (Table 2).

Endometrial cancer risk

A statistically significant inverse association between total coffee consumption and endometrial cancer risk was observed among heavy consumers (age-adjusted HR 0.44, 95% CI 0.29-0.67) (Table 3). This association was slightly attenuated after multivariate adjustment (HR 0.52, 95% CI 0.34-0.79).

When analyzing different brewing methods, there was an inverse association among heavy consumers of filtered coffee only and heavy consumers of filtered coffee combined with other coffee brewing methods (multivariate-adjusted HR 0.55, 95% CI 0.32-0.94 and HR 0.55, 95% CI 0.33-0.91 respectively). However, when frequency of filtered coffee consumption was analyzed as a continuous variable, no significant dose-response relationship was observed for filtered coffee alone (p for trend 0.07), or for filtered coffee combined with other brewing methods (p for trend 0.12). In boiled coffee drinkers we observed a significant inverse association only among heavy consumers (age-adjusted HR 0.42, 95% CI 0.19-0.91), but this inverse association was borderline significant after multivariate adjustment (HR 0.45, 95% CI 0.21-1.01). We did not observe any significant dose-response relationship when boiled coffee

Table 2 Baseline characteristics of postmenopausal NOWAC Study participants according to total coffee consumption

Characteristics	Average total coffee consumption, cups per day			
	≤ 1	2-3	4-7	≥ 8
Number of women (%)	15795 (16.1)	32200 (32.9)	36992 (37.8)	12939 (13.2)
Number of EC cases (%)	82 (17.7)	171 (37.1)	177 (37.8)	32 (6.9)
Mean (sd)				
Age at enrollment, years	47.5 (8.64)	48,7 (8.73)	47.9 (8.31)	43.7 (6.82)
Age at menarche, years	13.4 (1.45)	13.4 (1.37)	13.3 (1.36)	13.2 (1.39)
Age at first birth, years	24.8 (4.53)	24.4 (4.39)	23.6 (4.17)	22.7 (3.99)
Age at menopause, years	48.1 (5.03)	48.7 (8.74)	48.3 (4.66)	46.9 (4.95)
Duration of OC use, years	2.9 (4.56)	2.6 (4.29)	2.5 (4.12)	2.6 (4.01)
Number of children, %				
0	11.4	9.5	7.9	7.5
1-2	55.4	53.9	52.6	53.5
≥ 3	33.2	36.6	39.5	39.0
Years of total education, %				
< 9	16.6	21.2	28.9	36.7
10-12	30.2	33.3	36.3	38.3
13-16	32.5	29.9	24.3	19.3
> 17	20.8	15.6	10.5	5.9
Smoking status, %				
Current	17.7	21.8	39.3	61.8
Former	31.1	35.6	31.9	24.6
Never	51.2	42.6	28.8	13.7
BMI, %				
< 20	10.1	8.1	7.9	10.9
20-24.9	56.8	58.1	56.7	57.6
25-29.9	24.3	26.7	28.3	24.8
≥ 30	8.8	7.1	7.0	6.8
HRT use, %				
Never	79.7	79.9	83.3	91.1
Former	8.7	8.4	7.1	3.3
Current	11.6	11.7	9.7	5.6
Prevalence of diabetes, %	0.98	0.82	0.92	0.98

Abbreviations: *NOWAC* Norwegian Women and Cancer, *EC* Endometrial cancer, *SD* Standard deviation, *OC* Oral contraceptives, *BMI* Body mass index, *HRT* Hormone replacement therapy.
N = 97 926.

consumption alone, or boiled coffee combined with other brewing methods were included as continuous variables (p for trend 0.07 and 0.36 respectively).

Since BMI is a strong risk factor for endometrial cancer, and smoking has been reported to alter caffeine clearance, we examined additional effect modification by stratifying analyses by brewing method, BMI (<25 and ≥25, which is considered overweight) and smoking status (never, former, current). Total coffee consumption was significantly inversely associated with endometrial cancer only among overweight women who were heavy total coffee consumers or heavy consumers of filtered coffee (multivariate-adjusted HR 0.39, 95% CI 0.21-0.73 and HR 0.46, 95% CI 0.22-0.96, respectively) (Table 4). There was a significant reduction in endometrial cancer risk among current smokers who were heavy total coffee consumers (multivariate-adjusted HR 0.36, 95% CI 0.17-0.79). However, the association between smoking and endometrial cancer risk in relation to different brewing methods did not reach statistical significance, which could be due to limited

Table 3 HRs and 95% CIs of endometrial cancer related to total, filtered and boiled coffee among 97 926 postmenopausal NOWAC Study participants

No of cups	Total coffee[a]	Boiled coffee		Filtered coffee	
		Combined with other types[b]	Only boiled coffee	Combined with other types	Only filtered coffee
		Age-adjusted HR, CI 95%[c]			
≤ 1 cups/day	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
2-3 cups/day	0.87 (0.67-1.13)	1.12 (0.83-1.53)	1.03 (0.70-1.51)	0.98 (0.79-1.23)	0.93 (0.70-1.23)
4-7 cups/day	0.76 (0.59-0.99)	1.06 (0.78-1.44)	0.87 (0.59-1.26)	0.89 (0.71-1.13)	0.79 (0.60-1.06)
≥ 8 cups/day	0.44 (0.29-0.67)	0.43 (0.2-0.91)	0.42 (0.19-0.91)	0.48 (0.29-0.79)	0.45 (0.26-0.75)
p for trend	< 0.0001	0.21	0.02	0.002	0.03
		Multivariate-adjusted HR, CI 95%[d]			
≤ 1 cups/day	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
2-3 cups/day	0.91 (0.70-1.19)	1.08 (0.79-1.46)	1.04 (0.71-1.54)	0.99 (0.79-1.24)	0.99 (0.75-1.31)
4-7 cups/day	0.84 (0.65-1.1)	1.10 (0.81-1.50)	0.92 (0.62-1.36)	0.95(0.76-1.20)	0.91 (0.68-1.21)
≥ 8 cups/day	0.52 (0.34-0.79)	0.48 (0.23-1.03)	0.45 (0.21-1.01)	0.55 (0.33-0.91)	0.55 (0.32-0.94)
p for trend	0.003	0.36	0.07	0.12	0.07

[a]Total coffee is combination of responses on total coffee consumption in the total coffee version of the questionnaire and filtered, boiled and instant coffee consumption in the brewing methods version of the questionnaire. [b]Combined with other types: those women who positively responded to question about "total coffee consumption" or/and other brewing method (filtered, boiled, instant). [c]Basic model that was adjusted for age. [d]Multivariate model that was adjusted for parity, smoking status, BMI, duration of OC and HRT use. *Abbreviations*: *HR* Hazard ratio, *CI* Confidence interval, *NOWAC* Norwegian Women and Cancer, *BMI* Body mass index, *OC* Oral contraceptives, *HRT* Hormone replacement therapy.

statistical power. Stratified analysis by history of diabetes mellitus, physical activity and hormone replacement therapy use was hampered by small numbers. Nevertheless, when analyses were restricted to non-diabetic women, results were similar to those from the entire study sample. To eliminate the potential effects of early undiagnosed endometrial cancer, we repeated our analysis, excluding endometrial cancer diagnosed during the first year of follow-up. Results from this analysis did not differ from those for the entire cohort. Adjusting one coffee brewing method by another method did not affect any of the risk analyses (Additional file 1: Table S1). No significant differences between boiled coffee and filtered coffee were found (Additional file 2: Table S2).

Discussion

In this large population-based prospective study of postmenopausal Norwegian women, total coffee consumption was inversely associated with endometrial cancer risk, whereas there were no significant differences in this association by considered coffee brewing methods. The inverse association was stronger among overweight women, current smokers and heavy consumers. The current results show an overall association between total coffee consumption and endometrial cancer risk, which corroborates the findings of recent prospective studies [10,28-30] and supports the hypothesis that coffee and its compounds may play an important role in the chemoprevention of endometrial carcinogenesis.

There are several strengths in our study. The NOWAC cohort is well-characterized with a large number of participants who were randomly selected from the general population, giving our study enough statistical power to detect small differences in the studied subgroups. Lifestyle and dietary data were collected prior to endometrial cancer diagnosis. This minimizes the risk of recall bias, selection bias and reverse causation. In addition, we had a broad range of exposures in the cohort, and information on several coffee brewing methods. To our knowledge this is the largest cohort study to investigate the association between endometrial cancer risk and boiled/filtered coffee consumption. Finally, our cohort was based in a population with strong coffee consumption habits and low number of non-consumers.

Our study also has some limitations, such as the self-reporting of coffee consumption. Even though results from both the NOWAC Study [25] and other studies [31,32] have shown a satisfactory reproducibility and validity of information on coffee consumption, various biases may still arise when self-reported FFQs are used. However, this kind of misclassification would most probably weaken the studied association, and would not lead to significant bias. Other limitations are the lack of information on the newer types of coffee drinks that have been incorporated into the Norwegian diet in the last decade (e.g. cappuccino, mocha and latte), and the lack of information about decaffeinated coffee, which, however, was rare in Norway during the enrollment periods. Moreover, we lacked data on how coffee beans were roasted, which can also influence the level and properties of some coffee compounds [16,33]. Finally, for a

Table 4 HRs and 95% CIs of total, boiled and filtered coffee consumption stratified by BMI and smoking status in 97 926 postmenopausal NOWAC Study participants

	No of cups	Age-adjusted HR, CI 95%[a]			Multivariate-adjusted HR, CI 95%[b]		
		Total coffee	Boiled coffee	Filtered coffee	Total coffee	Boiled coffee	Filtered coffee
BMI < 25	≤ 1	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
	2-3	0.93 (0.63-1.38)	0.88 (0.53-1.47)	1.29 (0.93-1.79)	0.96 (0.65-1.42)	0.87 (0.52-1.46)	1.25 (0.90-1.74)
	4-7	0.78 (0.53-1.16)	0.88 (0.53-1.44)	1.05 (0.75-1.49)	0.86 (0.58-1.29)	0.97 (0.59-1.60)	1.11 (0.78-1.57)
	≥ 8	0.54 (0.31-0.96)	0.50 (0.19-1.36)	0.57 (0.29-1.14)	0.65 (0.36-1.17)	0.59 (0.22-1.63)	0.66 (0.33-1.32)
p for trend		0.009	0.13	0.39	0.008	0.31	0.77
BMI ≥ 25	≤ 1	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
	2-3	0.82 (0.57-1.17)	1.26 (0.85-1.85)	0.79 (0.58-1.09)	0.83 (0.58-1.19)	1.22 (0.83-1.80)	0.78 (0.57-1.08)
	4-7	0.72 (0.51-1.02)	1.13 (0.77-1.67)	0.72 (0.51-1.02)	0.78 (0.55-1.12)	1.21 (0.82-1.79)	0.83 (0.60-1.13)
	≥ 8	0.33 (0.18-0.61)	0.33 (0.10-1.02)	0.40 (0.19-0.83)	0.39 (0.21-0.73)	0.39 (0.12-1.23)	0.46 (0.22-0.96)
p for trend		0.0005	0.44	0.01	0.009	0.79	0.05
Never smokers	≤ 1	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
	2-3	1.01 (0.7-1.44)	1.09 (0.72-1.67)	1.15 (0.85-1.57)	1.05 (0.74-1.51)	1.07 (0.70-1.62)	1.19 (0.87-1.62)
	4-7	0.97 (0.67-1.41)	0.95 (0.57-1.58)	0.08 (0.76-1.53)	0.97 (0.67-1.42)	0.91 (0.55-1.53)	1.08 (0.76-1.53)
	≥ 8	0.54 (0.23-1.26)	0.36 (0.05-2.52)	0.67 (0.25-1.83)	0.49 (0.21-1.16)	0.33 (0.05-2.33)	0.63 (0.23-1.72)
p for trend		0.41	0.59	0.99	0.26	0.44	0.94
Former smokers	≤ 1	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
	2-3	0.75 (0.47-1.19)	1.05 (0.59-1.86)	0.69 (0.45-1.05)	0.76 (0.47-1.21)	1.04 (0.58-1.85)	0.70 (0.46-1.07)
	4-7	0.76 (0.48-1.21)	1.79 (1.12-2.85)	0.79 (0.52-1.18)	0.74 (0.46-1.18)	1.69 (1.06-2.72)	0.77 (0.51-1.16)
	≥ 8	0.59 (0.29-1.20)	0.25 (0.03-1.76)	0.66 (0.30-1.45)	0.57 (0.28-1.15)	0.23 (0.03-1.67)	0.64 (0.29-1.42)
p for trend		0.09	0.67	0.19	0.06	0.83	0.18
Current smokers	≤ 1	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
	2-3	0.73 (0.37-1.45)	1.16 (0.56-2.41)	1.04 (0.60-1.78)	0.76 (0.38-1.52)	1.16 (0.56-2.42)	1.04 (0.60-1.79)
	4-7	0.61 (0.32-1.15)	0.76 (0.39-1.48)	0.93 (0.58-1.50)	0.64 (0.34-1.21)	0.77 (0.39-1.50)	0.96 (0.59-1.55)
	≥ 8	0.36 (0.17-0.79)	0.69 (0.28-1.73)	0.40 (0.17-0.95)	0.37 (0.17-0.81)	0.69 (0.28-1.73)	0.40 (0.17-0.95)
p for trend		0.009	0.30	0.12	0.01	0.30	0.13

[a]Basic model that was adjusted for age. [b]Multivariate model that was adjusted for parity, smoking status, BMI, duration of OC and HRT use. *Abbreviations*: *HR* Hazard ratio, *CI* Confidence interval, *BMI* Body mass index, *NOWAC* Norwegian Women and Cancer, *OC* Oral contraceptives, *HRT* Hormone replacement therapy.

large part of the cohort there is no detailed information on consumption of tea, cola-type drinks, and chocolate, which contain some of the same possible bioactive compounds as coffee, and therefore these variables could not be adjusted for the present analysis.

Over the past two decades several studies have evaluated the association between coffee consumption and endometrial cancer risk. Only one prospective study from Sweden has investigated the association between two coffee brewing methods and endometrial cancer risk [19] and did not find any significant decrease in endometrial cancer risk associated with coffee consumption. Our results support the main hypothesis of recent prospective studies [10,28], which suggested a significant inverse association with total coffee consumption, in particular among overweight women. However, these studies reported a protective effect with moderate coffee consumption (3–4 cups/day) [10,28], whereas we only observed this effect at high consumption levels (≥8 cups/day). This discrepancy might be explained by the fact that Scandinavians used to drink far more coffee than people in other countries [34,35]. Recent investigations from the National Coffee Information Organization showed that in the period 1982–2009, which includes the enrolment period of the NOWAC Study, 90% of Norwegians aged over 40 years had an average coffee consumption of 4–5 cups/day [21]. It is possible that such a long tradition of heavy consumption changed mechanisms involved in the association between coffee consumption and cancer risk. This hypothesis however, needs further exploration. We cannot exclude the possibility that coffee consumption reported at enrollment decreases during follow-up for most participants.

There are several potential theories by which coffee may reduce endometrial cancer risk. The hormonal theory asserts that the metabolism of caffeine and other coffee compounds interact mainly with the metabolism

of estrogens. The oxidative metabolism of both caffeine and estrogens are regulated by the same family of cytochrome enzymes, and there is an interaction between them [36-39]. In addition, the hormonal mechanism focuses on the ability of coffee to regulate several hormone levels, the altered stimulation of which may lead to hyperinsulinemia and, therefore, increased proliferation of the endometrial stromal cells [40,41]. Thus, coffee increases the level of adiponectin, the deficiency of which leads to hyperinsulinemia [42], increases the level of free estrogens by decreasing the level of sex hormone-binding globulin (SHBG), and increases the level of circulating free insulin-like growth factor-1 by decreasing the levels of insulin-like growth factor-binding protein [9]. Another theory suggests that coffee compounds block the initiation phase of the carcinogenic process, decrease DNA damage and protect cells against reactive oxygen species by inducing the production of detoxifying enzymes through several mechanisms [43,44].

The question about which bioactive compounds in coffee have a higher antioxidant capacity remains open. Exposure to these coffee components is highly dependent on different conditions such as choice of beans and phase of administration, and is most likely also dependent on brewing method [16,33]. For a long time in Norway, boiled coffee, which is prepared by boiling water and adding coarse grounds in a pot, was the traditional brewing method. However, since boiled coffee may increase cholesterol levels [15], this brewing method was, to a large extent, replaced by filtered coffee starting in the 1990s. Based upon the market information from the National Coffee Information Organization [21], Norway has been mostly importing coffee Arabica, which is known to contain a higher percentage of diterpenes, but a lower concentration of caffeine in comparison to coffee Robusta [16]. In some studies it was highlighted that use of different methods and conditions can change the chemical composition of coffee and therefore open opportunities to modify and design various coffee brews with desired beneficial health effects [15]. Despite of this, we did not observe any significant favorable differences in the brewing methods we considered in our report. On another note, the negative effects of coffee drinking on health cannot be ignored. Indeed, high amounts of boiled coffee may increase cholesterol levels [45], although the beneficial effects of coffee on health might be expected at consumption levels that do not entail any increase in blood cholesterol [46].

In subgroup analyses, our data suggested that mechanisms involving interactions with BMI and smoking may be relevant in the association between coffee consumption and endometrial cancer risk. Indeed, overweight women with high coffee consumption have already been reported to have stronger protection against endometrial cancer in comparison to lean women [10,28]. This could be explained by the fact that obese women already have lower levels of SHBG, resulting in higher levels of bioavailable estrogen, hypoadiponectinemia, insulin resistance, hyperinsuliemia, and high levels of oxidative stress [47], all of which have been reported to improve in coffee drinkers [9,42]. It is also of interest that in our study there was a strong significant inverse association between coffee drinking and endometrial cancer risk among women who were current smokers. These results are consistent with the results from a recently published study [10], but the underlying mechanisms are not fully understood. It is known that caffeine, nicotine and estrogens are metabolized by the same family of cytochrome P450 enzymes [48,49]. As the half-life of caffeine is much lower in smokers than non-smokers, caffeine intake is usually higher in smokers. It was also reported that smoking induces the 2-hydroxylation pathway of estradiol metabolism, leading to decreased bioavailability at estrogen target tissues. Thus, it was suggested that caffeine in combination with nicotine enhances the clearance of estradiol by increasing CYP1A2 activity, and, hence, the combination of coffee drinking and smoking might offer more pronounced protection against hormone-dependent tumors [50]. In addition to high BMI and smoking, some studies on the association between endometrial cancer and coffee consumption have reported a stronger association among never users, or at least former users, of hormone replacement therapy [10,28]. We did not observe any significant differences in our subgroup analyses for ever and never users of hormone replacement therapy but our analysis was hampered by small numbers.

Conclusions

In conclusion, the findings of our study are in line with previous reports indicating that total coffee consumption may decrease endometrial cancer risk. In addition, we found no significant heterogeneity in risk when comparing different brewing methods (filtered and boiled coffee). Because our population generally has a high coffee consumption at inclusion, the observed decreased endometrial cancer risk among women consuming ≥8 cups/day should be considered with caution. Thus, these findings need confirmation in other populations with consistent heavy coffee consumption.

Additional files

Additional file 1: Age-adjusted and multivariate-adjusted HRs and 95% CIs. One coffee brewing method adjusted for other brewing methods.

Additional file 2: Test for heterogeneity. Comparison between heavy boiled coffee drinkers only and heavy filtered coffee drinkers only.

Abbreviations

BMI: Body mass index; CI: Confidence interval; HR: Hazard ratio; NOWAC: Norwegian Women and Cancer; SBHG: Sex hormone-binding globulin.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
OG carried out the interpretation of the data and drafted the manuscript. TB carried out the statistical analysis. EL developed the research plan, directed the analysis and contributed with critical revision of the manuscript. He is the principal investigator and designed the NOWAC Study. VD contributed with the interpretation of the data and helped to draft the manuscript. GS contributed with the planning of statistical analysis, consulted in nutrition questions and helped in revision of the manuscript. EW contributed with planning the statistical analysis, interpretation of the data, and revision of the manuscript. All authors read and approved the final manuscript.

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Paper II

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Lifetime number of years of menstruation as a risk index for postmenopausal endometrial cancer in the Norwegian Women and Cancer Study

Running headline: Menstruations and endometrial cancer

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Conflict of interest

The authors declare that they have no conflict of interest.

Abstract

Introduction. Lifetime number of years of menstruation (LNYM) reflects a woman's cumulative exposure to endogenous estrogen and can be used as a measure of the combined effect of reproductive factors related to endometrial cancer (EC) risk.

Material and methods. We aimed to study the association between LNYM and EC risk among postmenopausal women and calculate the population attributable fraction of EC for different LNYM categories. Our study sample consisted of 117 589 women from the Norwegian Women and Cancer (NOWAC) Study. All women were aged 30-70 years at enrollment and completed a baseline questionnaire between 1991 and 2006. Women were followed up for EC through December 2014 via linkages to national registries. We used Cox proportional hazards models to estimate hazard ratios with 95% confidence intervals (CIs), adjusted for potential confounders.

Results. Altogether, 720 women developed EC. We found a statistically significant, positive dose-response relationship between LNYM and EC, with a 9.1% higher risk for each additional year of LNYM (p for trend <0.001). Using the LNYM category ≥40 as a reference, the hazard ratios for LNYM <25, 25-29, 30-34, 35-39 were 0.17 (95% CI 0.22-0.27), 0.25

(95% CI 0.17-0.36), 0.43 (95% CI 0.32-0.58), and 0.68 (95% CI 0.51-0.92), respectively. The association between LNYM and EC was independent of incomplete pregnancies, menopausal hormone therapy, diabetes and body mass index. When considering population attributable fraction, 67% of EC was estimated to be attributable to LNYM ≥25.

Conclusions. Our study supports that increasing LNYM is an important and independent predictor of EC risk.

Keywords

endometrial cancer; number of menstruations; reproductive factors; prospective study; menopause

Abbreviations

BMI body mass index;

CI confidence interval;

EC endometrial cancer;

HR hazard ratio;

LNYM lifetime number of years of menstruation;

MHT menopausal hormone therapy;

NOWAC Study Norwegian Women and Cancer Study

PA physical activity

PAF population attributable fraction

Key Message

Higher number of years of menstruation is significantly associated with increased risk of endometrial cancer in Norwegian women.

Introduction

Endometrial cancer (EC) is the sixth most common cancer among women worldwide and the most common gynecological cancer in the Western World (1). In Norway, EC incidence rates have increased markedly in the last decades (2), with age-standardized rates of 19.7 per 100 000 person-years reported in the period 1982-1986 and 27.6 per 100 000 person-years in the period 2012-2016 (3). Further, EC incidence rates in Norway are predicted to increase by 57% in 2025, compared with the rates observed in 2005 (4).

Among women, menstrual and reproductive factors, such as earlier age at menarche (5-9), later age at menopause (5-8, 10, 11), nulliparity, and/or nulligravidity (5-9, 12-17), contribute to hormonal changes, the effects of which play an important role in the development of hormone-related cancers. Indeed, these factors might be linked to prolonged, excessive exposure of endometrial cells to estrogen, leading to an increased EC risk. Conversely, full-term pregnancies (9, 14), later age at last birth (5, 16, 17), and breastfeeding (5, 9, 12, 13) play a protective role in EC risk due to prolonged exposure to progesterone. Studies investigated relationship between incomplete pregnancies and EC risk provided controversial results, showing no association (5, 12, 18-21), inverse association (9, 16, 22) or even increased risk (8). Lifetime number of years of menstruation (LNYM) can be used as a composite variable to summarize the effect of the above-mentioned factors and indirectly measure cumulative exposure to endogenous hormones during a woman's reproductive years.

Several epidemiological studies have prospectively investigated the combined impact of menstrual and reproductive factors on EC risk. The most cited reports investigated the effect of number of years of ovulation or total menstrual lifespan (5, 8, 9). However, studies based

on postmenopausal populations from the USA (IOWA population) (8) and China (9) were limited by a small number of cancer cases, and they presented age-adjusted analyses only, failing to control for potential risk factors. The study by Dossus et al. (5) included both pre- and postmenopausal women with heterogeneous information on breastfeeding and number of full-term pregnancies from different European countries. That study presented risk estimates per year of menstruation, but did not show any association between increasing LNYM and EC.

When strong associations are observed between an outcome and a risk factor, population attributable fraction (PAF) is often used to measure the impact of that risk factor on a population level (23). Although recent studies have investigated the PAF of EC in relation to physical activity (PA), obesity, menopausal hormone therapy (MHT) use, parity, and breastfeeding (14, 24, 25), to our knowledge, there are no published cohort studies that have calculated PAF in regard to composite variable like LNYM, which covers cumulative menstrual and reproductive risk factors. Using a population-based cohort of Norwegian women, we aimed to study the association between LNYM and EC risk among postmenopausal women and calculate the PAF of EC for different LNYM categories.

Material and methods

The Norwegian Women and Cancer Study

The Norwegian Women and Cancer (NOWAC) Study is an ongoing, nationally-representative, prospective cohort study, which includes Norwegian women aged 30-70 years who were randomly selected from the Central Population Register of Norway (26). Selected women received a comprehensive, eight-page, self-administered questionnaire, which included questions on diet, medical history, and lifestyle; and an informed consent form. Women were recruited during two waves of data collection (1991/97 and 2003/06), with an overall response rate of 57% and 48.4% respectively. In total, more than 172,478 women completed the enrollment questionnaire. Follow-up questionnaires were sent at intervals of 6-

8 years. The external validity of the NOWAC Study is reported to be acceptable (27). Further details on the NOWAC Study and its design have been described in detail elsewhere (28).

Study sample

Women who reported that their periods stopped spontaneously (*Do you still have regular/irregular menstruation? Did menstruation stop? yes/no*) in either their baseline or follow-up questionnaire were categorized as postmenopausal and were eligible for inclusion (n = 159 246). We then excluded participants with prevalent cancer (*n* = 7246), those who reported hysterectomy or oophorectomy at baseline or follow-up (*n* = 12 221), and those who emigrated or died before the start of follow-up (*n* = 7). We further excluded women with missing information on years of menstruation (*n* = 11 113), which included missing information in age at menarche (*n* = 2274) and ever use of oral contraceptive and duration (*n* = 8839). Women with missing information on the selected confounders: height or weight (*n* = 2666) (24), smoking status (*n* = 557), and PA (*n* = 7847) were also excluded. Thus, the final study cohort included 117 589 postmenopausal women.

Assessment of covariates and calculation of lifetime number of years of menstruation

Information on the covariates age at menarche, age at menopause, number of full-term pregnancies, duration of breastfeeding, pregnancies shorter than 6 months of duration, height, weight, oral contraceptive use, smoking status, MHT use, diabetes and smoking status was taken from NOWAC questionnaires. Self-reported height and weight (29) were used to calculate body mass index (BMI) in kg/m^2. Parity and breastfeeding variables are generally reported to have good validity in the NOWAC Study (27). Missing information on age at menopause was treated according to smoking status, as women who smoke have been shown to have earlier menopause (30). Mean age at menopause for current and former smokers in our study (49 and 50 years, respectively) was used to complete missing data for participants who were current or former smokers. For non-smokers, missing data on age at menopause was set at 53 years, which has been used in the NOWAC Study before (31) and represents approximately 80% of women in our study population. Assessment of PA level was performed as in previous NOWAC reports (32, 33).

LNYM represented the cumulative duration of menstrual cycles in a woman's lifetime. However, we used a definition that was slightly different from that used in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study (34). Instead, we defined LNYM as the number of years between age at menarche and age at menopause, minus the cumulative duration of full-term pregnancies (calculated as the number of full-term pregnancies, including live and stillbirths, times 0.75 years), duration of breastfeeding (calculated as the cumulative number of months of breastfeeding in all pregnancies), and duration of oral contraceptive use. LNYM was then divided into 5 categories: <25, 25-29, 30-34, 35-39, ≥40. All the aforementioned variables were added on a continuous scale in years.

MHT is an established risk factor for EC (35) and is also associated with menstrual characteristics (36). However, we decided not to include MHT in the multivariable models, since this variable is included in the calculation of LNYM through age at menopause.

Identification of endometrial cancer

Women with EC were identified through linkage to the Cancer Registry of Norway via the unique identification number assigned to each resident of Norway. The registry provides detailed information on all cancer sites and histology, and covers the whole population of Norway (3). To identify topography, we used the International Classification of Diseases (ICD), Revision 7 and 10 (code 172 for corpus uteri cancer in ICD-7 or corresponding code C54 in ICD-10 version). Morphological codes were further classified according to the International Classification of Diseases for Oncology, Revision 2 and 3. Ninety-nine percent of identified EC cases were type 1 and 0.4% were type 2 (37, 38), with the following distribution of histological subtypes: 670 (93%) endometrioid adenocarcinoma, 38 (5.3%) adenocarcinoma with squamous metaplasia, and <1% other types, including five (0.7%) irregular plate epithelium, two (0.28%) adenocarcinoma UNS, one (0.14%) undifferentiated carcinoma, one (0.14%) combined small cell carcinoma, one (0.14%) papillary adenocarcinoma, one (0.14%) serous papillary adenocarcinoma, and one (0.14%) stromal sarcoma respectively.

Statistical analyses

As we studied postmenopausal women, age at inclusion into the present study was set as the age at menopause. Therefore, we calculated person-years from age at menopause to the date of any incident cancer diagnosis (except basal cell carcinoma), emigration, death, or the end of the study (31 December 2014), whichever came first.

We used Cox proportional hazards regression (39), with age as the underlying time scale, to estimate age-adjusted and multivariable-adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) for the associations between EC and LNYM. Multivariable-adjusted models included BMI (normal weight: <25, overweight: 25–29.9, obese: ≥30 kg/m^2), smoking status (never, former, current), and PA level (PA <5, PA ≥5). The proportional hazards assumption was checked by Schoenfeld residuals, and there was no evidence of deviation from proportionality. We further used Royston-Parmar flexible parametric proportional hazard models (40) to estimate the baseline HRs according to different LNYM categories (Figure 1). Cubic splines were used to show the dose-response associations between LNYM and EC risk. Adjusted HRs and 95% CIs (dashed lines) were constructed with four knots based on Harrell's default percentiles (41) (Supporting Information Figure S1). We then used a Wald-type test to check for any non-linear relationship between LNYM and EC risk.

We performed sensitivity analyses estimating the association between LNYM and EC risk in each BMI category (<25, 25–29.9, ≥30 kg/m^2) and in each PA category (PA <5, PA ≥5). We also estimated Cox regression with additional adjustment for diabetes and MHT separately and combined. Other sensitivity analyses were undertaken, which included information on incomplete pregnancies (abortion, yes/no; extra-uterine pregnancy, yes/no). There were 52 796 (48%) women with information on abortions (without separating into induced or spontaneous, defined as "abortion variable" in our analysis), 29 250 (27%) with information available on extra-uterine pregnancies (defined as "exu-variable" in our analysis), and 33 450 with information on both these variables. Therefore we constructed models with two new LNYM values, which were calculated in the same manner as LNYM above, but also subtracted 12 weeks for each incomplete pregnancy. The value LNYM_1 included both the abortion and exu-variables (n = 33 450), and LNYM_2 included just the

abortion variable (n = 65 548). Using Cox regression, we then estimated the association between LNYM_1 and EC risk, and between LNYM_2 and EC risk (data not shown). A final sensitivity analysis was restricted to women who never used oral contraceptives.

We calculated the PAF to estimate the proportion of EC that could have been prevented in the population if women had a lower LNYM, using the formula: PAF=Pe*(RRe-1)/[Pe*RR+(1-Pe)], where Pe is the proportion of LNYM in the study population, and RRe is the RR in the final baseline multivariable proportional hazards regression model, including all aforementioned confounders and BMI. We calculated two-sided 95% CIs for the PAFs using the PUNAF Stata module (42).

We constructed cumulative incidence rate (CIR) plots for EC in the NOWAC Study and compared them with those of the cumulative incidence rate in the general Norwegian female population (Supporting Information Figure S2).

All the analyses were done in 214 STATA version 14.0 (Stata Corp, College Station, TX, USA).

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. All the participants were informed about the study objectives and provided informed consent.

Results

Of the 117 589 included postmenopausal women, 720 developed EC during the study period. Age at EC diagnosis among our NOWAC participants ranged between 31 and 70 years, with a mean age of 62 (standard deviation [SD] 6.5) years. On average, participants reported age at menarche of 13 (SD 1.4) years and age at menopause of around 50 (SD 3.6) years (Table 1). With increasing LNYM, we observed the following linear change in baseline characteristics: decrease in age at menarche, increase in age at menopause, increase in nulliparity, decrease in duration of breastfeeding, younger age at last birth, increased number of incomplete pregnancies, increase in mean BMI and obesity (BMI ≥30), decrease in smoking, decrease in MHT and oral contraceptive use among ever users, and an increase in the number of women with diabetes (Table 1).

Our participants breastfed for on average around 7 months (SD 1.0), had a mean BMI of 24.3 (SD 3.9) kg/m^2, and almost half had at least two children and were ever users of oral contraceptives (n = 47 287) (Table 1). Interestingly, women who developed EC had a mean BMI of 26.5 (SD 5.2) kg/m^2, 306 women (42.5%) had two children, only 31 (4.3%) had diabetes, 197 (27.4%) had ever used oral contraceptives, and 159 (22.1%) were ever MHT users (data not shown).

We observed a significant dose-response association between LNYM and EC risk (p trend <0.0001). Compared to women with a LNYM >40 (reference group), the multivariable HR for those with LNYM <24, 25-29, 30-34, and 35-39 were 0.17 (95% CI 0.22-0.27), 0.25 (95% CI 0.17-0.36), 0.43 (95% CI 0.32-0.58) and 0.68 (95% CI 0.51-0.92), respectively. For every additional LNYM, women experienced a 9.1% higher EC risk. Using the lowest LNYM category (LNYM <25) as a reference, as was done in previous analogue reports, rendered a HR of 5.0 (95% CI 3.10-8.03) (Table 2).

Although the test for interaction between BMI and LNYM was not statistically significant ($p = 0.78$), we decided to look at the association between EC risk and BMI in 2 categories ≤24.9 and ≥25 (Table 3). When we did this, both age-adjusted and multivariable analysis showed a significant ($p = 0.0001$) increased EC risk with increasing LNYM.

Figure 1 illustrates age-specific HRs for EC by LNYM. All lines showed a sharp increase in hazards in the perimenopausal period and a peak in postmenopause (60-65 years), before levelling off after age 70. Cubic splines illustrating dose-response associations between LNYM and EC risk showed nonlinearity tests of $p = 0.001$, and the restricted cubic splines model showed a consistent increase in EC risk for each additional LNYM (Figure S1).

Sensitivity analyses restricted to never users of oral contraception, as well as models using the values LNYM_1 (that included both the abortion and exu-variables) and LNYM_2 (that included just the abortion variable), were of similar magnitude and in line with the main dose-response trend. Additional stratification for PA, MHT, and diabetes did not attenuate these results. Tests for interaction between PA (PA <5, PA ≥5) and BMI (BMI ≤24.9 and BMI ≥25) were not significant (Table 3).

PAF calculations showed that if women with LNYM ≥35 could decrease their LNYM <35 years, 48% of EC could be avoided. The proportion of avoided cases increased to 64% and 67%, if LNYM was decreased to 20 and 25 years, respectively (Supporting Information Table S1).

Discussion

To our knowledge, this is the first large, nationally-representative cohort study that estimated the fraction of EC in postmenopausal women attributable to LNYM. We observed a significant increase in EC risk with each additional LNYM. EC risk was more pronounced in women in aged 50-65 years, but this was no longer significant after approximately 70 years

of age, confirming the limited effect of reproductive factors in EC risk. Stratification for BMI, MHT use, and diabetes did not attenuate the association between LNYM and EC risk.

The PAF was interpreted as the proportion of overall ECs that would not occur in the average population if women with a LNYM ≥35 had a LNYM <35 (Table S1), assuming that the distribution of the adjustment variables remained unchanged. Our PAF estimates are consistent with other studies (14), showing that reproductive factors explain almost half of EC incidence.

Several studies have investigated the association between cumulative lifetime hormonal exposure and EC risk by merging the effects of several hormone-related factors (43-49). In 1986, Pettersson et al. were the first to present a clear, dose-response association and a 4-fold increased EC risk with a longer menstruation span (50). Thereafter, other studies looked at this association using a prospective design (5, 8, 9). All analogue cohort and case-control studies have substantial methodological heterogeneity in their construction of LNYM and in the number of potential confounders available for adjustment (46). In addition, and in contrast to other studies, we used the highest LNYM category (LNYM ≥40 years) as the reference category, as there were fewer EC in the lowest category of LNYM, and our intention was to show the distribution of risk estimates in 5-year intervals. When we ran analyses using our lowest LNYM category (LNYM <25) as a reference, women with >40 LNYM showed a five-fold increased EC risk (HR=5.0, 95% CI 3.10-8.03). Nevertheless, this methodological difference did not alter the significant dose-response association found in our study, which is in line with other earlier reports.

Despite the limited number of reports that directly investigated the association between EC risk and LNYM, there are numerous studies that indirectly confirmed this association by showing the effect of each individual component of LNYM. A woman's natural menstrual lifespan starts at menarche, is interrupted by pregnancies and breastfeeding periods, and ends with menopause (50). All these factors contribute to changes in lifetime exposure to natural

estrogen and progesterone and may, therefore, contribute to endometrial carcinogenesis. However, the possible long-term consequences of each reproductive factor differ substantially and vary across individuals (51).

In order to minimize the possible influence of lifestyle risk factors on the association between LNYM and EC risk, we took into account the effect of BMI, MHT use, and diabetes. Obesity and overweight are reported to contribute to about 40% of EC cases, and according to several reports, they confer a 4- to 6-fold increase in risk (52). However, when we adjusted for or stratified by BMI, the results and dose-response trend were lightly attenuated but remained significant, suggesting that LNYM and BMI have an independent effect on EC risk. These findings are in line with another recently published study, showing that, in comparison to genetic determinants, reproductive factors are less dependent on obesity and overweight in regard to EC risk (45). We did not observe any changes in the main association when we stratified by diabetes and MHT. The possible effect of MHT use in our study was also ruled out by including this variable in multivariable-adjustment analysis and in the calculation of LNYM.

The relationship between LNYM and EC risk is clearly biologically plausible. In terms of EC development, there are two possible key mechanisms. The first one relates to the widely proposed “estrogen window hypothesis”, which is based on incessant ovulation causing prolonged exposure to unopposed estrogen (53). The second mechanism supports the theory that the increased number of periods and, therefore, cycles, creates incessant repeated disruption of the uterine lining and increases the probability of genetic alterations (8). Previous studies reported a low incidence of breast and other estrogen-dependent malignancies among indigenous women. It has been shown that these women historically had fewer periods and ovulatory cycles during their life, due to multiple pregnancies and long periods of breastfeeding (54).

The main strength of our study is the population-based prospective design, as the NOWAC Study is representative of Norwegian middle-aged women. A good illustration of this are the cumulative incidence rate plots for EC constructed for both NORDCAN

(Norway) (2) and NOWAC, which are matched by age group (Figure S2). Another important strength of our study is the large sample size, which gave sufficient statistical power to investigate the association between LNYM and EC risk, as well as the effect of important confounding factors. Being population-based, our study is of particular interest in showing the independent association between reproductive factors and EC risk, which can likely be extrapolated to similar populations. We observed normal or slightly increased BMI and few cases of diabetes among our participants, allowing us to propose that other factors might also contribute to the continuous increase in EC in Norway. Along with other Scandinavian countries, and in contrast to several other countries in Europe, Norwegian women had earlier access to oral contraceptives, which were widely used in this study population (55). This allowed us to perform additional analyses among ever users and never users of oral contraceptives and conclude that the dose-response relationship we observed between LNYM and EC risk is independent of oral contraceptive use. Moreover, 99% of the EC cases in our study were type 1, which is believed to be more associated with reproductive factors (38), thus strengthening the plausibility of our findings. The results of sensitivity analyses also confirmed the validity of our LNYM variable, showing unchanged HRs regardless of which risk factors were included.

Our study also had several methodological limitations. First, we used information about past events in women's life, thus misclassification of exposures may have occurred. However, given the prospective nature of our design, if recall errors exist, we would expect them to be non-differential. Second, although we were able to include information on incomplete pregnancies for some women, a substantial proportion of women had missing data on these variables. However, several studies with higher statistical power showed no biological evidence that incomplete pregnancies produce the equivalent long-term decrease in estrogen levels that full-term pregnancies do in regard to hormone-dependent cancers (56). Third, due to lack information on menstrual regularity, bleeding volume, anovulation, and cycle length, we cannot rule out the possibility of residual confounding (57). Moreover, ovulatory cycles and LNYM might be independent risk factors (58), and we could not address the potential effect of other bleeding problems, like secondary amenorrhea, that some women might experience during their reproductive life. Finally, due to a limited number of premenopausal EC in NOWAC, our analysis was restricted to postmenopausal women, which, on the other hand, allowed us to look at the effect of the entire menstrual span.

In summary, the results indicate that a higher LNYM increases EC risk among postmenopausal women. Our results support the hypothesis that LNYM is an important tool that represents the cumulative effect of several risk factors and can be used to predict EC risk at a population level, which is, in our opinion, a better indicator of risk than each individual component.

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Supporting Information legends

Table S1. Population attributable fraction (PAF): proportion of endometrial cancer in the population that would be avoided if lifetime number of years of menstruation (LNYM) category decreased.

Figure S1. Spline regression models for lifetime number of years of menstruation in relation to endometrial cancer risk. Four knots determined by Harrell's default percentiles of lifetime number of years of menstruation. Solid lines – hazard ratios, dashed lines - 95% confidence intervals.

Figure S2. Cumulative incidence rate (CIR) for endometrial cancer in Norwegian Women and Cancer (NOWAC) Study and Cancer statistics for the Nordic countries (NORDCAN). PY – person-years.

Figure and Table legends

Table 1. Selected baseline characteristics of participants in the Norwegian Women and Cancer Study by lifetime number of years of menstruation (LNYM) (n=117 589). BMI, body mass index.

Table 2. Hazard ratios (HRs) and 95% confidence intervals (CI) for endometrial cancer by lifetime number of years of menstruation (LNYM) in the Norwegian Women and Cancer Study (n=117 589).

Table 3. Sensitivity analyses. Hazard ratios (HRs) and 95% confidence intervals (CI) for endometrial cancer by lifetime number of years of menstruation (LNYM) according to body mass index (BMI), physical activity (PA), diabetes and menopausal hormone therapy (MHT) use, abortions, extra-uterine pregnancies, and never oral contraceptive (OC) use in the Norwegian Women and Cancer Study.

Figure 1. Smoothed baseline hazard rate of endometrial cancer by lifetime number of years of menstruation category estimated with stpm2 models.

Table 1. Selected baseline characteristics of participants in the Norwegian Women and Cancer Study by LNYM (n=117 589)

Characteristics	LNYM				
	<25	**25-29**	**30-34**	**35-39**	**≥40**
Person-years at risk[a]	21 779	288 258	684 430	452 787	41 874
N of endometrial cancer cases	27	60	270	314	49
Age at menarche (mean, ±SD)[b]	13.6 (1.4)	13.6 (1.5)	13.5 (1.3)	12.8 (1.2)	12.0 (1.2)
Age at menopause (mean, ±SD)[c]	46.9 (5.5)	48.3 (3.5)	49.9 (2.2)	52.1 (2.5)	54.4 (2.1)
Age at first birth (mean, ± SD)	24.3 (4.9)	24.2 (4.5)	23.9 (4.3)	24.5 (4.6)	25.4 (5.1)
Age at last birth (mean, ±SD)	30.1 (5.6)	30.7 (5.2)	29.9 (5.1)	29.2 (5.1)	28.8 (5.2)
Number of full-term pregnancies (among parous women) (%)					
0	8.3	5.8	4.8	13.6	43.9
1	11.8	9.2	9.0	15.7	20.3
2	42.5	37.9	42.4	45.5	25.8
3	25.8	30.9	31.4	18.9	7.1
≥4	11.6	16.2	12.5	6.2	2.9
Cumulative duration of breastfeeding (%)					
0	50.3	43.9	48.9	66.9	89.8
≤1	29.4	30.7	31.9	25.3	9.0
1-3 years	16.4	20.4	17.5	7.7	1.2
>3 years	3.9	4.9	1.7	0.2	0.0
Cumulative duration of breastfeeding (years) (mean, ±SD)	0.96 (1.39)	1.08 (1.29)	0.82 (0.98)	0.49 (0.71)	0.21 (0.47)
Number of ectopic pregnancies (%)[d]					
Ever	1.2	1.3	1.3	1.2	0.8
Never	98.8	98.7	98.7	98.8	99.2
Number of abortions (%)[e]					
0	71.5	69.3	65.9	68.8	75.2
1	18.7	20.4	22.7	21.2	16.0
≥2	9.7	10.3	11.4	10.0	8.8

BMI[f] (%)					
<20	6.9	6.2	5.6	4.9	4.3
20-24.9	54.8	53.7	53.6	50.2	41.6
25-29.9	29.9	31.2	31.9	33.6	36.2
≥30	8.4	8.9	8.9	11.4	17.9
BMI (mean, ±SD)	24.2 (3.8)	24.3 (3.8)	24.1 (3.8)	24.4 (4.1)	25.5 (4.7)
Oral contraceptive use (%)					
Never	13.2	31.8	64.9	82.3	89.9
Ever	86.9	68.2	35.1	17.7	10.2
Smoking status (%)					
Never	25.1	27.2	25.9	52.9	73.0
Former	38.9	39.5	38.7	28.9	18.6
Current	36.1	33.4	35.3	18.5	8.4
Menopausal hormone therapy use (%)[g]					
Never	53.9	56.3	62.3	66.1	71.5
Former	17.8	17.3	14.6	13.3	10.2
Current	18.9	17.8	17.1	16.2	14.4
Diabetes (%)[h]					
Yes	1.6	1.7	1.7	2.1	3.4

[a] Total person-years=1,685,143; average follow-up time 14.3 years (sd 7.1)

[b] SD-standard deviation

[c] Age at menopause is the start-age of follow-up in the present study

[d] Information available in limited number of questionnaires (n= 35,540)

[e] Without separating into spontaneous or induced. Information available in limited number of questionnaires (n= 65,548)

[f] BMI: Body mass index. Measured at baseline

[g] Number of total missing 22,147(19%)

[h] Number of total missing 7,336 (6.2%)

Table 2. Hazard ratios (HRs) and 95% confidence intervals (CI) for endometrial cancer by lifetime number of years of menstruation (LNYM) in the Norwegian Women and Cancer Study (n=117 589)

LNYM	N of cases	Age-adjusted analyses HR (95% CI)	Multivariable analyses[a] HR (95% CI)
0-24	27	0.17 (0.22-0.27)	0.20 (0.12-0.32)
25-29	60	0.25 (0.17-0.36)	0.29 (0.19-0.42)
30-34	270	0.43 (0.32-0.58)	0.49 (0.36-0.68)
35-39	314	0.68 (0.51-0.92)	0.75 (0.55-1.01)
≥40	49	1.00	1.00
P for trend		0.00	0.00
Risk per year of menstruation		1.09 (1.08-1.11)	1.09 (1.07-1.11)

[a] Multivariable model adjusted for smoking, body mass index, and physical activity.

Table 3. Sensitivity analyses. Hazard ratios (HRs) and 95% confidence intervals (CI) for endometrial cancer by lifetime number of years of menstruation (LNYM) according to body mass index (BMI), physical activity (PA), diabetes and menopausal hormone therapy (MHT) use, abortions, extra-uterine pregnancies, and never oral contraceptive (OC) use in the Norwegian Women and Cancer Study

LNYM	Multivariable-adjusted analyses, HR (95% CI)						
	BMI[a] (N = 68 158)		Physical activity (PA)[b] (N = 28 847)		Diabetes+MHT[c] (N = 117 589)	Abortions[d] + extra-uterine pregnancy[e] (N = 33 540)	Never OC users[f] (N = 28 847)
	BMI <25	BMI ≥25	PA <5	PA ≥5			
≤24	0.25 (0.11-0.56), *n* = 13	0.17 (0.09-0.32), *n* = 14	0.22 (0.10-0.45), *n* = 11	0.19 (0.10-0.36), *n* = 16	0.20 (0.13-0.33), *n* = 27	0.07 (0.01-0.53), *n* = 1	0.12 (0.04-0.38), *n* = 3
25-29	0.34 (0.16-0.69), *n* = 26	0.26 (0.16-0.42), *n* = 34	0.29 (0.16-0.54), *n* = 23	0.28 (0.17-0.47), *n* = 37	0.29 (0.19-0.43), *n* = 60	0.26 (0.10-0.68), *n* = 9	0.29 (0.18-0.49), *n* = 26
30-34	0.56 (0.31-1.06), *n* = 110	0.46 (0.32-0.66), *n* = 160	0.43 (0.69-1.26), *n* = 86	0.55 (0.36-0.88), *n* = 184	0.49 (0.37-0.68), *n* = 270	0.46 (0.23-0.91), *n* = 78	0.51 (0.36-0.72), *n* = 186
35-39	0.98 (0.53-1.83), *n* = 126	0.65 (0.46-0.92), *n* = 188	0.65 (1.06-2.03), *n* = 106	0.83 (0.55-1.23), *n* = 208	0.75 (0.56-1.02), *n* = 314	0.81 (0.42-1.55), *n* = 121	0.81 (0.58-1.12), *n* = 267
≥40	1.00, *n* = 11	1.00, *n* = 38	1.00, *n* = 22	1.00, *n* = 27	1.00, *n* = 49	1.00, *n* = 10	1.00, *n* = 41
P for trend	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Risk per year of menstruation	1.09 (1.06-1.12)	1.10 (1.07-1.12)	1.09 (1.06-1.13)	1.09 (1.07-1.12)	1.09 (1.07-1.11)	1.12 (1.07-1.17)	1.10 (1.07-1.13)

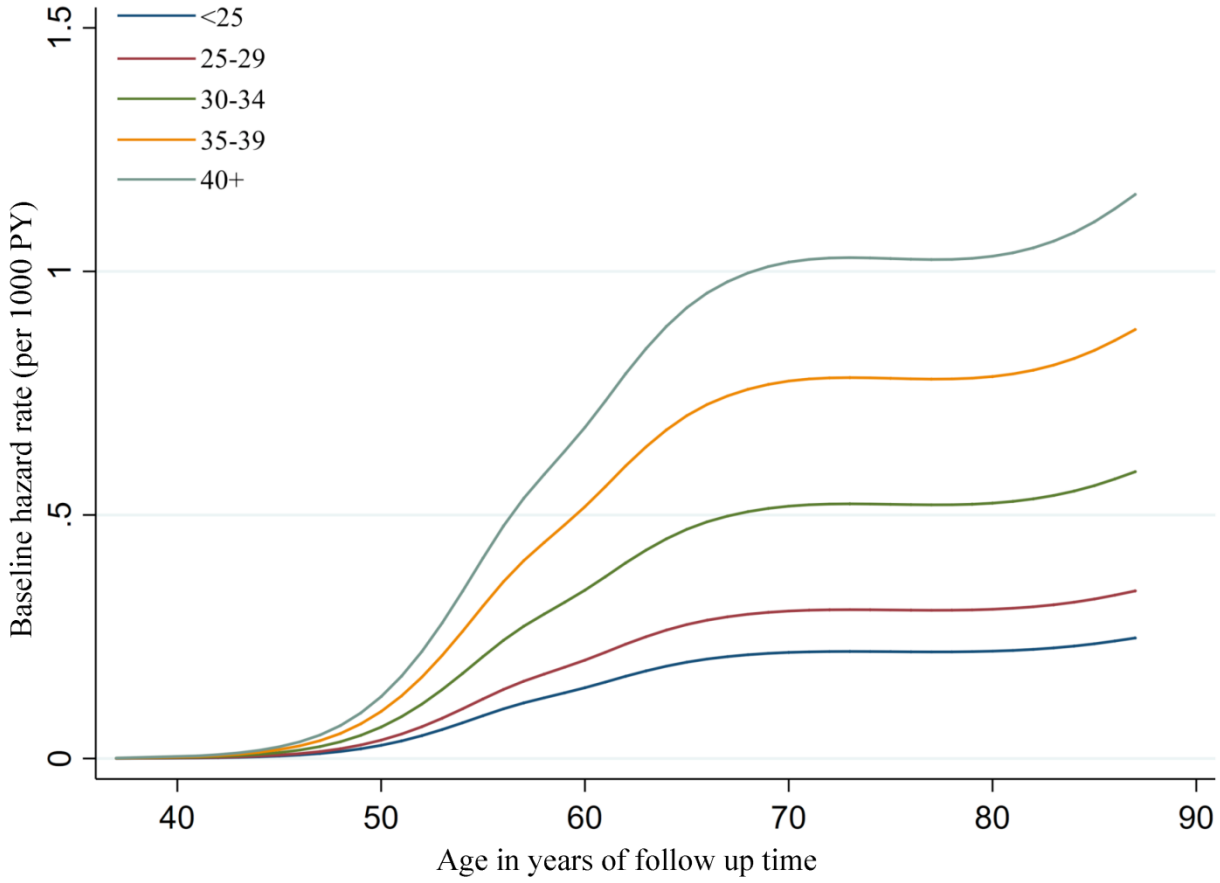
[a] Stratification analysis according to BMI. Multivariable model adjusted for smoking, physical activity.

[b] Stratification according to PA. Multivariable analysis adjusted for smoking and BMI.

[c] Multivariable model adjusted for smoking, BMI, physical activity, diabetes, and MHT use.

[d,e] Model with new LNYM_1, which includes information on both abortions ("abortion variable" in the text)[4] and information on extra-uterine pregnancy ("exu-variable" in the text)[5]. Multivariable analysis adjusted for smoking, BMI and physical activity.

[f] Model for never users of OC. Multivariate analysis adjusted for physical activity, smoking and BMI.



Paper III

Gene expression profiling of peripheral blood and endometrial cancer risk factors: systems epidemiology approach in the NOWAC Postgenome Cohort Study.

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Keywords: Endometrial cancer; gene expression; systems epidemiology; risk factors; prospective study;

Abbreviations:

LNYM - lifetime number of years of menstruation

BMI – body mass index

CI – confidence interval

EC – endometrial cancer

MHT - menopausal hormone therapy

NOWAC – The Norwegian Women and Cancer Study

OC – oral contraceptives

FDR – false discovery rate

GSEA - gene set enrichment analysis

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Author's contributions:

OG, EL, VD, and IS designed the study and interpreted the results. EL is a PI of the NOWAC Study. OG and IS constructed the tables and drafted the manuscript. MH and LH carried out the statistical analyses and contributed to drafting the manuscript. HMB participated in initial statistical analysis and critically revised the manuscript. VD and JCT contributed to the interpretation of the data, and the drafting and revision of the manuscript. All authors read and approved the final manuscript.

Abstract

Background:

Increasing incidence of endometrial cancer (EC), the most common gynecologic cancer in the world, requires extensive search for novel preventive tools and early intervention approaches. Several factors, including parity status, breastfeeding duration, use of oral contraceptives

(OC), coffee consumption, BMI, use of hormone replacement therapy (HRT), and lifetime number of years of menstruation have previously been reported to modify EC risk. However, establishment of reliable predictive models is impossible without knowledge on genetic changes prior to diagnosis. In this work, we aimed to establish if known EC risk factors influence peripheral blood gene expression in a prospective design.

Methods:

First, we selected variables that were shown to have an impact on EC risk in the whole Norwegian Women and Cancer (NOWAC) cohort (165 000 women). Then, we tested the association between these variables and changes in gene expression profiles in blood in a nested case-control study (79 case-control pairs) of women from the NOWAC postgenome cohort. Lastly, we undertook a gene set enrichment analysis (GSEA).

Results:

When we looked at overall gene expression, we found no difference between EC cases and controls. Introduction of parity status into the statistical model, revealed changes in expression of 1379 genes (false discovery rate (FDR) 20%) in controls, while we did not observe any expression changes in cases. 27 genes (FDR 20%) were associated with BMI increase in controls, whereas there was no association between changes in BMI and gene expression in women with EC. In GSEA, the major part of significantly enriched gene sets (2407, FDR 20%) were attributed to parity increase among cancer-free women.

Conclusions:

We found that increased number of parities and elevated BMI do not change peripheral blood gene expression in women diagnosed with EC later in life. The descriptive study design does not allow us to provide accurate explanation of our findings in biologic terms but this work brings solid background for further research on the development of predictive EC risk models.

Introduction

Endometrial cancer (EC) is s the most common and second most lethal gynecological cancer among women worlwide with 774 new cases registered in 2016 in Norway [1, 2]. While the incidence and mortality rates of several other cancers have plateaued or decreased in the last decade, the incidence of EC has been rising globally, with the highest increase found in countries that have undergone a drastic change in living standards and lifestyles [3, 4]. In particular, changes in reproductive factors (eg declines in parity) combined with increase in obesity prevalence might explain the rise in EC incidence associated with socioeconomic transition [5].

Both descriptive and analytic epidemiological approaches can help health officials appropriately target prevention and control activities, but provide only limited information on the biological processes underlying the cause-effect relationship between a risk factor and the disease. Technological advances in genomic profiling provide epidemiologists with the opportunity to integrate molecular data into etiologic studies in order to decypher the carcinogenic process.

So far, most studies have been focused on the development of tools to reclassify endometrial tumors according to their molecular features and stratify patients according to risk of metastases and recurrence [6-8]. We recently showed that an in-depth analysis of multi-tissue genomic profiles could be a crucial addition to the personalized assessment of an individual's biology and health [9].

As a major defense and transport system, blood cells can adjust expression of their genes in response to various environmental factors and pathological conditions. We previously highlighted several specific behavioral programs, such as metabolism or signaling, deregulated in the individual's blood cells that are associated with biological and/or

pathological responses to a given condition in the general population (eg smoking) [10], cancer-related risk factors (article in press), and health status (eg breast cancer diagnosis) [12, 13].

In this study, we aim to investigate the associations between known EC risk factors and gene expression changes in blood cells differential between women who will develop EC and controls. This will provide insight into biological processes underlying lifestyle/exposure EC risk factors that might explain incidence of EC.

Material and methods

The NOWAC Study

The NOWAC Study is a national population-based cohort study which include Norwegian women aged between 30-70 randomly drawn from the Norwegian Central Population Register [14]. Starting from 1991 and within 4-6 years intervals, these women filled in questionnaires with focus on lifestyle and health. Of this original cohort of about 172 000 women, more than 45 000 women born between 1943 and 1957 provided blood samples between 2003 and 2006 and filled an additional 2-page questionnaire to constitute the NOWAC Postgenome Cohort [14]. PAXgene tubes (PreAnalytiXGmbH, Hembrechtikon, Switzerland) were used to prevent RNA degradation after blood sampling and allowed genome–wide analyses of blood gene expression profiles. Through the linkage to the Cancer Registry of Norway and Register of death certificates in Statistics Norway, we have identified 88 women from the NOWAC Postgenome Cohort who developed EC between the time of blood sampling and December 31, 2008 (end of follow-up). Of these 88 individuals, four were excluded, as blood samples were not received and stored at -80C within 4 days after blood collection . To ensure the same storage time and age between cases and controls, the controls, who did not receive any cancer

diagnosis, were drawn at random from the NOWAC Postgenome Cohort but matched by time of blood collection and birth year. Matched case-control pairs of blood samples (n=84 pairs) were sent to the Genomics Core Facility at the Norwegian University of Science and Technology (NTNU) for microarray gene expression profiling in January 2011.

Assessment of covariates and calculation of lifetime number of years of menstruation

Information on the covariates age at menarche, age at menopause, number of full-term pregnancies, duration of breastfeeding, height, weight, oral contraceptive use, and smoking status was taken from NOWAC questionnaires (series from years 2002-2005). Self-reported height and weight were used to calculate BMI in kg/m^2. Parity and breastfeeding variables are generally reported to have a good validity in NOWAC Study [15]. The lifetime number of years of menstruation (LNYM) count the number of years between age at menarche and age at menopause, minus the cumulative duration of full-term pregnancies (calculated as the number of full-term pregnancies, including live and stillbirths, times 0.75 years), duration of breastfeeding (calculated as the cumulative number of months of breastfeeding in all pregnancies), and duration of OC use [16].

Laboratory procedures

In order to minimize the technical variability, each control sample was processed together with the matching case sample throughout RNA extraction, amplification and hybridization. Total RNA was isolated using the PAXgene Blood RNA Isolation Kit (Preanalytix, Qiagen, Hilden, Germany) following the manufacturer's instructions. RNA quantity and purity were assessed by the NanoDrop ND1000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA) and Agilent bioanalyzer (Agilent Technologies, Palo Alto, CA, USA RNA amplification was performed in 96-well plates using 300 ng of total RNA and the Illumina® TotalPrep™-96 RNA Amplification Kit (Ambion Inc., Austin, TX, USA). Genome-wide RNA profiles were obtained using IlluminaHumanHT-12 chips version 4.

Outlier removal

Initial quality control made at the NTNU laboratory revealed two technical outliers (one 5S type degradation and one failed cRNA synthesis). These were removed along with their matching samples. Further, one case was later found to have two cancer diagnoses and was excluded along with its matching control. The data was carefully investigated to identify and remove technical outliers using the standard operating procedure for outlier removal described in [17]. In particular, probes related to genes in the human leukocyte antigen (HLA) systems were excluded (38 probes). These genes are known to be expressed strongly and have a high variance, which could affect multivariate analyses used in the outlier search. For individuals that were borderline outlier candidates, we excluded the ones with quality measures outside the range of the following thresholds: RIN value <7, 260/280 ratio <2, 260/230 ratio <1.7, and 50 < RNA < 500. Based on this outlier search we identified two additional individuals as technical outliers and excluded them along with their matching individual. In total, we investigated blood profiles from 79 women who developed EC and 79 age-matched controls.

Microarray data preprocessing and normalization

Microarray data preprocessing and analysis were performed using R v3.3.1 (http://cran.r-project.org), and tools from the Bioconductor project (http://www.bioconductor.org), adapted to our needs.

Expression profiles including 47 248 probes were adjusted for background noise using the negative control probes [18]. The data was further log2-transformed using a variance stabilizing technique [19] and finally normalized using quantile normalization. We retained probes present in at least 70% of the samples. If a gene was represented by more than one probe, the average expression of the probes was used as expression value for the gene. The probes were translated to genes using the lumiHumanIDMapping database [20]. In total, the

final data set included the expression for 7104 unique genes. Finally, we computed the differences in log2 gene expression levels for each case-control pair.

Statistical methods

We used linear models (Bioconductor R-package Limma [21] to evaluate the significance of gene-wise expression differences between cases and controls using the following intercept-only model:

$$Y_{g,c} = \alpha + \varepsilon_{g,c}$$

where $Y_{g,c} = Y_{g,c}^{case} - Y_{g,c}^{ctrl}$ is the difference in log2 gene expression level for case-control pair c and gene g and $\varepsilon_{g,c}$ is normally distributed with zero expectation.

Using the same appraoach, we tested whether gene expression changes between cases and controls were associated with EC risk factors (parity, LNYM, coffee, BMI, age of menopause, or OC use) using the following model:

$$Y_{gc} = \alpha + \beta_V^{ctrl} V_c^{ctrl} + \beta_V^{case} V_c^{case} + \varepsilon_{g,c}$$

where V_c^{ctrl} and V_c^{case} are the parity, coffee, LNYM, BMI, age of menopause, or OC use variable for case-control pair c in control and cases, respectively.

Similarly, we applied a gene set approach to determine changes in gene signatures between EC cases and controls associated with known risk factors. In this approach, the dependent variable is the difference in enrichment scores for case-control pair c and a defined gene set. The enrichment scores for eight collections (C1-C7 and H) of gene sets from the Molecular Signatures Database v6.1 (http://software.broadinstitute.org/gsea/index.jsp) [22] are obtained using the GSVA Bioconductor/R package [23]. P-values were adjusted for multiple testing using the Benjamini-Hochberg procedure for controlling FDR [26].

Distributions of known EC risk factors were compared between cases and matched controls using independent two-sided sample t-tests, Mann-Whitney U tests, and Chi square tests (R statistical package).

Ethics Statement

The NOWAC study was approved by the Norwegian Data Inspectorate and the Regional Ethical Committee of North Norway (REK). The study was conducted in compliance with the Declaration of Helsinki and all participants gave written informed consent. The linkages of the NOWAC database to national registries such as the Cancer Registry of Norway and registries on death and emigration was approved by the Directorate of Health. The women were informed about these linkages. Furthermore, the collection and storing of human biological material was approved by the REK in accordance with the Norwegian Biobank Act. Women were informed in the letter of introduction that the blood samples would be used for gene expression analyses.

Results

Study population

In total, blood gene expression profiles were analyzed from 79 women diagnosed with EC after blood collection and 79 women, matched by year of birth and time of blood sampling, who did not receive any cancer diagnosis within the same interval after blood collection. There was no significant difference in age, menarche onset, and number of children between the two groups (Table 1). In both group, about half of the women were premenopausal. In general, cases had later occurrence of menopause compared to controls. Additionally, we observed a difference in LNYM with trend towards increase in cases. In our study, cumulative breastfeeding duration was the lowest in controls. This can be explained by a large spread in reported time of breastfeeding by women with EC. There were more OC users among cases. Our study population was overweight, particularly, 64.6% of women with EC diagnosis had BMI >25. Controls drank slightly more coffee.

Differential blood gene expression profiles associated with EC diagnosis and risk factors in cases and controls

After preprocessing, the study dataset included expression values for 7104 genes. In overall analysis we were unable to identify any significant differences in gene expression profiles between cases and controls. The same negative result was obtained when we performed separate analysis for each year before diagnosis.

Then, we tested the hypothesis that the expression of some genes in the blood of either cases or controls might be influenced by a set of variables modulating EC risk. Among the cases, there was no relationship between any of the variables used and log gene expression. In controls, we observed no differentially expressed genes when using a model that included either of the following: coffee consumption, age of menopause, use of OC. Variations in BMI and number of pregnancies had the strongest impact on the gene expression in controls. Increasing parity was related to expression differences of 1379 genes (FDR 20%). Higher BMI altered the expression of 8, 17, and 27 genes at FDR 10%, 15%, and 20% respectively. Of note, for both BMI and parity, the major number of top 10 genes were downregulated (Table 2).

Gene set enrichment analysis

For GSEA, we used all collections available at MSigDB (Molecular Signatures Database, http://software.broadinstitute.org/gsea/index.jsp) [27]. In women diagnosed with EC, totally, we identified 3 significantly enriched gene sets (FDR 20%), of which 1 was parity-associated; 1 was enriched along with BMI increase, and 1 was linked to the use of OC (Table S1). Among the controls, GSEA revealed 2415 enriched gene sets (FDR 20%), where 2407 were attributed to parity (Figure 2). Remarkably, the biggest part of these gene sets (786 at FDR 15% and 1184 at FDR 20%) were from C7 collection (immunologic gene sets).

Discussion

In a large prospective cohort, we studied the possibility to trace blood gene expression changes prior EC diagnosis. We did not observe any significant differences in expression signatures between cancer-free controls and women with EC when compared directly. Interestingly, BMI and parity, also known to be associated with EC incidence, were associated with significant changes in blood expression profiles in controls but not in cases. To our knowledge, this is the first study demonstrating pre-diagnostic blood gene expression differences between EC cases and matched controls utilizing systems epidemiology approach.

Association between high BMI and increased risk of EC development is well established [28]. Moreover, overweight patients with EC have higher risk of death with relative risk up to 6.25 in patients with BMI>40 compared to normal weight women [29]. The main obesity-associated pathways contributing to EC development include augmented estrogen and estrogen metabolites synthesis, presence of chronic inflammation, and insulin resistance [28]. In our single gene analysis, unexpectedly, we did not observed changes in expression associated with BMI increase in EC cases. In turn, we observed a number of genes demonstrating differential expression in controls. This finding might be explained by the fact that the difference in BMI between cases and controls in the study cohort was modest (Table 1). In GSEA, we identified 3 significantly enriched gene sets from C2 collection (1 in cases and 2 in controls), and 1 gene set from H collection. Notably, "HALLMARK_TGF_BETA_SIGNALING" gene set from H collection was enriched when the BMI of controls increased. This finding is in line with other studies demonstrating the involvement of disturbed TGFβ signaling in EC development and progression [30, 31].

Not surprisingly, the major disparity on both single gene and gene sets levels in our study was connected to the number of pregnancies. There is a large body of evidence showing the negative correlation between parity and risk of EC [32-34]. However, recent meta-analysis

report nonlinear association between number of children and RR [5]. Indeed, in the entire NOWAC cohort, we found decrease in EC incidence rate in women with 1, 2 or 3 children compared to nulliparous (Figure S1). The elevated incidence rate of EC in women with 4 and more children is attributed to the low number of women with high number of pregnancies in the cohort and, therefore, limited sample size. Similar observations were reported by other studies [33]. Reduced time of estrogen exposure with increased parity is considered a major protective mechanism [35]. Additionally, shedding of the endometrium resulting in mechanical elimination of potentially premalignant cells is well described [5]. In the current study, we observed significant enrichment of a large number of immunologic gene sets (C7 collection in MSigDB) in controls with growing number of parities. Based on this finding, it is possible to assume that changes in the immune system associated with pregnancy may be yet another explanation of parity-dependent protection against EC. Moreover, this protective effect grows cumulatively with every new child. Previously, our group published similar observations on parity and BC protection in a larger cohort (article in press). Nevertheless, taking into account the complexity of gene sets data, limited sample size, and per se explorative design of this study, it is impossible to provide clear hypothesis on how the immune system changes in pregnancy contribute to EC protection. Therefore, further studies using both laboratory and epidemiologic design, which address the long-term effects of immune processes on endometrial tumorigenesis, are warranted.

In cases, only "HALLMARK_DNA_REPAIR" gene set was significantly associated with high parity (FDR 10%). Alterations in DNA repair machinery are well known to play a major role in EC carcinogenesis [36]. Thus, it is possible to hypothesize that mutations influencing DNA repair mechanisms could abrogate protective effect of parity on EC and lead to cancer development even in women who have given several births.

In current work, other factors with published evidences of involvement in EC development had relatively week impact on pre-diagnostic blood gene expression.

It has been demonstrated in NOWAC and by others that increased coffee consumption inversely associated with EC risk [9, 17]. Here we identified one significant gene set "HALLMARK_IL2_STAT5_SIGNALING" (FDR 20%) related to coffee drinking in controls. Recently, Gotthardt and co-authors demonstrated that maintenance of stable STAT5 level is necessary for tumor surveillance by NK cells [37]. STAT5 depleted NKs were shown to promote tumor development. Hence, impact of coffee compounds on STAT5 metabolism in immune cells can be an additional biologic substrate of protective functions.

In OC users among cases, we revealed significant enrichment of "REACTOME_HYALURONAN_METABOLISM" gene set (FDR 20%). Interestingly, despite the low significance level, second gene set from the top "REACTOME_HYALURONAN_UPTAKE_AND_DEGRADATION" was also related to hyaluronic acid metabolism. Elevated levels of hyaluronic acid in both tumor tissue and serum have been demonstrated to be involved in EC progression [38, 39]. Therefore, monitoring of hyaluronan in the blood of women using OC might be a valuable tool in endometrial cancer screening.

To what extent circulating blood cells can reflect processes that occur in tumors is still an open question. Being an easily accessible tissue, blood may serve as an ideal tool for disease prognosis, monitoring and assessment of the treatment. In this work, we attempted to discover gene expression changes in circulating cells that can be identified long before diagnosis of EC. It is important to emphasize that findings reported here need further investigation as most of the information available on role of different genes in tumorigenesis is based on tissue studies and, therefore, cannot be entirely extrapolated to the blood cells.

The main strengths of the study include prospective design, population representativeness of the cohort, complete information on cancer status, emigration and mortality obtained from national registries. The systems approach of testing epidemiologic data in the sub cohort using gene expression profiles reduces the probability of false positive findings.

Relatively small sample size and the lack of a common algorithm for the gene expression analysis are limitations of this work. In addition, FDR levels we accepted were higher than recommended but this can be justified by relatively small sample size and low p-values of genes and gene sets included.

In conclusion, we identified a number of differences in gene set enrichment profiles between cancer-free women and women with EC prior diagnosis in relation to known risk factors for EC. We believe that this integrated analysis may provide a promising background for developing a new multilevel prediction model of EC risk at population level. However, this approach should be further tested in a bigger sample size and in different populations.

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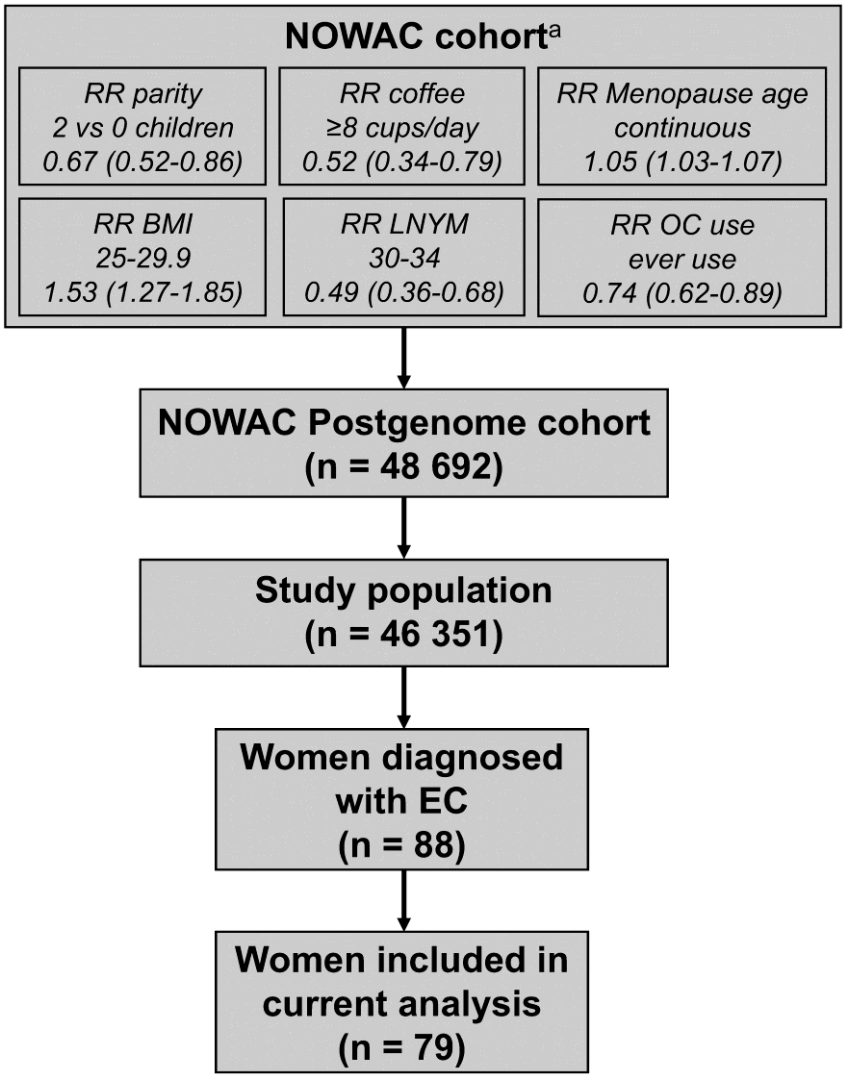
Figure 1. Study population.



Notes: a. Relative risk estimates of EC in the Norwegian Women and Cancer Study calculated using multivariable model adjusted for BMI, use of HRT, use of OC, smoking and alcohol consumtion

Figure 2. Number of significantly enriched gene sets in association with increasing parity.

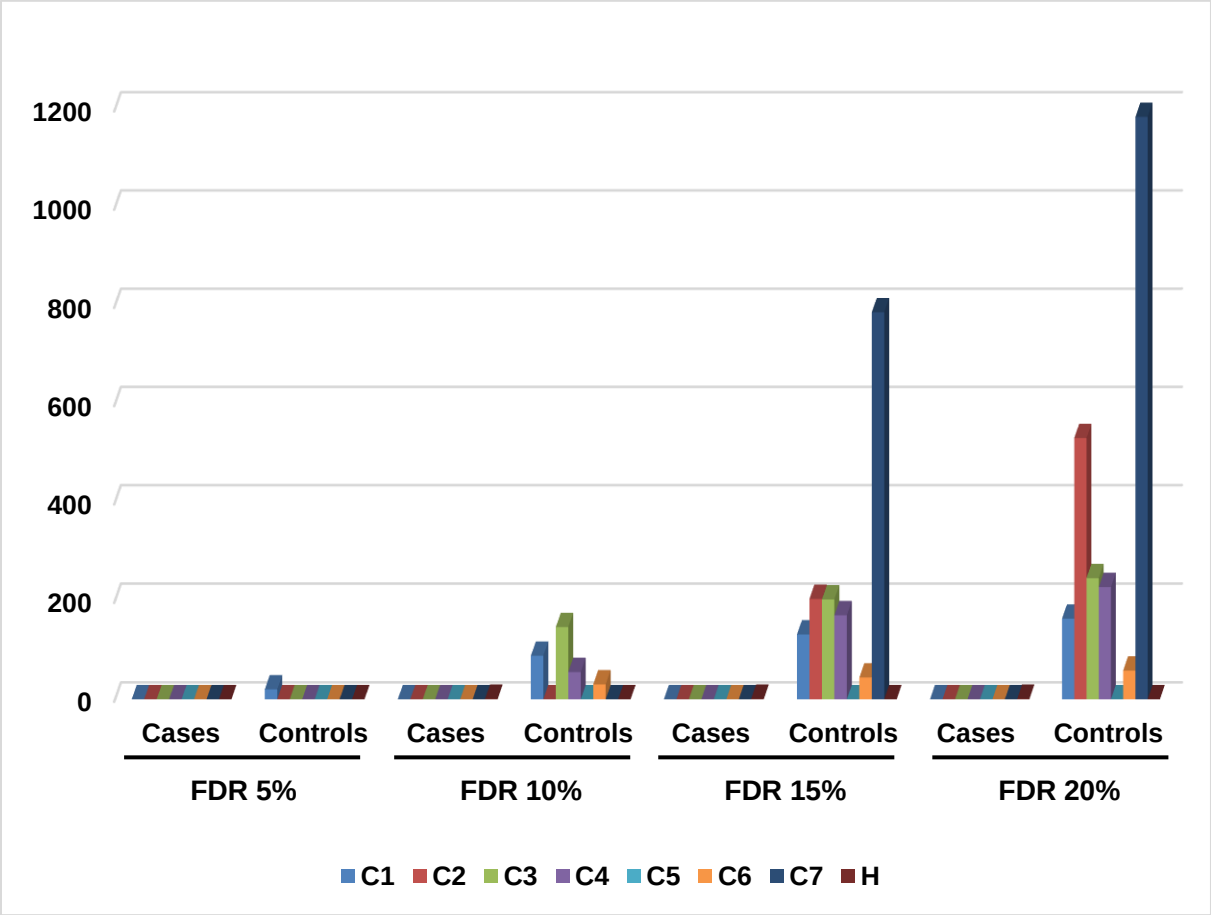


Table 1. Baseline characteristics of the study (N = 79 case/control pairs)

Characteristics	**EC cases**	**Controls**
Age (mean, ±SE)	49.3 (6.3)	49.6 (6.5)
Menopausal status *Premenopausal* *Postmenopausal*	 38 (56 %) 30 (44 %)	 41 (46 %) 49 (54 %)
Age at menopause (mean, ±SE)	49.9 (4.2)	47.5 (5.5)
Age at menarche (mean, ±SE)	12.9 (1.4)	13.2 (1.3)
Parity 0 1 2 3 4 5	 8 11 38 17 5 0	 8 9 35 17 9 1
Cumulative duration of breastfeeding (mean, ±SE)	13.5 (17.0)	10.4 (7.2)
Ever consumption of oral contraceptives (%)	43%	30.7%
LNYM <25 25-29 30-34 35-39 40+	 10.7% 10.7% 36.0% 34.7% 8.0%	 15.4% 14.1% 41.0% 26.9% 2.6%
Body mass index (mean, ±SE) < 25 (%) > 25 (%)	27.5(5.4) 35.4% 64.6%	25.8 (4.9) 58.2% 41.8%
Ever coffee consumption (%)	92.4%	93.7%

Table 2. Top 10 differentially expressed genes associated with parity and BMI.

Parity				
Gene	**logFC[a]**	**p-value**	**q-value[b]**	**Function**
NUDT22	-0.081	8.15E-05	0.163	UDP-glucose and UDP-galactose hydrolase
SH2B2	-0.104	8.34E-05	0.163	Regulator of tyrosine kinase receptor activity
NUP188	-0.056	0.000123829	0.163	Nuclear pore complex involved in the flow of various substances between the cytoplasm and nucleoplasm
TRIP12	0.134	0.000157622	0.163	E3 ubiquitin-protein ligase, involved in regulation of DNA repair
APBA3	-0.094	0.000204851	0.163	Involved in signal transduction and synaptic transmission
CYB5A	0.055	0.000221835	0.163	Electron carrier, regulates hemoglobin metabolism
CEP250	-0.053	0.000252363	0.163	Required for interphase progression of the cell cycle
NRM	-0.073	0.000258652	0.163	Encodes protein residing within the inner nuclear membrane. May be involved in apoptosis
PLRG1	0.097	0.000348695	0.163	Regulator of alternative spicing
TWF2	-0.083	0.000479402	0.163	involved in motile processes and endocytosis regulation
BMI				
Gene	**logFC[a]**	**p-value**	**q-value[b]**	**Function**
ALS2	0.016	2.69E-05	0.099	GTPase regulator. Involved in the development of spinal neurons
TAOK1	0.026	3.11E-05	0.099	Involved in p38 MAPK signaling, apoptosis regulation and cytoskeleton maintenance
ZZEF1	-0.019	7.34E-05	0.099	Involved in calcium ion binding.
DNAJB1	-0.020	7.35E-05	0.099	Stimulates ATP hydrolysis and promotes folding and unfolding of proteins
PROSC	-0.017	7.87E-05	0.099	Involved in homeostasis regulation of pyridoxal 5-phosphate (active form of B6 vitamin)
H2AFY	-0.023	8.35E-05	0.099	Histone-coding gene that represses transcription and inactivates X chromosome

EDEM1	-0.021	0.000109136	0.099	Involved in protein processic in endoplasmic reticulum.
ZBTB44	0.024	0.000111261	0.099	Zinc finger protein realted to nucleic acid binding
SFRS9	-0.019	0.000132804	0.105	Regulates mRNA maturation.
ANKRD11	-0.020	0.000156181	0.110	Inhibits ligand-dependent activation of transcription

Notes: [a]LogFC is the estimated log-fold change in gene expression when the parity increases continuously. [b]q-value is an FDR adjusted p-value.

1

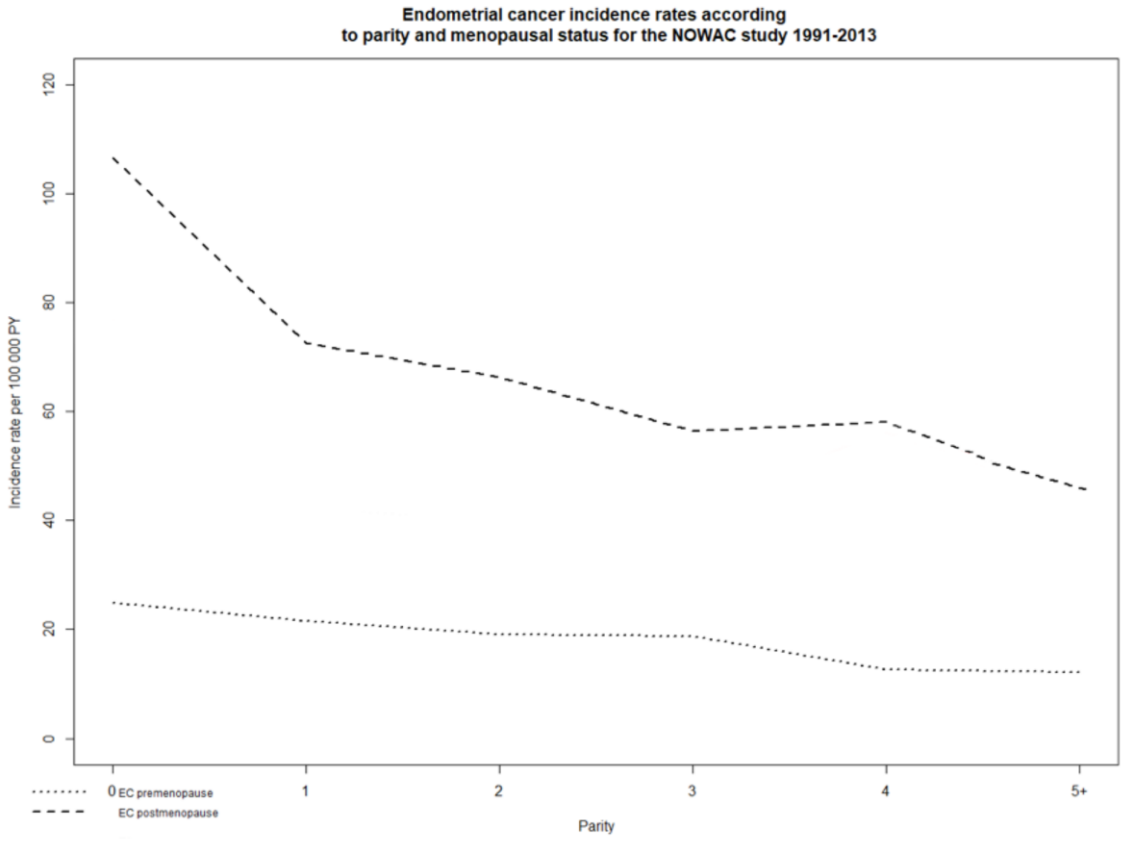
Supplementary Table 1. Gene set enrichment analysis.

	Number of significant gene sets (FDR 5% – 10% – 15% – 20%)	
Parity	**Cases**	**Controls**
C1	0 - 0 - 0 - 0	20 - 88 - 131 - 163
C2	0 - 0 - 0 - 0	0 - 0 - 203 - 530
C3	0 - 0 - 0 - 0	0 - 146 - 202 - 245
C4	0 - 0 - 0 - 0	0 - 55 - 170 - 227
C5	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C6	0 - 0 - 0 - 0	0 - 30 - 44 - 58
C7	0 - 0 - 0 - 0	0 - 0 - 786 - 1184
H	0 - 1 - 1 - 1	0 - 0 - 0 - 0
Coffee		
C1	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C2	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C3	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C4	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C5	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C6	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C7	0 - 0 - 0 - 0	0 - 0 - 0 - 0
H	0 - 0 - 0 - 0	0 - 0 - 0 - 1
BMI		
C1	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C2	1 - 1 - 1 - 1	0 - 0 - 2 - 2
C3	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C4	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C5	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C6	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C7	0 - 0 - 0 - 0	0 - 0 - 0 - 0
H	0 - 0 - 0 - 0	1 - 1 - 1 - 1
LNYM		

C1	0 - 0 - 0 - 0	0 - 1 - 1 - 2
C2	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C3	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C4	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C5	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C6	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C7	0 - 0 - 0 - 0	0 - 0 - 0 - 0
H	0 - 0 - 0 - 0	0 - 0 - 1 - 1
Age of menopause		
C1	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C2	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C3	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C4	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C5	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C6	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C7	0 - 0 - 0 - 0	0 - 0 - 0 - 0
H	0 - 0 - 0 - 0	0 - 0 - 0 - 0
OC use		
C1	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C2	0 - 0 - 0 - 1	0 - 0 - 0 - 0
C3	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C4	0 - 0 - 0 - 0	1 - 1 - 1 - 1
C5	0 - 0 - 0 - 0	0 - 0 - 1 - 1
C6	0 - 0 - 0 - 0	0 - 0 - 0 - 0
C7	0 - 0 - 0 - 0	0 - 0 - 0 - 0
H	0 - 0 - 0 - 0	0 - 0 - 0 - 0

1

Supplementary Figure 1. EC risk according to parity



Appendix I

KVINNER OG KREFT

KONFIDENSIELT

Vi ber deg fylle ut spørreskjemaet så nøye som mulig, se orienteringen på brosjyren for nærmere opplysninger.

Sett kryss for JA i ruten ved siden av hvis du samtykker i å være med. Dersom du ikke ønsker å delta, sett kryss for NEI og returner skjemaet i vedlagte svarkonvolutt, så slipper du å bli purret på.

Med vennlig hilsen

Eiliv Lund
Professor dr. med.

11 KK/1996
1.000 50-69 år
130000 – 130999
Skj-type IV - 2 sider

Jeg samtykker i å delta i undersøkelsen JA ☐ NEI ☐

Hvor mange års skolegang/yrkesutdannelse har du i alt, ta med folkeskole og ungdomsskole? ..... år

Hvor mange personer er det i ditt hushold? Antall: .....

Hvor mange inntekter er det i husholdet? .....

Hvor høy er bruttoinntekten i husholdet pr. år?

☐ under 150 000 kr ☐ 151 000–300 000 kr
☐ 301 000–450 000 kr ☐ 451 000–600 000 kr
☐ over 600 000 kr

Menstruasjonsforhold

Hvor gammel var du da du fikk menstruasjon første gang? ..... år

Har du menstruasjon fremdeles? ☐ Ja ☐ Nei

Hvis nei, alder da menstruasjonen opphørte? .....år

Graviditeter, fødsler og amming

Fyll ut for hvert barn opplysninger om fødselsår og antall måneder du ammet hvert barn (fylles også ut for dødfødte eller for barn som er døde senere i livet). Dersom du ikke har født barn, fortsetter du ved neste spørsmål.

Barn	Fødselsår	Antall måneder med amming
1		
2		
3		
4		
5		
6		

Hormonbruk

HORMONTABLETTER/PLASTER/KREM/STIKKPILLER

Har du noen gang brukt hormontabletter/plaster? ☐ Ja ☐ Nei

Hvis Ja;

Hvor lenge har du brukt hormontabletter/plaster i alt? ..... år

Hvor gammel var du første gang du brukte hormontabletter/plaster? ..... år

HORMONPREPARAT TIL LOKAL BRUK I SKJEDEN

Har du noen gang brukt krem/stikkpille? ☐ Ja ☐ Nei

Hvis Ja; hvor lenge har du brukt krem/stikkpille i alt? ..... år

Hvor gammel var du første gang du brukte hormonkrem/stikkpille? ..... år

Bruker du krem/stikkpille nå? ☐ Ja ☐ Nei

Vi vil be deg om å besvare spørsmålene om bruk av hormontablett/ plaster/krem/stikkpille (hormonpreparater) mer nøye. For hver periode med sammenhengende bruk av samme hormonpreparat håper vi du kan si oss hvor gammel du var da du startet, hvor lenge du brukte det samme hormonpreparatet og navnet på dette.
Dersom du har tatt opphold eller skiftet merke, skal du besvare spørsmålene for en ny periode. Dersom du ikke husker navnet på hormonpreparatet sett usikker. For å hjelpe deg til å huske navnet på hormonpreparatene ber vi deg bruke den vedlagte bjosjyre som viser bilder av hormonpreparater som har vært solgt i Norge. Vennligst oppgi også nummer på hormontabletten/plasteret/kremen/stikkpillen som står i brosjyren.

Periode	Alder ved start	Brukt samme hormon-tablett/plaster/krem/ stikkpille Sammenhengende år	måned	Hormontablett/ plaster/krem stikkpille (se brosjyre) Nr.	Navn
Første					
Andre					
Tredje					

P-Piller

Har du noen gang brukt p-piller, minipiller inkludert? Ja ☐ Nei ☐

Hvis Ja;

Hvor lenge har du brukt p-piller i alt? .....år

Hvor gammel var du første gang du brukte p-piller? .....år

Bruker du p-piller nå? Ja ☐ Nei ☐

Vi vil be deg om å besvare spørsmålene om p-pille bruk mer nøye. For hver periode med sammenhengende bruk av samme p-pille merke håper vi du kan si oss hvor gammel du var da du startet, hvor lenge du brukte det samme p-pille merket og navnet på p-pillene.
Dersom du har tatt opphold eller skiftet merke, skal du besvare spørsmålene for en ny periode. Dersom du ikke husker navnet på p-pille merket, sett usikker. For å hjelpe deg til å huske navnet på p-pille merkene ber vi deg bruke den vedlagte brosjyre som viser bilder av p-pille merker som har vært solgt i Norge. Vennligst oppgi også nummeret på p-pillen som står i brosjyren.

Periode	Alder ved start	Brukt samme p-pille sammenhengende år	måneder	P-pillene (se brosjyren) Nr.	Navn
Første					
Andre					
Tredje					
Fjerde					
Femte					
Sjette					

Brystkreft i nærmeste familie

Har mor hatt brystkreft; Ja ☐ Nei ☐

Undersøkelser for brystkreft

Hvor ofte undersøker du brystene dine selv?
(Sett ett kryss)

Aldri..... ☐
Uregelmessig..... ☐
Regelmessig (omtrent hver måned)..... ☐

Går du til regelmessig undersøkelse av brystene dine med mammografi? Ja ☐ Nei ☐

Høyde og vekt

Hvor høy er du? ..... cm
Hvor mye veier du i dag? ..... kg
Hvor mye veide du da du var 18 år? ..... kg

Røykevaner

Har du noen gang røkt? Ja ☐ Nei ☐

Hvis du røker eller har røkt ber vi deg om å fylle ut for hver alders periode i livet hvor mange sigaretter du i gjennomsnitt røkte pr. dag i den perioden.

	Antall sigaretter hver dag						
Alder	0	1-4	5-9	10-14	15-19	20-24	25+
10-19							
20-29							
30-39							
40-49							
50-59							
60-69							

Røker du nå? Ja ☐ Nei ☐

Fysisk aktivitet

Vi ber deg angi din fysiske aktivitet etter en skala fra svært lite til svært mye ved 14 års alder og i dag. Skalaen nedenfor går fra 1-10. Med fysisk aktivitet mener vi både arbeid i hjemmet og i yrkeslivet, samt trening og annen fysisk aktivitet som turgåing o.l. Sett ring rundt det tallet som best angir ditt nivå av fysisk aktivitet.

Alder	Svært lite									Svært mye
14 år	1	2	3	4	5	6	7	8	9	10
I dag	1	2	3	4	5	6	7	8	9	10

Kosthold

For hver matsort nedenfor ber vi deg krysse av i den ruten som passer hvor ofte du i gjennomsnitt i løpet av siste år har spist slik mat.

	6-10 pr dag	4-5 pr dag	2-3 pr dag	1 pr uke	5-6 pr uke	2-4 pr uke	1 pr uke	1-3 pr måned	Nesten aldri
Helmelk (glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lettmelk (glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Kaffe (kopper)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Brød (skiver)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ost (skiver)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Poteter	☐	☐	☐	☐	☐	☐	☐	☐	☐
Appelsiner o.l.	☐	☐	☐	☐	☐	☐	☐	☐	☐

Middag	5+ pr uke	4 pr uke	3 pr uke	2 pr uke	1 pr uke	2-3 pr mnd	1 pr mnd	Nesten aldri
Rent kjøtt	☐	☐	☐	☐	☐	☐	☐	☐
Oppmalt kjøtt	☐	☐	☐	☐	☐	☐	☐	☐
Fet fisk (makrell, laks o.l.)	☐	☐	☐	☐	☐	☐	☐	☐
Mager fisk (torsk o.l.)	☐	☐	☐	☐	☐	☐	☐	☐
Fiskeboller/pudding/kake	☐	☐	☐	☐	☐	☐	☐	☐
Ris, spaghetti	☐	☐	☐	☐	☐	☐	☐	☐
Pizza	☐	☐	☐	☐	☐	☐	☐	☐
Grøt	☐	☐	☐	☐	☐	☐	☐	☐

Hvorfor spiser du ikke mer fisk	Lite viktig	Viktig	Meget viktig
– for høy pris	☐	☐	☐
– for lite utvalg	☐	☐	☐
– for ujevn tilgang	☐	☐	☐
– kvaliteteten varierer	☐	☐	☐
– uten tilgang på ferdigretter	☐	☐	☐
– lukt ved tilberedning	☐	☐	☐
– vanskelig å tilberede	☐	☐	☐
– smaken	☐	☐	☐
– familien liker ikke fisk	☐	☐	☐

– annet, angi .....

Alkohol

Er du total avholdskvinne? ☐ Ja ☐ Nei

Hvis Nei, hvor ofte og hvor mye drakk du i gjennomsnitt siste året?

	6-10 pr dag	4-5 pr dag	2-3 pr dag	1 pr dag	5-6 pr uke	2-4 pr uke	1 pr uke	1-3 pr mnd	Nesten aldri
Øl (1/2 lite)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vin (glass)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Brennevin (drinker)	☐	☐	☐	☐	☐	☐	☐	☐	☐

Takk for at du ville delta i undersøkelsen!

LUNDBLAD GRAFISK AS, TROMSØ - TLF. 77 67 51 01 - Hormon 2

Appendix II

Høst 1998

KVINNER OG KREFT

KONFIDENSIELT

Hvis du samtykker i å være med, sett kryss for JA i ruten ved siden av. Dersom du ikke ønsker å delta kan du unngå purring ved å sette kryss for NEI og returnere skjemaet i vedlagte svarkonvolutt.

Hvis du vil være med, så ber vi deg fylle ut spørreskjemaet så nøye som mulig, se orienteringen på brosjyren for nærmere opplysninger.

Med vennlig hilsen

Eiliv Lund
Professor dr. med

uts. 28 + 29

Jeg samtykker i å delta i spørreskjema-undersøkelsen JA ☐ NEI ☐

I hvilken kommune har du bodd lengre enn ett år?

Kommune:	Alder			
1. Fødested:	Fra	0 år	til	år
2.	Fra	år	til	år
3.	Fra	år	til	år
4.	Fra	år	til	år
5.	Fra	år	til	år
6.	Fra	år	til	år
7.	Fra	år	til	år

Menstruasjonsforhold

Er menstruasjonen din;

☐ Regelmessig (naturlig)
☐ Uregelmessig
☐ Uteblitt pga. legemiddelbruk, sykdom, trening, annet
☐ Sluttet/stoppet

Hvis du ikke har menstruasjon;

har den stoppet av seg selv? ..... ☐
operert vekk begge eggstokkene? ..... ☐
operert vekk livmoren? ..... ☐
annet, angi ..... ☐

Alder da menstruasjonen opphørte? ..... år

Graviditeter etter 1991

Fyll ut for hvert barn du har født etter 1991 fødselsår og antall måneder du ammet (fylles også ut for dødfødte eller for barn som er døde senere i livet). Dersom du ikke har født barn, fortsetter du ved neste spørsmål.

Barn Nr.:	Fødselsår	Antall måneder med amming

P-Pillebruk etter 1991

Har du noen gang brukt p-piller, minipiller inkludert, etter 1991? ☐ Ja ☐ Nei

Bruker du p-piller nå? ☐ Ja ☐ Nei

Vi vil be deg om å besvare spørsmålene om p-pillebruk etter 1991 mer nøye. For hver periode med sammenhengende bruk av samme p-pille merke håper vi du kan si oss hvor gammel du var da du startet, hvor lenge du brukte det samme p-pillemerket og navnet på p-pillene.
Dersom du har tatt opphold eller skiftet merke, skal du besvare spørsmålene for en ny periode. Dersom du ikke husker navnet på p-pillen, sett usikker. For å hjelpe deg til å huske navnet på p-pille merkene ber vi deg bruke den vedlagte brosjyren som viser bilder av p-pille- merker som har vært solgt i Norge. Vennligst oppgi også nummeret på p-pillen som står i brosjyren.

Årstall	Alder ved start	Brukt samme p-pille sammenhengende år	måneder	Nr.	P-pillene (se brosjyren) Navn

Hormonspiral

Har du noengang brukt hormonspiral (Levonova)? ☐ Ja ☐ Nei

Hvis Ja; hvor lenge har du brukt hormonspiral i alt? ..... år

Hvor gammel var du første gang du du fikk innsatt hormonspiral? ..... år

Bruker du hormonspiral nå? ☐ Ja ☐ Nei

Holdning til bruk av østrogen

Hvilket av følgende alternativer dekker best ditt syn på østrogenbehandling i forbindelse med overgangsalderen (sett ett kryss)

Positivt - en hjelp som bør tilbys alle kvinner ☐
Et nødvendig onde- bør bare brukes av de med store plager ☐
Negativt- bør ikke «klusse med naturen» ☐

Bruk av hormonpreparater med østrogen i overgangsalderen

Har du noen gang brukt østrogentabletter/plaster?

☐ Ja ☐ Nei

Hvis Ja; hvor lenge har du brukt østrogentabletter/plaster i alt? ..... år

Hvis du har brukt østrogenpreparater i *kun 1 år eller mindre;* hvorfor har du brukt midlene så kort tid?

- Har nettopp startet behandlingen ☐
- Er kvitt plagene ☐
- Redd for skadevirkninger ☐
- Fikk plagsomme bivirkninger ☐
- Annet ☐

Hvor gammel var du første gang du brukte østrogentabletter/plaster? ..... år

Hvorfor begynte du å bruke østrogentabletter/plaster?

- Lindre plager i overgangsalderen (hetetokter, uopplagthet, underlivsplager mm) ☐
- Forebygge benskjørhet (osteoporose) ☐
- Forebygge hjerte/kar sykdom ☐
- Annet ☐

Bruker du tabletter/plaster nå? ☐ Ja ☐ Nei

UTFYLLENDE SPØRSMÅL TIL ALLE SOM HAR BRUKT ELLER BRUKER PREPARATER MED ØSTROGEN I FORM AV TABLETTER ELLER PLASTER.

For hver periode med sammenhengende bruk av samme østrogenpreparat håper vi du kan si oss hvor gammel du var da du startet, hvor lenge du brukte det samme østrogenpreparatet, og navnet på dette. Dersom du har tatt opphold eller skiftet merke, skal du besvare spørsmålene for en ny periode. Dersom du ikke husker navnet på østrogenpreparatet sett «usikker». For å hjelpe deg til å huske navnet på østrogenpreparatene ber vi deg bruke den vedlagte brosjyren som viser bilder av østrogenpreparater som har vært solgt i Norge. Vennligst oppgi også nummer på østrogentabletten/plasteret som står i brosjyren.

Periode	Alder ved start	Brukt samme østrogen-tablett/plaster Sammenhengende år	måned	Østrogentablett/ plaster (se brosjyre) Nr.	Navn
Første					
Andre					
Tredje					
Fjerde					
Femte					

Har østrogenpreparatene gitt deg bivirkninger? ☐ Ja ☐ Nei

Hvis Ja; kryss av for hvilke bivirkninger:

- Uregelmessige blødninger ☐
- Brystspenning ☐
- Kvalme/magesmerter ☐
- Hodepine ☐
- Hudreaksjoner ☐
- Vektøkning ☐ ☐ Ant kg
- Annet ..... ☐

Førte de overnevnte bivirkninger til at du forandret østrogenbehandlingen din? ☐ Ja ☐ Nei

Hvis ja;

- Skiftet østrogenpreparat ☐
- Sluttet ☐
- Annet, angi ☐

Østrogenpreparat til lokal bruk i skjeden

Har du noen gang brukt østrogenkrem/stikkpille? ☐ Ja ☐ Nei

Bruker du krem/stikkpille nå? ☐ Ja ☐ Nei

Selvopplevd helse

Oppfatter du din egen helse som; (Sett ett kryss)

☐ meget god ☐ god ☐ dårlig ☐ meget dårlig

Sykdom

Har du eller har du hatt noen av følgende sykdommer?

	Ja	Nei	Hvis Ja: Alder ved start
Høyt blodtrykk	☐	☐	
Hjertesvikt/hjertekrampe	☐	☐	
Årebetennelse	☐	☐	
Blodpropp i legg eller lår	☐	☐	
Hjerteinfarkt	☐	☐	
Slag	☐	☐	
Migrene	☐	☐	
Epilepsi	☐	☐	
Sukkersyke (diabetes)	☐	☐	
Endometriose	☐	☐	
Hypothyreose	☐	☐	
Depresjon (oppsøkt lege)	☐	☐	

For følgende tilstander kryss av for hvilket år tilstanden oppsto eller angi årstall for perioden før 1991.

	før 91	91	92	93	94	95	96	97	98
Muskelsmerter (myalgi)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fibromyalgi/Fibrositt	☐	☐	☐	☐	☐	☐	☐	☐	☐
Kronisk tretthetssyndrom	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ryggsmerter ukjent årsak	☐	☐	☐	☐	☐	☐	☐	☐	☐
Nakkeslengskade	☐	☐	☐	☐	☐	☐	☐	☐	☐
Osteoporose/(b.skjørhet)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Brudd									
Underarmen (håndledd)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ryggvirvel (kompresjon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Andre brudd angi :.....	☐	☐	☐	☐	☐	☐	☐	☐	☐

Sosiale forhold

Er du: (Sett ett kryss) ☐ gift ☐ samboer ☐ annet

Hvor mange personer er det i ditt hushold? .....

Yrke? .....

Hvor høy er bruttoinntekten i husholdet pr. år?

☐ under 150 000 kr ☐ 151 000–300 000 kr
☐ 301 000–450 000 kr ☐ 451 000–600 000 kr
☐ over 600 000 kr

Røykevaner

	Ja	Nei
Har du noen gang røkt?	☐	☐

Hvis Ja, ber vi deg om å fylle ut hvor mange sigaretter du i gjennomsnitt røkte pr. dag i perioden 1991-1998.

	Antall sigaretter hver dag						
Årstall	0	1-4	5-9	10-14	15-19	20-24	25+
1991-94							
1995-98							

	Ja	Nei
Røker du daglig nå?	☐	☐
Bor du sammen med noen som røker?	☐	☐

Hvis Ja, hvor mange sigaretter røker de til sammen pr. dag? .....

Brystkreft i nærmeste familie

Har noen nære slektninger hatt brystkreft;

	Ja	Nei'	Vet ikke	Alder ved start
datter	☐	☐	☐	
mor	☐	☐	☐	
mormor	☐	☐	☐	
farmor	☐	☐	☐	
søster	☐	☐	☐	

Hvor mange helsøsken har du? ☐ **Søstre** ☐ **Brødre**
(oppgi antall)

Hvilket nummer i søskenflokken er du? Nummer ☐

Undersøkelser for kreft

Hvor ofte undersøker du brystene dine selv?
(sett ett kryss)

Aldri ..... ☐
Uregelmessig ..... ☐
Regelmessig (omtrent hver måned) ..... ☐

Går du til regelmessig undersøkelse av brystene dine med mammografi? (sett ett kryss)

Nei ..... ☐
Ja, med to års mellomrom eller mindre ..... ☐
Ja, med to års mellomrom ..... ☐

Fysisk aktivitet

Vi ber deg angi din fysiske aktivitet etter en skala fra svært lite til svært mye. Skalaen nedenfor går fra 1-10. Med fysisk aktivitet mener vi både arbeid i hjemmet og i yrkeslivet, samt trening og annen fysisk aktivitet som turgåing o.l. Sett ring rundt det tallet som best angir ditt nivå av fysisk aktivitet.

Alder	Svært lite									Svært mye
30 år	1	2	3	4	5	6	7	8	9	10
I dag	1	2	3	4	5	6	7	8	9	10

Hvor mange timer pr. dag i gjennomsnitt går eller spaserer du utendørs?

	mindre enn ½ time	½-1 time	1-2 timer	mer enn 2 timer
Vinter				
Vår				
Sommer				
Høst				

	Ja	Nei
Arbeider du utendørs i yrkessammenheng?	☐	☐

Hvis ja:
hvor mange timer pr. uke? .....Sommer .....vinter

Høyde og vekt

Hvor høy er du? ..... cm

Hvor mye veier du i dag? ..... kg

Kosthold

Vi er interessert i å få kjennskap til hvordan kostholdet ditt er **vanligvis.** Kryss av for hvert spørsmål om hvor ofte du **i gjennomsnitt siste året** har brukt den aktuelle matvaren, og hvor mye du pleier å spise/drikke hver gang.

Hvor mange glass melk drikker du vanligvis av hver type? (Sett ett kryss pr. linje)

	aldri/ sjelden	1-4 pr. uke	5-6 pr. uke	1 pr. dag	2-3 pr. dag	4+ pr. dag
Helmelk (søt, sur)	☐	☐	☐	☐	☐	☐
Lettmelk (søt, sur)	☐	☐	☐	☐	☐	☐
Skummet (søt, sur)	☐	☐	☐	☐	☐	☐

Hvor mange kopper kaffe drikker du vanligvis av hver sort? (Sett ett kryss for hver linje)

	aldri/ sjelden	1-6 pr. uke	1 pr. dag	2-3 pr. dag	4-5 pr. dag	6-7 pr. dag	8+ pr. dag
Kokekaffe	☐	☐	☐	☐	☐	☐	☐
Traktekaffe	☐	☐	☐	☐	☐	☐	☐
Pulverkaffe	☐	☐	☐	☐	☐	☐	☐

Hvor mange glass juice, saft og brus drikker du vanligvis? (Sett ett kryss for hver linje)

	aldri/ sjelden	1-3 pr. uke	4-6 pr. uke	1 pr. dag	2-3 pr. dag	4+ pr. dag
Appelsinjuice	☐	☐	☐	☐	☐	☐
Saft/brus med sukker	☐	☐	☐	☐	☐	☐
Saft/brus sukkerfri	☐	☐	☐	☐	☐	☐

Hvor ofte spiser du yoghurt (1 beger)? (Sett ett kryss)

☐ aldri/sjelden ☐ 1 pr. uke ☐ 2-3 pr. uke ☐ 4+ pr. uke

Hvor ofte har du i gjennomsnitt siste året spist kornblanding, havregryn eller müsli? (Sett ett kryss)

☐ aldri/nesten aldri ☐ 1-3 pr. uke ☐ 4-6 pr. uke ☐ 1 pr. dag

Hvor mange skiver brød/rundstykker og knekkebrød/skonrokker spiser du vanligvis?
(1/2 rundstykke = 1 brødskive) (Sett ett kryss for hver linje)

	aldri/ sjelden	1-4 pr. uke	5-7 pr. uke	2-3 pr. dag	4-5 pr. dag	6+ pr. dag
Grovt brød						
Fint brød						
Knekkebrød o.l.						

Nedenfor er det spørsmål om bruk av ulike påleggstyper. Vi spør om hvor mange brødskiver med det aktuelle pålegget du pleier å spise. Dersom du også bruker matvarene i andre sammenhenger enn til brød (f. eks. til vafler, frokostblandinger, grøt), ber vi om at du tar med dette når du besvarer spørsmålene.

På hvor mange brødskiver bruker du? (Sett ett kryss pr. linje)

	0 pr. uke	1-3 pr. uke	4-6 pr. uke	1 pr. dag	2-3 pr. dag	4+ pr. dag
Syltetøy og annet søtt pålegg						
Brun ost, helfet						
Brun ost, halvfet/mager						
Hvit ost, helfet						
Hvit ost, halvfet/mager						
Kjøttpålegg, leverpostei						

Videre kommer spørsmål om fiskepålegg.
På hvor mange brødskiver pr. uke har du i gjennomsnitt siste året spist? (Sett ett kryss pr. linje)

	0 pr. uke	1 pr. uke	2-3 pr. uke	4-6 pr. uke	7-9 pr. uke	10+ pr. uke
Makrell i tomat, røkt makrell						
Kaviar						
Annet fiskepålegg						

Hva slags fett bruker du vanligvis på brødet?
(Sett gjerne flere kryss)

☐ bruker ikke fett på brødet
☐ smør
☐ hard margarin (f. eks. Per, Melange)
☐ myk margarin (f. eks. Soft)
☐ smørblandet margarin (f. eks. Bremykt)
☐ Brelett
☐ lettmargarin (f. eks. Soft light, Letta)

Dersom du bruker fett på brødet, hvor tykt lag pleier du smøre på? (En kuvertpakke med margarin veier 12 gram).
(Sett ett kryss)

☐ skrapet (3 g) ☐ tynt lag (5 g) ☐ godt dekket (8 g)
☐ tykt lag (12 g)

Hvor ofte spiser du frukt? (Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-4 pr. uke	5-6 pr. uke	1 pr. dag	2+ pr. dag
Epler/pærer							
Appelsiner o.l.							
Bananer							
Annen frukt (f.eks. druer, fersken)							

Hvor ofte spiser du ulike typer grønnsaker?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2 pr. uke	3 pr. uke	4-5 pr. uke	6-7 pr. uke
Gulrøtter							
Kål							
Kålrot							
Broccoli/blomkål							
Blandet salat							
Grønnsakblanding (frossen)							
Andre grønnsaker							

For de grønnsakene du spiser, kryss av for hvor mye du spiser hver gang. (Sett ett kryss for hver sort)

- gulrøtter ☐ 1/2 stk. ☐ 1 stk. ☐ 1 1/2 stk. ☐ 2+ stk.
- kål ☐ 1/2 dl ☐ 1 dl ☐ 1 1/2 dl ☐ 2+ dl
- kålrot ☐ 1/2 dl ☐ 1 dl ☐ 1 1/2 dl ☐ 2+ dl
- broccoli/blomkål ☐ 1-2 buketter ☐ 3-4 buketter ☐ 5+ buketter
- blandet salat ☐ 1 dl ☐ 2 dl ☐ 3 dl ☐ 4+ dl
- grønnsakblanding ☐ 1/2 dl ☐ 1 dl ☐ 2 dl ☐ 3+ dl

Hvor mange poteter spiser du vanligvis (kokte, stekte, mos)? (Sett ett kryss)

☐ spiser ikke/spiser sjelden poteter

☐ 1-4 pr. uke ☐ 5-6 pr. uke

☐ 1 pr. dag ☐ 2 pr. dag

☐ 3 pr. dag ☐ 4+ pr dag

Hvor ofte bruker du ris og spaghetti/makaroni ?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2 pr. uke	3+ pr. uke
Ris					
Spaghetti, makaroni					

Hvor ofte spiser du risengrynsgrøt? (Sett ett kryss)

☐ aldri/sjelden ☐ 1 pr. mnd ☐ 2-3 pr. mnd ☐ 1+ pr. uke

Hva slags fett blir vanligvis brukt til matlaging i din husholdning? (Sett gjerne flere kryss)

☐ smør

☐ hard margarin (f. eks. Per, Melange)

☐ myk margarin (f. eks. Soft)

☐ smørblandet margarin (f. eks. Bremykt)

☐ soyaolje ☐ olivenolje ☐ maisolje

Fisk

Vi vil gjerne vite hvor ofte du pleier å spise fisk, og ber deg fylle ut spørsmålene om fiskeforbruk så godt du kan. Tilgangen på fisk kan variere gjennom året. Vær vennlig å markere i hvilke årstider du spiser de ulike fiskeslagene.

	aldri/ sjelden	like mye hele året	vinter	vår	sommer	høst
Torsk, sei, hyse, lyr						
Steinbit, flyndre, uer						
Laks, ørret						
Makrell						
Sild						

Med tanke på de periodene av året der du spiser fisk, hvor ofte pleier du å spise følgende? (Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd	2-3 pr. mnd	1 pr. uke	2 pr. uke	3+ pr. uke
Kokt torsk, sei, hyse, lyr						
Stekt torsk, sei, hyse, lyr						
Steinbit, flyndre, uer						
Laks, ørret						
Makrell						
Sild						

Dersom du spiser fisk, hvor mye spiser du vanligvis pr. gang? (1 skive/stykke = 150 gram)
(Sett ett kryss for hver linje)

- kokt fisk (skive) ☐ 1 ☐ 1,5 ☐ 2 ☐ 3+
- stekt fisk (stykke) ☐ 1 ☐ 1,5 ☐ 2 ☐ 3+

Hvor mange ganger pr. år spiser du fiskeinnmat?
(Sett ett kryss pr. linje)

	0	1-3	4-6	7-9	10+
Rogn	☐	☐	☐	☐	☐
Fiskelever	☐	☐	☐	☐	☐

Dersom du spiser fiskelever, hvor mange spiseskjeer pleier du å spise hver gang? (Sett ett kryss)

☐ 1 ☐ 2 ☐ 3-4 ☐ 5-6 ☐ 7+

Hvor ofte bruker du følgende typer fiskemat?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd	2-3 pr. mnd	1 pr. uke	2+ pr. uke
Fiskekaker/pudding/ boller					
Plukkfisk, fiskegrateng					
Frityrfisk, fiskepinner					
Andre fiskeretter					

Hvor stor mengde pleier du vanligvis å spise av de ulike rettene? (Sett ett kryss for hver linje)

- fiskekaker/pudding/boller (stk.) (2 fiskeboller=1 fiskekake) ☐ 1 ☐ 2 ☐ 3 ☐ 4+
- plukkfisk, fiskegrateng (dl) ☐ 1-2 ☐ 3-4 ☐ 5+
- frityrfisk, fiskepinner (stk.) ☐ 1-2 ☐ 3-4 ☐ 5-6 ☐ 7+

Hvor ofte spiser du skalldyr (f. eks. reker, krabbe)**?**
(Sett ett kryss)

aldri/ sjelden	1 pr. mnd	2-3 pr mnd	1+ pr. uke
☐	☐	☐	☐

I tillegg til informasjon om fiskeforbruk er det viktig å få kartlagt hvilket tilbehør som blir servert til fisk. Hvor ofte bruker du følgende til fisk? (Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd	2-3 pr. mnd	1 pr. uke	2+ pr. uke
Smeltet eller fast margarin/fett					
Seterrømme (35%)					
Lettrømme (20%)					
Saus med fett (hvit/brun)					
Saus uten fett (hvit/brun)					

For de ulike typene tilbehør du bruker til fisk, vær vennlig å kryss av for hvor mye du vanligvis pleier spise.

- smeltet/fast fett (ss) ☐ 1/2 ☐ 1 ☐ 2 ☐ 3 ☐ 4+
- seterrømme (ss) ☐ 1/2 ☐ 1 ☐ 2 ☐ 3 ☐ 4+
- lettrømme (ss) ☐ 1/2 ☐ 1 ☐ 2 ☐ 3 ☐ 4+
- saus med fett (dl) ☐ 1/4 ☐ 1/2 ☐ 3/4 ☐ 1 ☐ 2+
- saus uten fett (dl) ☐ 1/4 ☐ 1/2 ☐ 3/4 ☐ 1 ☐ 2+

Andre matvarer

Hvor ofte spiser du følgende kjøtt- og fjærkreretter?
(Sett ett kryss for hver rett)

	aldri/ sjelden	1 pr. mnd	2-3 pr. mnd	1 pr. uke	2+ pr. uke
Steik (okse, svin, får)					
Koteletter					
Biff					
Kjøttkaker, karbonader					
Pølser					
Gryterett, lapskaus					
Pizza m/kjøtt					
Kylling					
Andre kjøttretter					

Dersom du spiser følgende retter, oppgi mengden du vanligvis spiser: (Sett ett kryss for hver linje)

- steik (skiver) ☐ 1 ☐ 2 ☐ 3 ☐ 4+
- koteletter (stk.) ☐ 1/2 ☐ 1 ☐ 1,5 ☐ 2+
- kjøttkaker, karbonader (stk.) ☐ 1 ☐ 2 ☐ 3 ☐ 4+
- pølser (stk. à 150g) ☐ 1/2 ☐ 1 ☐ 1,5 ☐ 2+
- gryterett, lapskaus (dl) ☐ 1-2 ☐ 3 ☐ 4 ☐ 5+
- pizza m/kjøtt (stykke à 100 g) ☐ 1 ☐ 2 ☐ 3 ☐ 4+

Hvor mange egg spiser du vanligvis i løpet av en uke (stekte, kokte, eggerøre, omelett)? (Sett ett kryss)

☐ 0 ☐ 1 ☐ 2 ☐ 3-4 ☐ 5-6 ☐ 7+

Vi ber deg fylle ut hovedrettene til middag en gang til som en oppsummering. Kryss av i den ruten som passer hvor ofte du i gjennomsnitt i løpet av siste år har spist slik mat til middag

	5+ pr. uke	4 pr. uke	3 pr. uke	2 pr. uke	1 pr. uke	2-3 pr. mnd	1 pr. mnd	nesten aldri
Rent kjøtt	☐	☐	☐	☐	☐	☐	☐	☐
Oppmalt kjøtt	☐	☐	☐	☐	☐	☐	☐	☐
Fet fisk (makrell, laks o.l.)	☐	☐	☐	☐	☐	☐	☐	☐
Mager fisk (torsk o.l.)	☐	☐	☐	☐	☐	☐	☐	☐
Fiskemat	☐	☐	☐	☐	☐	☐	☐	☐

Hvor ofte spiser du iskrem (til dessert, krone-is osv.)?
(Sett ett kryss for hvor ofte du spiser iskrem om sommeren, og ett kryss for resten av året)

	aldri/ sjelden	1-3 pr mnd	1 pr. uke	2-3 pr. uke	4+ pr. uke
– om sommeren	☐	☐	☐	☐	☐
– resten av året	☐	☐	☐	☐	☐

Hvor mye is spiser du vanligvis pr. gang? (Sett ett kryss)

☐ 1 dl ☐ 2 dl ☐ 3 dl ☐ 4+ dl

Hvor ofte spiser du bakervarer som boller, kaker, wienerbrød, vafler, småkaker? (Sett ett kryss)

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-3 pr. uke	4-6 pr. uke	7+ pr. uke
Gjærbakst(boller)						
Kaker						
Pannekaker						
Vafler						
Småkaker						

Hvor ofte spiser du dessert? (Sett ett kryss)

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-3 pr. uke	4-6 pr. uke	7+ pr. uke
Pudding Sjokolade/karamell						
Riskrem, fromasj						
Kompott, fruktgrøt hermetisk frukt						

Hvor ofte spiser du sjokolade? (Sett ett kryss)

☐ aldri/sjelden ☐ 1-3 pr. mnd ☐ 1 pr. uke
☐ 2-3 pr. uke ☐ 4-6 pr. uke ☐ 1+ pr. dag

Dersom du spiser sjokolade, hvor mye pleier du vanligvis å spise hver gang? Tenk deg størrelsen på en Kvikk-Lunsj sjokolade, og oppgi hvor mye du spiser i forhold til den.

☐ 1/4 ☐ 1/2 ☐ 3/4 ☐ 1 ☐ 1,5 ☐ 2+

Hvor ofte spiser du salt snacks? (Sett ett kryss)

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-3 pr. uke	4-6 pr. uke	7+ pr. uke
Potetchips						
Peanøtter						

Tilberedningsmåte

Har du mikrobølgeovn? ☐ Ja ☐ Nei

Hvis Ja; hvor mange ganger pr. uke bruker du mikrobølgeovnen til ganger pr. uke

middagslaging? .....
annet? .....

Hvilken farve foretrekker du på stekeskorpen?

☐ Lys brun ☐ Middels ☐ Mørk brun

Hvor ofte spiser du stekt eller grillet mat?

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-3 pr. uke	4-6 pr. uke	7+ pr. uke
Mørkt kjøtt (biff ol.)						
Lyst kjøtt (kylling ol.)						
Oppmalt kjøtt (kjøttkaker ol.)						
Bacon						
Fisk						

Bruker du stekefettet eller sjyen etter steking?

☐ nei, aldri ☐ av og til
☐ som oftest ☐ ja, alltid

Tran og fiskeoljekapsler

Bruker du tran (flytende)? ☐ Ja ☐ Nei

Hvis ja; hvor ofte tar du tran?
Sett ett kryss for hver linje.

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-6 pr. uke	daglig
- om vinteren	☐	☐	☐	☐	☐
- resten av året	☐	☐	☐	☐	☐

Hvor mye tran pleier du å ta hver gang?

☐ 1 ts ☐ 1/2ss ☐ 1+ss

Bruker du tranpiller/kapsler? ☐ Ja ☐ Nei

Hvis ja; hvor ofte tar du tranpiller/kapsler?
Sett ett kryss for hver linje.

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-6 pr. uke	daglig
- om vinteren	☐	☐	☐	☐	☐
- resten av året	☐	☐	☐	☐	☐

Hvilken type tranpiller/kapsler bruker du vanligvis, og hvor mange pleier du å ta hver gang?

	ja	antall pr. gang
Møllers trankapsler	☐	.....
Møllers omega-3 kapsler	☐	.....
Møllers dobbel	☐	.....
annet, navn	☐	.....

Bruker du fiskeoljekapsler? ☐ Ja ☐ Nei

Hvis ja; hvor ofte tar du fiskeoljekapsler?

aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-6 pr. uke	daglig
☐	☐	☐	☐	☐

Hvilken type fiskeoljekapsler bruker du vanligvis, og hvor mange pleier du å ta hver gang?

	ja	antall pr. gang
Triomar	☐	.....
Almarin	☐	.....
Nycomed Omega-3	☐	.....
annet, navn	☐	.....

Kosttilskudd

Bruker du annet kosttilskudd (eks. vitaminer, mineraler)? ☐ Ja ☐ Nei

Hvis ja; hvor ofte tar du slike kosttilskudd?

aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-6 pr. uke	daglig
☐	☐	☐	☐	☐

Navn .....

Alkohol

Er du total avholdskvinne? ☐ Ja ☐ Nei

Hvis Nei, hvor ofte og hvor mye drakk du i gjennomsnitt siste året? (Sett ett kryss for hver linje)

	aldri/ sjelden	1 pr. mnd	2-3 pr. mnd	1 pr. uke	2-4 pr. uke	5-6 pr. uke	1+ pr. dag
Øl (½ L)	☐	☐	☐	☐	☐	☐	☐
Vin (glass)	☐	☐	☐	☐	☐	☐	☐
Brennevin (drinker)	☐	☐	☐	☐	☐	☐	☐

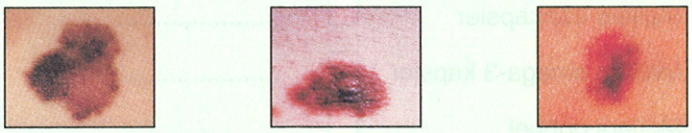
Solvaner

Får du fregner når du soler deg? ☐ Ja ☐ Nei

Hvor mange føflekker har du sammenlagt på begge armer (fra fingertuppene til skuldrene)?

☐ 0 ☐ 1-10 ☐ 11-50 ☐ 51+

Hvor mange uregelmessige føflekker større enn 5 mm har du sammenlagt på begge armene (fra fingrene til armhulene)? Tre eksempler på føflekker større enn 5 mm med uregelmessig form er vist i nedenfor.



5 mm

☐ 0 ☐ 1 ☐ 2-3 ☐ 4-6 ☐ 7-12 ☐ 13-24 ☐ 25+

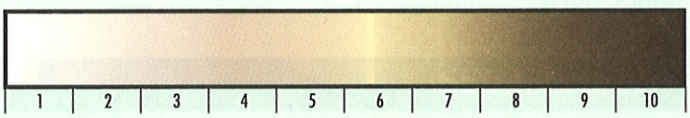
Hvor mange små, regelmessige føflekker har du sammenlagt på begge armene (fra fingrene til armhulene)?

☐ 0 ☐ 1-10 ☐ 11-50 ☐ 51+

Hva er din opprinnelige hårfarge? (sett ett kryss)

☐ mørkbrunt, svart ☐ brun ☐ blond, gul ☐ rød

For å kunne studere effekten av soling på risiko for hudkreft ber vi deg gi opplysninger om hudfarge
Sett ett kryss på den fargen som best passer din hudfarge (uten soling)



Hvor ofte dusjer eller bader du?

	Mer enn 1 g dagl	1 g dagl	4-6 g pr. uke	2-3 g pr. uke	1 g pr. uke	2-3 g pr. mnd.	Sjelden aldri
Med såpe/shampo							
Uten såpe/shampo							

Hvor mange ganger pr. år er du blitt forbrent av solen slik at du har fått svie og blemmer med avflassing etterpå? (ett kryss for hver aldersgruppe)

Årstall	Aldri	Høyst 1 gang pr. år	2-3 g. pr. år	4-5 g. pr. år	6 eller flere ganger
1991-94					
1995-98					

Hvor mange uker soler du deg pr. år i syden?

Årstall	Aldri	1 uke	2-3 uker	4-5 uker	7 uker eller mer
1991-94					
1995-98					

Hvor mange uker pr. år soler du deg i Norge eller utenfor syden?

Årstall	Aldri	1 uke	2-3 uker	4-5 uker	7 uker eller mer
1991-94					
1995-98					

Når bruker du krem med solfaktor (sett evt. flere kryss):

☐ påsken ☐ i Norge eller utenfor syden ☐ solferie i syden

Hvilke solfaktorer bruker du i disse periodene?

	påsken	i Norge eller utenfor syden	solferie i syden
- I dag	.....	.....	.....
- For 10 år siden	.....	.....	.....

Hvilke solkremmerker bruker du? Angi faktor hvis du husker.

	Ja	faktor		Ja	faktor
Piz Buin	☐	....	Cosmica	☐	....
Ambre Solairé	☐	....	Natusan	☐	....
HTH	☐	....	Delial	☐	....
Andre, angi navn.....		....			

Hvor ofte har du solt deg i solarium?

Alder	Aldri	Sjelden	1 gang pr. mnd.	2 ganger pr. mnd.	3-4 ganger pr. mnd	oftere enn1 gang pr. uke
1991-94						
1995-98						

Til slutt vil vi spørre deg om ditt samtykke til å kontakte deg på nytt pr. post.
Vi vil hente adressen fra det sentrale personregister.

☐ Ja ☐ Nei

Takk for at du ville delta i undersøkelsen

LUNDBLAD GRAFISK AS, TROMSØ - TLF. 77 67 51 01

Appendix III

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

Bilder av hormoner til bruk i og etter overgangsalderen (østrogen)

Denne brosjyren er et hjelpemiddel for å huske riktig navn på de hormontabletter/plaster 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 du har brukt. Eldre avregistrerte preparater er ikke gjengitt med bilder, det gjelder:

Nr. 104 **Etifollin** 50 mcg tabletter, solgt fra 1953-2000
Nr. 121 **Menorest** 37,5 mcg/24t plaster, solgt fra 1996-2002
Nr. 122 **Menorest** 50 mcg/24t plaster, solgt fra 1996-2002
Nr. 123 **Menorest** 75 mcg/24t plaster, solgt fra 1996-2002
Nr. 124 **Menorest** 100 mcg/24t plaster, solgt fra 1996-2002
Nr. 196 **Primolut** tabletter, solgt fra 1958-
Nr. 197 **Perlutex** tabletter, solgt fra 1960-
Nr. 199 **Provera** 5 og 10 mg tabletter, solgt fra 1964-
Nr. 202 **Diethylstilbøstrol** 0,1 mg tabletter solgt fra1980-85
Nr. 204 **Primodos** tabletter solgt fra 1961-74
Nr. 205 **Østriol** 1 mg tabletter solgt fra 1975-95
Nr. 206 **Østriol** 0,25 mg tabletter solgt fra 1961-83

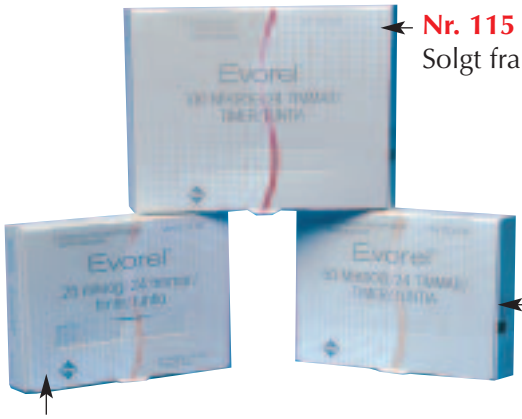
Nr. 110
Solgt fra 1994-2002

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. 116
Solgt fra
1995

Nr. 117
Solgt fra
1994

Nr. 101 Solgt fra 1978

Nr. 102
Solgt fra 1978

Nr. 103
Solgt fra
1978

Nr. 105 Solgt fra 1988

Nr. 118
Solgt fra
1989

Nr. 119 Solgt fra 1989

Nr. 120
Solgt fra
1989

Nr. 107 (2mg)
Solgt fra 1967

Nr. 106 (1mg) Solgt fra 1970

Nr. 125
Solgt fra 1996.

Nr. 136 Vagifem
Solgt fra 2000

Nr. 126
Solgt fra 1997.

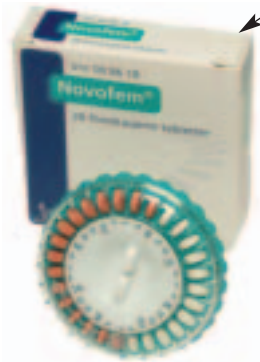
Nr. 127
Solgt fra 1997.

Nr. 138
Climodien
Solgt fra 2001

Nr. 140 Oestriol
Solgt fra 1999

Nr. 128
Livial
Solgt fra 1999

Nr. 141
Novofem
Solgt fra 2002



Nr. 143
Estradot 50 mg

Nr. 142
Estradot
37,5 mg

Nr. 144
Estradot 75 mg

Solgt fra 2002

Nr. 145
Estradot 100 mg

Nr. 130
Indivina 1mg/2,5 mg Solgt fra 2001

Nr. 132
Indivina 2mg/5 mg
Solgt fra 2001

Nr. 131
Indivina 1mg/5 mg
Solgt fra 2001

Nr. 146
Estalis
Solgt fra 2002

Nr. 147
Estalis Sekvens
Solgt fra 2003

Nr. 133
Diviseq
Solgt fra 2001

Nr. 134
Climen
Solgt fra 1999

Nr. 135 Activelle
Solgt fra 1999

Nr. 148
Totelle Sekvens
Solgt fra 2003

Bilder av P-pille merker i salg 1965-2003

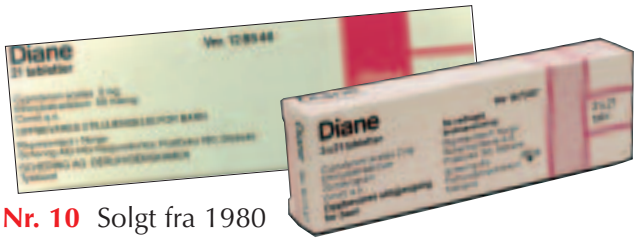
Denne brosjyren er et hjelpemiddel for å huske riktig navn på de p-piller du har brukt. Under bildene er det oppgitt hvilke år p-pillene var i salg. For noen p-piller finnes det esker med samme utseende, men med ulik størrelse, anhengig av om de inneholder p-piller for en eller flere måneder. Vi ber deg tenke nøye gjennom navnet på de p-pillene du har brukt. Av noen p-piller/ merker 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. 5. **Anovlar Mite,** solgt fra 1967–69
Nr. 8. **Consan,** solgt fra 1968–70
Nr. 9. **Delpregnin,** solgt fra 1968–71
Nr. 14. **Kombikvens,** solgt fra 1971–75
Nr. 20. **Micronor,** solgt fra 1971–79
Nr. 22. **Norlestrin,** solgt fra 1965–80
Nr. 23. **Nyo-Kon,** solgt fra 1968–70
Nr. 26. **Ortho-Novin Mite,** solgt fra 1968–72
Nr. 39. **Implanon,** solgt fra 2002-

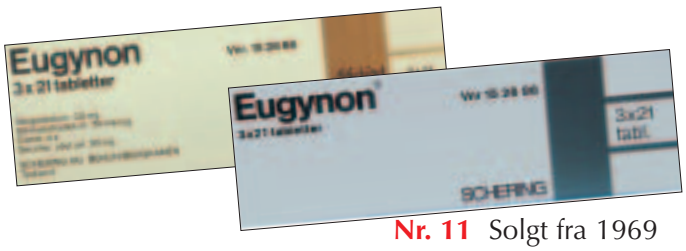
Nr. 4 Solgt fra 1965-68

Nr. 6. Solgt fra 1980

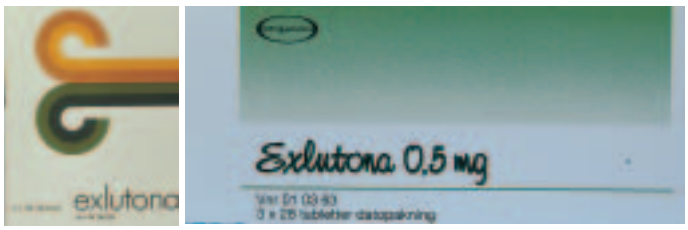
Nr. 7 Solgt fra 1971



Nr. 10 Solgt fra 1980



Nr. 11 Solgt fra 1969



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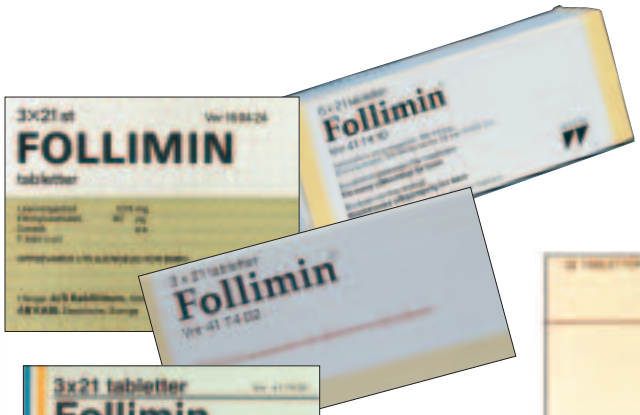


Nr. 13 Solgt fra 1978

Nr. 15 Solgt fra 1966-72

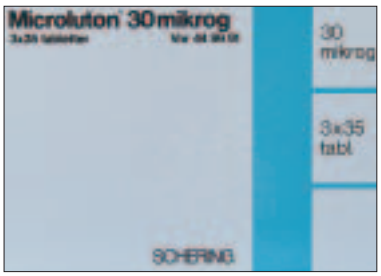
Nr. 16 Solgt fra 1965

Nr. 17 Solgt fra 1985

Nr. 18 Solgt fra 1975

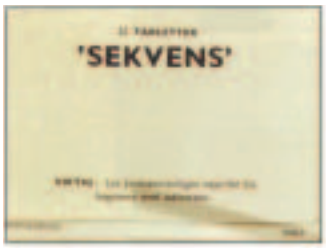
Nr. 29 Solgt fra 1973-82

Nr. 30 Solgt fra 1968-84

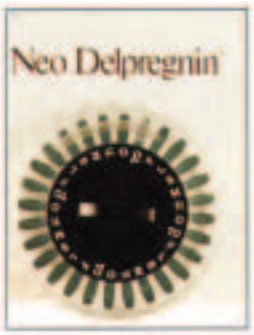


Nr. 19 Solgt fra 1973

Nr. 31 Solgt fra 1977

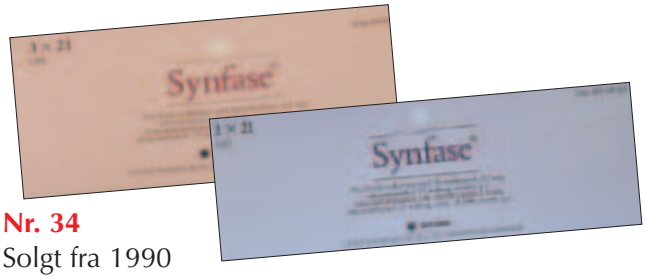


Nr. 32 Solgt fra 1969-70



Nr. 21 Solgt fra 1971-79

Nr. 33 Solgt fra 1967-69



Nr. 34
Solgt fra 1990

Nr. 24 Solgt fra 1971-81

Nr. 35
Solgt fra 1981

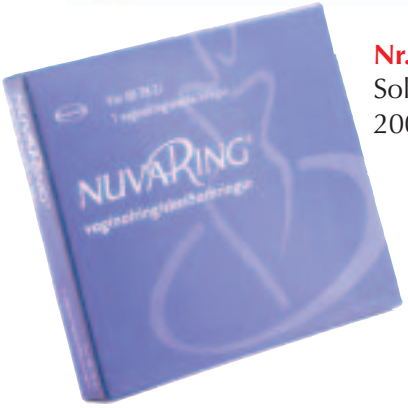
Nr. 36 Solgt fra 1981

Nr. 25 Solgt fra 1966-69

Nr. 37
Solgt fra 2001

Nr. 38 Solgt fra 2002

Nr. 27 Solgt fra 1965-71



Nr. 40
Solgt fra 2003

Nr. 28 Solgt fra 1970

TAKK FOR INNSATSEN!

Appendix IV

Høst 2004

KVINNER OG KREFT

KONFIDENSIELT

Hvis du samtykker i å være med, sett kryss for JA i ruten ved siden av.
Dersom du ikke ønsker å delta kan du unngå purring ved å sette kryss for NEI og returnere skjemaet i vedlagte svarkonvolutt.
Vi ber deg fylle ut spørreskjemaet så nøye som mulig.

Skjemaet skal leses optisk. Vennligst bruk blå eller sort penn.
Du kan ikke bruke komma, forhøy 0,5 til 1. Bruk blokkbokstaver.

Med vennlig hilsen
Eiliv Lund

Jeg samtykker i å delta i spørreskjemaundersøkelsen JA ☐ NEI ☐

Overgangsalder

Har du regelmessig menstruasjon fremdeles?

- ☐ Ja
- ☐ Har uregelmessig menstruasjon
- ☐ Vet ikke (menstruasjon uteblitt pga. sykdom o.l.)
- ☐ Vet ikke (bruker hormonpreparat med østrogen)
- ☐ Nei

Hvis Nei;

har den stoppet av seg selv? ☐
har du operert vekk eggstokkene? ☐
har du operert vekk livmoren? ☐
annet? ☐

Alder da menstruasjonen opphørte ☐

Graviditeter, fødsler og amming

Har du noen gang vært gravid? Ja ☐ Nei ☐

Hvis Ja; hvor mange barn har du født i alt ☐

Hvor gammel var du ved siste fødsel? ☐

P-pillebruk

Har du brukt p-piller eller minipiller? Ja ☐ Nei ☐

Hvis ja, hvor mange år har du brukt p-piller i alt? ☐

Bruker du p-piller nå? Ja ☐ Nei ☐

Bruk av hormonpreparater med østrogen i overgangsalderen

Har du noen gang brukt østrogentabletter/plaster? Ja ☐ Nei ☐

Hvis Ja; hvor mange år har du brukt østrogentabletter/plaster i alt? ☐

Hvor gammel var du første gang du brukte østrogentabletter/plaster? ☐

Bruker du tabletter/plaster nå? Ja ☐ Nei ☐

UTFYLLENDE SPØRSMÅL TIL ALLE SOM HAR BRUKT PREPARATER MED ØSTROGEN I FORM AV TABLETTER ELLER PLASTER FRA 1998 OG FREM TIL I DAG.

Har du svart «ja», ber vi deg utdype dette nærmere ved å svare på spørsmålene nedenfor. For hver periode med sammenhengende bruk av samme hormonpreparat håper vi du kan si oss hvor gammel du var da du startet, hvor lenge du brukte det samme hormonpreparatet og navnet på dette. Dersom du har hatt opphold eller skiftet merke skal du besvare spørsmålene for en ny periode. Dersom du ikke husker navnet på hormonpreparatet, sett «usikker». For å hjelpe deg til å huske navnet på hormonpreparatene ber vi deg bruke den vedlagte brosjyre som viser bilder av hormonpreparater som har vært solgt i Norge. Vennligst oppgi også nummer på hormontabletten/plasteret som står i brosjyren.

Periode	Alder ved start	Brukt samme hormontablett/ plaster/ sammenhengende fra 1998 år	måned	Nr.	Navn på hormontablett/plaster/ (se brosjyre)
1.					
2.					
3.					
4.					
5.					

Østrogenpreparat til lokal bruk i skjeden

Har du noen gang brukt østrogen-krem/stikkpille? ..... Ja ☐ Nei ☐

Hvis Ja;
bruker du krem/stikkpille nå? ..... Ja ☐ Nei ☐

Hormonspiral

Har du noen gang brukt hormonspiral (Levonova)? Ja ☐ Nei ☐

Hvis Ja; hvor mange hele år har du brukt hormonspiral i alt? ☐

Hvor gammel var du første gang du fikk innsatt hormonspiral? ☐

Bruker du hormonspiral nå? Ja ☐ Nei ☐

Selvopplevd helse

Oppfatter du din egen helse som; (Sett ett kryss)

Meget god ☐ God ☐ Dårlig ☐ Meget dårlig ☐

Sykdom

Har du eller har du hatt noen av følgende sykdommer?
(sett ett eller flere kryss)

	Ja	Nei	Hvis ja: Alder ved start
Kreft	☐	☐	☐
Høyt blodtrykk	☐	☐	☐
Hjertesvikt/hjertekrampe	☐	☐	☐
Hjerteinfarkt	☐	☐	☐
Slag	☐	☐	☐
Sukkersyke (diabetes)	☐	☐	☐
Depresjon (oppsøkt lege)	☐	☐	☐
Hypothyreose/lavt stoffskifte	☐	☐	☐

For følgende tilstander ber vi deg krysse av for hvilket år tilstanden oppsto første gang.

	før 98	98	99	00	01	02	03
Muskelsmerter (myalgi)	☐	☐	☐	☐	☐	☐	☐
Fibromyalgi/Fibrositt	☐	☐	☐	☐	☐	☐	☐
Kronisk tretthetssyndrom	☐	☐	☐	☐	☐	☐	☐
Ryggsmerter ukjent årsak	☐	☐	☐	☐	☐	☐	☐
Nakkeslengskade	☐	☐	☐	☐	☐	☐	☐
Osteoporose (b.skjørhet)	☐	☐	☐	☐	☐	☐	☐
Brudd							
Underarmen (håndledd)	☐	☐	☐	☐	☐	☐	☐
Lårhalsen	☐	☐	☐	☐	☐	☐	☐
Ryggvirvel (kompresjon)	☐	☐	☐	☐	☐	☐	☐

Andre legemidler

Bruker du noen av disse legemidlene daglig nå?

Fontex, Fluoxetin	Ja ☐	Nei ☐
Cipramil, Citalopram, Desital	Ja ☐	Nei ☐
Seroxat, Paroxetin	Ja ☐	Nei ☐
Zoloft	Ja ☐	Nei ☐
Fevarin	Ja ☐	Nei ☐
Cipralex	Ja ☐	Nei ☐

Hvis Ja; hvor lenge har du brukt dette legemidlet sammenhengede? Måneder ☐ År ☐

Har du benyttet noen av disse legemidlene tidligere? Ja ☐ Nei ☐

Hvis Ja; hvor lenge har du benyttet disse legemidlene i alt? År ☐

Høyde og vekt

Hvor høy er du?(i hele cm) ☐

Hvor mye veier du i dag?(i hele kg) ☐

Hvor mye veide du da du var 18 år?(i hele kg) ☐

Kroppstype i 1. klasse. (Sett ett kryss)

Veldig tynn ☐ Tynn ☐ Normal ☐ Tykk ☐ Veldig tykk ☐

Røykevaner

Har du i løpet av livet røykt mer enn 100 sigaretter til sammen? Ja ☐ Nei ☐

Hvis Ja, ber vi deg fylle ut for de siste fem årene hvor mange sigaretter du i gjennomsnitt røykte pr. dag i denne perioden.

Antall sigaretter pr. dag

0	1-4	5-9	10-14	15-19	20-24	25+
☐	☐	☐	☐	☐	☐	☐

Hvor gammel var du da du tok din første sigarett? ☐

Røyker du daglig nå? Ja ☐ Nei ☐

Hvis Nei, hvor gammel var du da du sluttet? ☐

Røykte noen av dine foreldre da du var barn? Ja ☐ Nei ☐

Hvis Ja, hvor mange sigaretter røykte de til sammen pr. dag? (antall) ☐

Brystkreft i nærmeste familie

Har noen nære slektninger hatt brystkreft?

	Ja	Nei	Vet ikke	Alder ved start
Datter	☐	☐	☐	
Mor	☐	☐	☐	
Søster	☐	☐	☐	

Mammografiundersøkelse

Har du vært til undersøkelse av brystene med mammografi Ja ☐ Nei ☐

Hvis Ja;
hvor gammel var du første gangen? (hele år)

Hvor mange ganger har du vært undersøkt?

-etter invitasjon fra Mammografiprogrammet

-etter henvisning fra lege

-uten henvisning fra lege

Fysisk aktivitet

Vi ber deg angi din fysiske aktivitet etter en skala fra svært liten til svært mye ved 14 års alder, ved 30 års alder og i dag. Skalaen nedenfor går fra 1-10. Med fysisk aktivitet mener vi både arbeid i hjemmet og i yrkeslivet samt trening og annen fysisk aktivitet som turgåing ol.

Alder	Svært lite									Svært mye
14 år	1	2	3	4	5	6	7	8	9	10
30 år	1	2	3	4	5	6	7	8	9	10
I dag	1	2	3	4	5	6	7	8	9	10

Hvor mange timer pr. dag i gjennomsnitt går eller spaserer du utendørs?

	sjelden/ aldri	mindre enn 1/2 time	1/2-1 time	1-2 timer	mer enn 2 timer
Vinter	☐	☐	☐	☐	☐
Vår	☐	☐	☐	☐	☐
Sommer	☐	☐	☐	☐	☐
Høst	☐	☐	☐	☐	☐

Hvor mange trapper (hele etasjer) går du i gjennomsnitt pr. dag

For hver av følgende aktiviteter du deltar i, ber vi deg oppgi hvor mange minutter pr. dag du bruker i gjennomsnitt til hver av aktivitetene.

Aktivitet	Minutter: Vinter	Vår	Sommer	Høst
Se på TV				
Lesing				
Håndarbeid				
Hagearbeid				
Dusj/bad/egenpleie				
Trening/jogging				
Sykling				

Hvor mange hele timer pr. dag bruker du på arbeidsplassen i gjennomsnitt til å

	Timer:
Sitte	
Stå	
Gå	
Løfte	
Tunge løft/pleie	

Kosthold

Påvirker noen av følgende forhold kostholdet ditt?
(sett gjerne flere kryss)

- ☐ Er vegetarianer/veganer
- ☐ Spiser ikke norsk kost til daglig
- ☐ Har allergi/intoleranse
- ☐ Kronisk sykdom
- ☐ Har anoreksi
- ☐ Har bulimi
- ☐ Prøver å gå ned i vekt
- ☐ Lav glykemisk mat

Vi er interessert i å få kjennskap til hvordan kostholdet ditt er vanligvis. Kryss av for hvert spørsmål om hvor ofte du i gjennomsnitt siste året har brukt den aktuelle matvaren, og hvor mye du pleier å spise/drikke hver gang.

Drikke

Hvor mange glass melk drikker du vanligvis av hver type? (Sett ett kryss pr. linje)

	aldri/ sjelden	1-4 pr. uke	5-6 pr. uke	1 pr. dag	2-3 pr. dag	4+ pr.dag
Helmelk (søt, sur)	☐	☐	☐	☐	☐	☐
Lettmelk (søt, sur)	☐	☐	☐	☐	☐	☐
Ekstra lettmelk	☐	☐	☐	☐	☐	☐
Skummet (søt, sur)	☐	☐	☐	☐	☐	☐

Hvor mange kopper kaffe/te drikker du vanligvis av hver sort? (Sett ett kryss for hver linje)

	aldri/ sjelden	1-6 pr. uke	1 pr. dag	2-3 pr. dag	4-5 pr. dag	6-7 pr. dag	8+ pr.dag
Kokekaffe	☐	☐	☐	☐	☐	☐	☐
Traktekaffe	☐	☐	☐	☐	☐	☐	☐
Pulverkaffe	☐	☐	☐	☐	☐	☐	☐
Svart te	☐	☐	☐	☐	☐	☐	☐
Grønn te	☐	☐	☐	☐	☐	☐	☐

Bruker du følgende i kaffe eller te:

	Kaffe		Te	
Sukker (ikke kunstig søtstoff)	☐ Ja	☐ Nei	☐ Ja	☐ Nei
Melk eller fløte	☐ Ja	☐ Nei	☐ Ja	☐ Nei

Hvor mange glass vann drikker du vanligvis?
(Sett ett kryss for hver linje)

	aldri/ sjelden	1-6 pr. uke	1 pr. dag	2-3 pr. dag	4-5 pr. dag	6-7 pr. dag	8+ pr.dag
Springvann/ flaskevann	☐	☐	☐	☐	☐	☐	☐

Hvor mange glass appelsinjuice, saft og brus drikker du vanligvis? (Sett ett kryss for hver linje)

	aldri/ sjelden	1-3 pr. uke	4-6 pr. uke	1 pr. dag	2-3 pr. dag	4+ pr. dag
Appelsinjuice	☐	☐	☐	☐	☐	☐
Annen juice	☐	☐	☐	☐	☐	☐
Saft/brus med sukker	☐	☐	☐	☐	☐	☐
Saft/brus sukkerfri	☐	☐	☐	☐	☐	☐

Yoghurt/kornblanding

Hvor ofte spiser du yoghurt (1 beger)? (Sett ett kryss)

☐ Aldri/sjelden ☐ 1 pr. uke
☐ 2-3 pr. uke ☐ 4+ pr. uke

Hvor ofte spiser du kornblanding, havregryn eller müsli? (Sett ett kryss)

☐ Aldri/sjelden ☐ 1-3 pr. uke
☐ 4-6 pr. uke ☐ 1 pr. dag

Brødmat

Hvor mange skiver brød/rundstykker og knekkebrød/ skonrokker spiser du vanligvis?
(1/2 rundstykke = 1 brødskive) (Sett ett kryss for hver linje)

	aldri/ sjelden	1-4 pr. uke	5-7 pr. uke	2-3 pr. dag	4-5 pr. dag	6+ pr. dag
Grovt brød	☐	☐	☐	☐	☐	☐
Kneipp/halvfint	☐	☐	☐	☐	☐	☐
Fint brød/baguett	☐	☐	☐	☐	☐	☐
Knekkebrød o.l.	☐	☐	☐	☐	☐	☐

I neste spalte er det spørsmål om bruk av ulike påleggstyper. Vi spør om hvor mange brødskiver med det aktuelle pålegget du pleier å spise. Dersom du også bruker matvarene i andre sammenhenger enn til brød (f. eks. til vafler, frokostblandinger, grøt), ber vi om at du tar med dette når du besvarer spørsmålene.

På hvor mange brødskiver bruker du? (Sett ett kryss pr. linje)

	Aldri/ sjelden	1-3 pr. uke	4-6 pr. uke	1 pr. dag	2-3 pr. dag	4+ pr.dag
Syltetøy	☐	☐	☐	☐	☐	☐
Brunost, helfet	☐	☐	☐	☐	☐	☐
Brunost, halvfet/mager	☐	☐	☐	☐	☐	☐
Hvitost, helfet	☐	☐	☐	☐	☐	☐
Hvitost, halvfet/mager	☐	☐	☐	☐	☐	☐
Kjøttpålegg, Leverpostei	☐	☐	☐	☐	☐	☐
Rekesalat, italiensk o.l.	☐	☐	☐	☐	☐	☐

På hvor mange brødskiver pr. uke har du i gjennomsnitt siste året spist? (Sett ett kryss pr. linje)

	Aldri/ sjelden	1 pr.uke	2-3 pr.uke	4-6 pr.uke	7-9 pr.uke	10+ pr.uke
Makrell i tomat, røkt makrell	☐	☐	☐	☐	☐	☐
Kaviar	☐	☐	☐	☐	☐	☐
Sild/Ansjos	☐	☐	☐	☐	☐	☐
Laks (gravet/røkt)	☐	☐	☐	☐	☐	☐
Annet fiskepålegg	☐	☐	☐	☐	☐	☐

Hva slags fett bruker du vanligvis på brødet?
(Sett gjerne flere kryss)

☐ Bruker ikke fett på brødet
☐ Smør
☐ Hard margarin (f. eks. Per, Melange)
☐ Myk margarin (f. eks. Soft, Vita, Solsikke)
☐ Smørblandet margarin (f.eks. Bremyk)
☐ Brelett
☐ Lettmargarin (f. eks. Soft light, Letta, Vita Lett)
☐ Middels lett margarin (f. eks. Olivero, Omega)

Dersom du bruker fett på brødet, hvor tykt lag pleier du å smøre på? (En kuvertpakke med margarin veier 12 gram).
(Sett ett kryss)

☐ Skrapet (3 g) ☐ Tynt lag (5 g)
☐ Godt dekket (8 g) ☐ Tykt lag (12 g)

Frukt og grønnsaker

Hvor ofte spiser du frukt? (Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr.mnd.	1 pr.uke	2-4 pr.uke	5-6 pr.uke	1 pr.dag	2+ pr. dag
Epler/pærer	☐	☐	☐	☐	☐	☐	☐
Appelsiner o.l.	☐	☐	☐	☐	☐	☐	☐
Bananer	☐	☐	☐	☐	☐	☐	☐
Annen frukt	☐	☐	☐	☐	☐	☐	☐

Hvor ofte spiser du ulike typer grønnsaker?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr.mnd.	1 pr.uke	2 pr.uke	3 pr.uke	4-5 pr.uke	6-7 pr. uke
Gulrøtter	☐	☐	☐	☐	☐	☐	☐
Kål	☐	☐	☐	☐	☐	☐	☐
Kålrot	☐	☐	☐	☐	☐	☐	☐
Brokkoli/blomkål	☐	☐	☐	☐	☐	☐	☐
Blandet salat	☐	☐	☐	☐	☐	☐	☐
Tomat	☐	☐	☐	☐	☐	☐	☐
Grønnsak-blanding (frossen)	☐	☐	☐	☐	☐	☐	☐
Løk	☐	☐	☐	☐	☐	☐	☐
Andre grønn-saker	☐	☐	☐	☐	☐	☐	☐

For de grønnsakene du spiser, kryss av for hvor mye du spiser hver gang. (Sett ett kryss for hver sort)

Gulrøtter	☐ 1/2 stk	☐ 1 stk	☐ 1 1/2 stk	☐ 2+ stk.
Kål	☐ 1/2 dl	☐ 1 dl	☐ 1 1/2 dl	☐ 2+ dl
Kålrot	☐ 1/2 dl	☐ 1 dl	☐ 1 1/2 dl	☐ 2+ dl
Brokkoli/ blomkål	☐ 1-2 buketter	☐ 3-4 buketter	☐ 5+ buketter	
Blandet salat	☐ 1 dl	☐ 2 dl	☐ 3 dl	☐ 4+ dl
Tomat	☐ 1/4 stk	☐ 1/2 stk	☐ 1stk	☐ 2+ stk
Grønnsak-blanding	☐ 1/2 dl	☐ 1 dl	☐ 2 dl	☐ 3+ dl

Hvor mange poteter spiser du vanligvis (kokte, stekte, mos)? (Sett ett kryss)

☐ Spiser ikke/spiser sjelden poteter ☐ 1-4 pr. uke
☐ 5-6 pr. uke ☐ 1 pr. dag ☐ 2 pr. dag
☐ 3 pr. dag ☐ 4+ pr. dag

Ris, spaghetti, grøt, suppe

Hvor ofte bruker du ris og spaghetti/makaroni?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2 pr. uke	3+ pr.uke
Ris	☐	☐	☐	☐	☐
Spagetti, makaroni, nudler	☐	☐	☐	☐	☐

Hvor ofte spiser du grøt ? (Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2-6 pr. uke	1+ pr. dag
Risengrynsgrøt	☐	☐	☐	☐	☐	☐
Annen grøt (havre o.l.)	☐	☐	☐	☐	☐	☐

Hvor ofte spiser du suppe?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2 pr. uke	3+ pr.uke
Som hovedrett	☐	☐	☐	☐	☐
Som forrett, lunsj eller kveldsmat	☐	☐	☐	☐	☐

Fisk

Vi vil gjerne vite hvor ofte du pleier å spise fisk, og ber deg fylle ut spørsmålene om fiskeforbruk så godt du kan. Tilgangen på fisk kan variere gjennom året. Vær vennlig å markere i hvilke årstider du spiser de ulike fiskeslagene.

	aldri/ sjelden	like mye hele året	vinter	vår	sommer	høst
Torsk, sei, hyse, lyr	☐	☐	☐	☐	☐	☐
Steinbit, flyndre, uer	☐	☐	☐	☐	☐	☐
Laks, ørret	☐	☐	☐	☐	☐	☐
Makrell	☐	☐	☐	☐	☐	☐
Sild	☐	☐	☐	☐	☐	☐
Annen fisk	☐	☐	☐	☐	☐	☐

Med tanke på de periodene av året der du spiser fisk, hvor ofte pleier du å spise følgende til middag?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2+ pr. uke
Kokt torsk, sei, hyse, lyr	☐	☐	☐	☐	☐
Stekt torsk, sei, hyse, lyr	☐	☐	☐	☐	☐
Steinbit, flyndre, uer	☐	☐	☐	☐	☐
Laks, ørret	☐	☐	☐	☐	☐
Makrell	☐	☐	☐	☐	☐
Sild	☐	☐	☐	☐	☐
Annen fisk	☐	☐	☐	☐	☐

Dersom du spiser fisk, hvor mye spiser du vanligvis pr. gang? (1 skive/stykke = 150 gram)

Kokt fisk (skive)	☐ 1	☐ 1,5	☐ 2	☐ 3+
Stekt fisk (stykke)	☐ 1	☐ 1,5	☐ 2	☐ 3+

Hvor mange ganger pr. år spiser du fiskeinnmat?
(Sett ett kryss pr. linje)

	0	1-3	4-6	7-9	10+
Rogn	☐	☐	☐	☐	☐
Fiskelever	☐	☐	☐	☐	☐

Dersom du spiser fiskelever, hvor mange spiseskjeer pleier du å spise hver gang? (Sett ett kryss)

☐ 1 ☐ 2 ☐ 3-4 ☐ 5-6 ☐ 7+

Hvor ofte bruker du følgende typer fiskemat?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2+ pr. uke
Fiskekaker/pudding/boller	☐	☐	☐	☐	☐
Plukkfisk/fiskegrateng	☐	☐	☐	☐	☐
Frityrfisk/fiskepinner	☐	☐	☐	☐	☐

Hvor stor mengde pleier du vanligvis å spise av de ulike rettene? (Sett ett kryss for hver linje)

Fiskekaker/pudding/boller (stk.) (2 fiskeboller=1 fiskekake)	☐ 1	☐ 2	☐ 3	☐ 4+
Plukkfisk, fiskegrateng (dl)	☐ 1-2	☐ 3-4	☐ 5+	
Frityrfisk, fiskepinner (stk.)	☐ 1-2	☐ 3-4	☐ 5-6	☐ 7+

I tillegg til informasjon om fiskeforbruk er det viktig å få kartlagt hvilket tilbehør som blir servert til fisk. Hvor ofte bruker du følgende til fisk?

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2+ pr. uke
Smeltet/fast smør	☐	☐	☐	☐	☐
Smeltet/fast margarin/fett	☐	☐	☐	☐	☐
Seterrømme (35%)	☐	☐	☐	☐	☐
Lettrømme (20%)	☐	☐	☐	☐	☐
Saus med fett (hvit/brun)	☐	☐	☐	☐	☐
Saus uten fett (hvit/brun)	☐	☐	☐	☐	☐

For de ulike typene tilbehør du bruker til fisk, vær vennlig å kryss av for hvor mye du vanligvis pleier å spise.

Smeltet/ fast smør (ss)	☐ 1/2	☐ 1	☐ 2	☐ 3	☐ 4+
Smeltet/ fast margarin (ss)	☐ 1/2	☐ 1	☐ 2	☐ 3	☐ 4+
Seterrømme (ss)	☐ 1/2	☐ 1	☐ 2	☐ 3	☐ 4+
Lettrømme (ss)	☐ 1/2	☐ 1	☐ 2	☐ 3	☐ 4+
Saus med fett (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+
Saus uten fett (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+

Hvor ofte spiser du skalldyr (f. eks. reker, krabbe og skjell)? (Sett ett kryss)

☐ Aldri/sjelden ☐ 1 pr. mnd ☐ 2-3 pr. mnd ☐ 1+ pr. uke

Kjøtt

Hvor ofte spiser du reinkjøtt?

☐ Aldri/sjelden ☐ 1 pr. mnd. ☐ 2-3 pr. mnd. ☐ 1 pr. uke
☐ 2-3 pr. uke ☐ 4+ pr. uke

Hvor ofte spiser du følgende kjøtt- og fjærkreretter? (Sett ett kryss for hver rett)

	aldri/ sjelden	1 pr.mnd.	2-3 pr.mnd.	1 pr.uke	2+ pr.uke
Steik (okse, svin, får)	☐	☐	☐	☐	☐
Koteletter	☐	☐	☐	☐	☐
Biff	☐	☐	☐	☐	☐
Kjøttkaker, karbonader	☐	☐	☐	☐	☐
Pølser	☐	☐	☐	☐	☐
Gryterett, lapskaus	☐	☐	☐	☐	☐
Pizza med kjøtt	☐	☐	☐	☐	☐
Kylling	☐	☐	☐	☐	☐
Bacon, flesk	☐	☐	☐	☐	☐
Andre kjøttretter	☐	☐	☐	☐	☐

Dersom du spiser følgende retter, oppgi mengden du vanligvis spiser: (Sett ett kryss for hver linje)

Steik (skiver)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5+
Koteletter (stk.)	☐ 1/2	☐ 1	☐ 1,5	☐ 2+	
Kjøttkaker, karbonader (stk.)	☐ 1	☐ 2	☐ 3	☐ 4+	
Pølser (stk. à 150g)	☐ 1/2	☐ 1	☐ 1,5	☐ 2+	
Gryterett, lapskaus (dl)	☐ 1-2	☐ 3	☐ 4	☐ 5+	
Pizza m/kjøtt (stykke à 100 g)	☐ 1	☐ 2	☐ 3	☐ 4+	

Hvilke sauser bruker du til kjøttretter og pastaretter? (Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2+ pr. uke
Brun saus	☐	☐	☐	☐	☐
Sjysaus	☐	☐	☐	☐	☐
Tomatsaus	☐	☐	☐	☐	☐
Saus med fløte/rømme	☐	☐	☐	☐	☐

Hvor mye bruker du vanligvis av disse sausene?

Brun saus (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+
Sjysaus (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+
Tomatsaus (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+
Saus med fløte/ rømme (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+

Andre matvarer

Hvor mange egg spiser du vanligvis i løpet av en uke?(stekte, kokte, eggerøre, omelett) (Sett ett kryss)

☐ 0 ☐ 1 ☐ 2 ☐ 3-4
☐ 5-6 ☐ 7+

Hvor ofte spiser du iskrem? (til dessert, Krone-is osv.) Sett ett kryss for hvor ofte du spiser iskrem om sommeren, og ett kryss for resten av året

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2+ pr.uke
Om sommeren	☐	☐	☐	☐	☐
Resten av året	☐	☐	☐	☐	☐

Hvor mye is spiser du vanligvis pr. gang? (Sett ett kryss)

☐ 1dl ☐ 2 dl ☐ 3 dl ☐ 4+ dl

Hvor ofte spiser du bakevarer som boller, kaker, wienerbrød eller småkaker (Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2-3 pr uke	4-6 pr. uke	1+ pr.dag
Gjærbakst (boller o.l.)	☐	☐	☐	☐	☐	☐
Wienerbrød, kringle	☐	☐	☐	☐	☐	☐
Kaker	☐	☐	☐	☐	☐	☐
Pannekaker	☐	☐	☐	☐	☐	☐
Vafler	☐	☐	☐	☐	☐	☐
Småkaker, kjeks	☐	☐	☐	☐	☐	☐
Lefser, lomper	☐	☐	☐	☐	☐	☐

Hvor ofte spiser du dessert? (Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr mnd.	1 pr uke	2-3 pr. uke	4+ pr. uke
Pudding sjokolade/karamell	☐	☐	☐	☐	☐	☐
Riskrem, fromasj	☐	☐	☐	☐	☐	☐
Kompott, fruktgrøt, hermetisk frukt	☐	☐	☐	☐	☐	☐
Jordbær (friske, frosne)	☐	☐	☐	☐	☐	☐
Andre bær (friske, frosne)	☐	☐	☐	☐	☐	☐

Hvor ofte spiser du sjokolade? (Sett ett kryss)

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2-3 pr uke	4-6 pr. uke	1+ pr.dag
Mørk sjokolade	☐	☐	☐	☐	☐	☐
Lys sjokolade	☐	☐	☐	☐	☐	☐

Dersom du spiser sjokolade, hvor mye pleier du vanligvis å spise hver gang? Tenk deg størrelsen på en Kvikk-Lunsj sjokolade, og oppgi hvor mye du spiser i forhold til den.

☐ 1/4 ☐ 1/2 ☐ 3/4 ☐ 1 ☐ 1,5 ☐ 2+

Hvor ofte spiser du snacks? (Sett ett kryss)

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2-3 pr. uke	4-6 pr. uke	1+ pr. dag
Potetchips	☐	☐	☐	☐	☐	☐
Peanøtter	☐	☐	☐	☐	☐	☐
Andre nøtter	☐	☐	☐	☐	☐	☐
Annen snacks	☐	☐	☐	☐	☐	☐

Tran og fiskeoljekapsler

Bruker du tran (flytende)? Ja ☐ Nei ☐

Hvis ja; hvor ofte tar du tran?
Sett ett kryss for hver linje.

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2-6 pr. uke	daglig
Om vinteren	☐	☐	☐	☐	☐
Resten av året	☐	☐	☐	☐	☐

Hvor mye tran pleier du å ta hver gang?

☐ 1 ts. ☐ 1/2 ss. ☐ 1+ ss.

Bruker du tranpiller/fiskeoljekapsler? Ja ☐ Nei ☐

Hvis ja; hvor ofte tar du tranpiller/fiskeoljekapsler?
Sett ett kryss for hver linje.

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2-6 pr. uke	daglig
Om vinteren	☐	☐	☐	☐	☐
Resten av året	☐	☐	☐	☐	☐

Hvilken type tranpiller/fiskeoljekapsler bruker du vanligvis, og hvor mange pleier du å ta hver gang?

Antall

Navn _____ ☐

Kosttilskudd

Bruker du kosttilskudd? Ja ☐ Nei ☐

Hvis ja, hvor ofte bruker du kosttilskudd?
(Sett ett kryss pr. linje)

Navn på kosttilskudd	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2-6 pr. uke	daglig
_____	☐	☐	☐	☐	☐
_____	☐	☐	☐	☐	☐
_____	☐	☐	☐	☐	☐
_____	☐	☐	☐	☐	☐

Bruker du soyapreparater mot plager i overgangsalderen? Ja ☐ Nei ☐

Varm mat

Hvor mange ganger i løpet av en måned spiser du varm mat?

Til frokost ☐ Til middag ☐

Til lunsj ☐ Til kvelds ☐

Kosthold som barn

Hvor mye melk drakk du som barn hver dag? (sett ett kryss)

☐ drakk ikke melk ☐ 1-3 glass ☐ 4-6 glass ☐ 7 glass eller me

Hvor ofte spiste du grønnsaker til middag som barn?
(sett ett kryss)

☐ aldri ☐ 1 gang i uken eller mer sjelden
☐ 2-3 ganger i uken ☐ 4 eller flere ganger pr. uke

Hvor ofte spiste du fisk til middag som barn? (sett ett kryss)

☐ aldri ☐ 1 gang i uken eller mer sjelden
☐ 2-3 ganger i uken ☐ 4 eller flere ganger pr. uke

Alkohol

Er du totalavholdskvinne? Ja ☐ Nei ☐

Hvis Nei; hvor ofte og hvor mye drakk du i gjennomsnitt siste året? (Sett ett kryss for hver linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2-4 pr. uke	5-6 pr. uke	1 pr. dag	2+ pr. dag
Øl (1/2 l.)	☐	☐	☐	☐	☐	☐	☐	☐
Vin (glass)	☐	☐	☐	☐	☐	☐	☐	☐
Brennevin (drink)	☐	☐	☐	☐	☐	☐	☐	☐
Likør/Hetvin (glass)	☐	☐	☐	☐	☐	☐	☐	☐

Sosiale forhold

Er du idag: (Sett ett kryss)

☐ gift ☐ samboer ☐ ugift ☐ skilt ☐ enke

Hvor mange personer er det i ditt hushold? ☐

Hvor høy er bruttoinntekten i husholdet pr. år?

☐ inntil 150.000 kr. ☐ 151.000-300.000 kr.
☐ 301.000-450.000 kr. ☐ 451.000-600.000 kr.
☐ 601.000-750.000 kr. ☐ over 750.000 kr.

Hva er din arbeidssituasjon? (sett ett eller flere kryss)

☐ Arbeider heltid ☐ Arbeider deltid ☐ Pensjonist
☐ Hjemmearbeidende ☐ Under utdanning ☐ Uføretrygdet
☐ Under attføring ☐ Arbeidssøkende

Arbeider du utendørs i yrkessammenheng? Ja ☐ Nei ☐

Hvis Ja;
hvor mange timer pr. uke? ☐ Sommer ☐ Vinter

Solvaner

Får du fregner når du soler deg? Ja ☐ Nei ☐

For å kunne studere effekten av soling på risiko for hudkreft, ber vi deg gi opplysninger om hudfarge. Sett ett kryss på det tallet under fargen som best passer din naturlige hudfarge (uten soling).

1	2	3	4	5	6	7	8	9	10

Hvor mange ganger pr. år er du blitt forbrent av solen slik at du har fått svie eller blemmer med avflassing etterpå? (ett kryss for hver aldersgruppe)

Alder	Aldri	Høyst 1 gang pr. år	2-3 g. pr. år	4-5 g. pr. år	6 eller flere ganger pr. år
40-49 år	☐	☐	☐	☐	☐
50 + år	☐	☐	☐	☐	☐

Hvor mange uker i gjennomsnitt pr. år har du vært på badeferie i syden eller i Norge?

Alder	Aldri	1 uke	2-3 uker	4-5 uker	7 uker eller mer
40-49 år	☐	☐	☐	☐	☐
50 + år	☐	☐	☐	☐	☐
Siste 12 mnd.	☐	☐	☐	☐	☐

Hvor ofte har du solt deg i solarium?

Alder	Aldri	Sjelden	1 gang pr. mnd.	2 ganger pr. mnd.	3-4 ganger pr. mnd	oftere enn 1 gang pr. uke
40-49 år	☐	☐	☐	☐	☐	☐
50+ år	☐	☐	☐	☐	☐	☐
Siste 12 mnd.	☐	☐	☐	☐	☐	☐

Hvor ofte dusjer eller bader du?

	mer enn 1 g. dagl.	1 g. dagl.	4-6 g. pr. uke	2-3 g. pr. uke	1 g. pr. uke	2-3 g. pr. mnd	sjelden/ aldri
Med såpe/ shampo	☐	☐	☐	☐	☐	☐	☐
Uten såpe/ shampo	☐	☐	☐	☐	☐	☐	☐

Når bruker du krem med solfaktor? (sett evt. flere kryss):

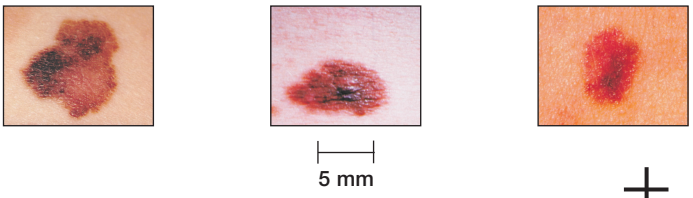
☐ i påsken ☐ i Norge eller utenfor syden
☐ solferie i syden ☐ aldri

Hvilken solfaktor bruker du i disse periodene?

	Ingen	1-4	5-9	10-14	15+
Påsken	☐	☐	☐	☐	☐
I Norge eller utenfor syden	☐	☐	☐	☐	☐
Solferie i syden	☐	☐	☐	☐	☐

Hvor mange uregelmessige føflekker større enn 5 mm har du sammenlagt på begge beina (fra tærne til lysken)? Tre eksempler på føflekker større enn 5 mm med uregelmessig form er vist nedenfor.

☐ 0 ☐ 1 ☐ 2-3 ☐ 4-6 ☐ 7-12 ☐ 13-24 ☐ 25+



Hvor ofte bruker du følgende hudpleiemidler?

(Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr.mnd.	1 pr.uke	2-4 pr.uke	5-6 pr.uke	1 pr.dag	2+ pr. dag
Ansiktskrem	☐	☐	☐	☐	☐	☐	☐
Håndkrem	☐	☐	☐	☐	☐	☐	☐
Body lotion	☐	☐	☐	☐	☐	☐	☐
Parfyme	☐	☐	☐	☐	☐	☐	☐

Til slutt vil vi spørre deg om ditt samtykke til å kontakte deg på nytt pr. post. Vi vil hente adressen fra det sentrale personregister.

Ja ☐ Nei ☐

Er du villig til å avgi en blodprøve?

Ja ☐ Nei ☐

Takk for at du ville delta i undersøkelsen

Appendix V

KVINNER OG KREFT

Du sendte i 2003 eller 2004 et utfylt spørreskjema til Institutt for samfunnsmedisin som del av den landsdekkende undersøkelsen "Kvinner og kreft". Spørsmålene var særlig rettet mot kosthold.
Vi ønsker å studere hvilken betydning våre matvaner har for kreftutvikling hos kvinner. I følgeskrivet til spørreskjemaet informerte vi om at en del kvinner senere ville bli forespurt om de var villig til å avgi blodprøve. Blodprøvene vil bli avidentifisert ved ankomst Institutt for samfunnsmedisin.

Formålet med blodprøven vil være:

- Måle nivå av vitaminer, mineraler og andre stoffer i blodet som kan settes i forbindelse med kostholdet.
- I fremtiden kunne studere de såkalte genetiske markører dvs. egenskaper i arvestoffet som kan disponere for kreft.
- Teste nye ideer eller hypoteser som oppstår i fremtiden.

Det er frivillig om du vil delta. Du kan trekke deg uten begrunnelse, og du kan be om at opplysninger du har gitt blir slettet, uten at dette vil få konsekvenser for deg.
Blodprøven vil kun bli benyttet til forskning og ingen resultater vil bli utlevert til deg eller noen andre. Blodprøven vil bli lagret i 30 år.

Ansvarlig for undersøkelsen er professor Eiliv Lund. Undersøkelsen er tilrådd av Regional komité for medisinsk forskningsetikk, Nord-Norge (REK NORD), og Datatilsynet har gitt konsesjon for oppbevaring av opplysninger.
Fremtidige forskningsprosjekter som vil benytte de lagrete blodprøvene vil forelegges Regional komité for medisinsk forskningsetikk, Nord-Norge (REK NORD).

Du kan finne mer informasjon om "Kvinner og kreft"og om forskningsresultatene på våre nettsider: www.ism.uit.no/kk/

Med vennlig hilsen

Eiliv Lund
professor dr.med.

Bente A. Augdal
prosjektmedarbeider

" ----------

Ønsker du ikke å delta og vil slippe påminning pr. brev ber vi deg fylle ut svar-slippen og returnere denne sammen med utstyret tilbake til oss (forseglet utstyr **må** ikke åpnes).

Jeg ønsker **ikke** å delta i blodprøvetakingen. .....

Underskrift

Appendix VI

NOWAC questionnaire that accompanies the blood samples

2005
CONFIDENCIAL

The questionnaire must be answered in connection with the blood draw.

ID:

The questionnaire MUST accompany the blood sample

LAB:

I have read the information concerning the blood sample donation and I concent to participate:

Yes ☐

Blood draw

When was the blood sample drawn?

Date (day, month) ☐☐ ☐☐
Time (hour,minute) ☐☐ ☐☐

When was your latest meal before blood draw?

Date (day, month) ☐☐ ☐☐
Time (hour,minute) ☐☐ ☐☐

Posture during blood draw:

Sitting ☐
Laying down ☐

Menstruation

Do you have menstruations?

Yes ☐
No ☐
Irregular ☐
Pregnant ☐

If yes, please provide the date for the first day of your last menstruation:
(day, month) ☐☐ ☐☐

Smoking during the past week

Have you smoked during the past week?

Yes ☐
No ☐

If yes, how many cigarettes did you smoke

Yesterday ☐
Today ☐

Weight/height

What do you weigh today? kg ☐☐☐
How tall are you? cm ☐☐☐

Were weight and height measured at the doctor's office today?

Yes ☐
No ☐

Medication during the past week

Have you used oral contraceptives during the past week?
Yes ☐
No ☐

If yes, please provide the date for the last tablet taken: (day, month) ☐☐ ☐☐

Have you used hormone tablets/patches (estrogen, gestagen) for climacteric complaints during the past week?
Yes ☐
No ☐

If yes, please provide the date when the last tablet was taken:
(day, month) ☐☐ ☐☐

Product name: _____
Product name: _____

Have you used any other medication during the past week?

Yes ☐
No ☐

If yes, please provide the date when the medication was last taken:

(day, month) ☐☐ ☐☐

Product name: _____

(day, month) ☐☐ ☐☐

Product name: _____

(day, month) ☐☐ ☐☐

Product name: _____

Dietary supplements use during the past week

Have you taken cod liver oil (liquid) during the past week?
Yes ☐
No ☐

If yes, please provide the date for the last dose
(day, month) ☐☐ ☐☐

How much did you take at that time?
1 teaspoon ☐
1/2 tablespoon ☐
≥ 1 tablespoon ☐

Have you taken capsules containing cod liver oil/omega-3/fish oil during the past week?
Yes ☐
No ☐

If yes, please provide the date for the last dose
(day, month) ☐☐ ☐☐

How many capsules did you take at that time?
1 ☐
2 ☐
≥ 3 ☐

Product name: _____

Have you taken soy supplements during the past week?
Yes ☐
No ☐

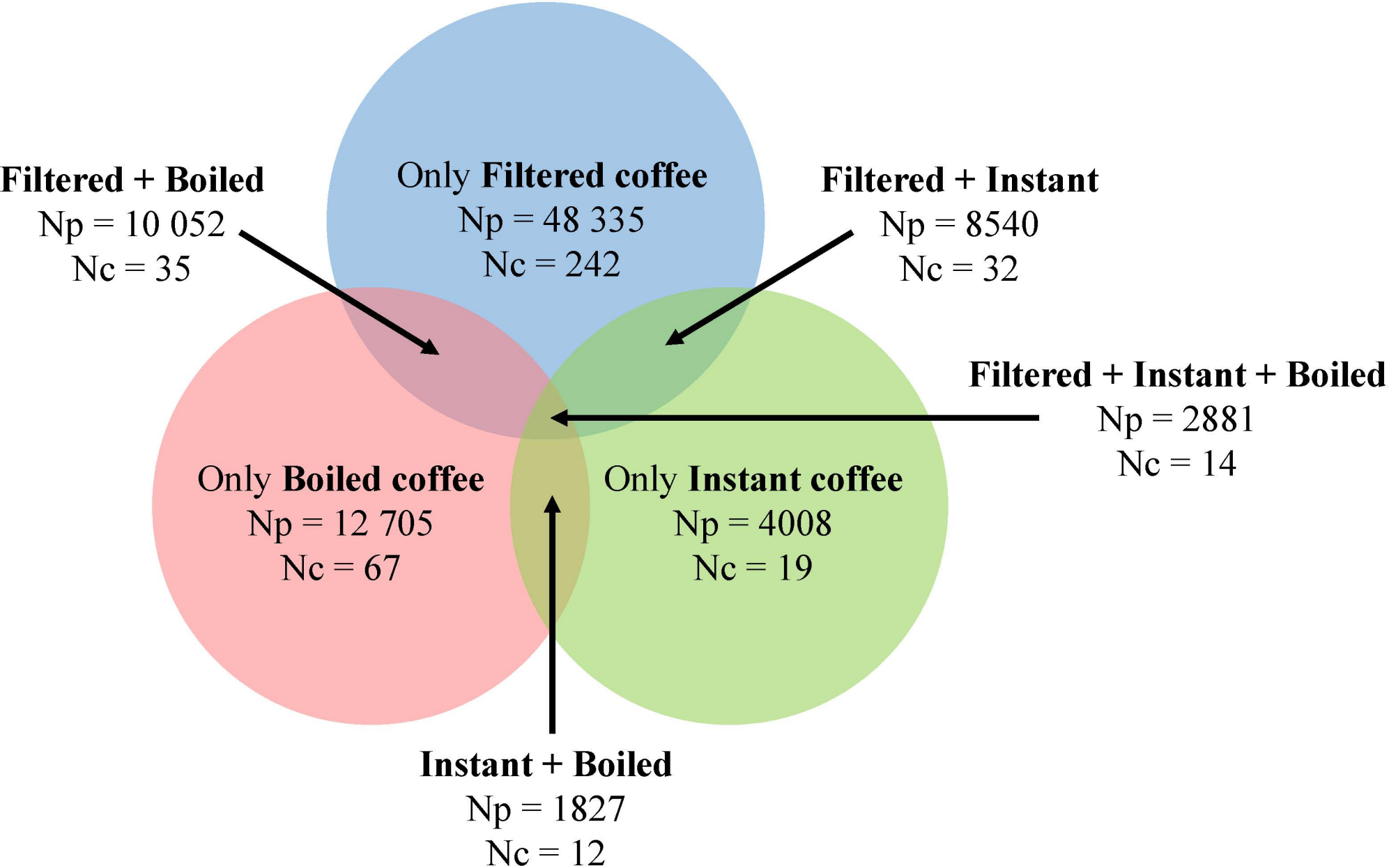
Product name: _____
Product name: _____

Have you taken any other dietary supplements (vitamins/minerals) during the past week?
Yes ☐
No ☐

If yes, please provide the date for the last dose
(day, month) ☐☐ ☐☐

Product name: _____
Product name: _____

Appendix VII


Only **Filtered coffee**
Np = 48 335
Nc = 242
Filtered + Boiled
Np = 10 052
Nc = 35
Filtered + Instant
Np = 8540
Nc = 32
Filtered + Instant + Boiled
Np = 2881
Nc = 14
Only **Boiled coffee**
Np = 12 705
Nc = 67
Only **Instant coffee**
Np = 4008
Nc = 19
Instant + Boiled
Np = 1827
Nc = 12

Appendix VIII

Appendix 8. Modification of coffee categories for analysis based on available information from NOWAC questionnaires.

Series 1-5, 8, 9
Year 1991-1992
«How many glasses of coffe do you ussually drink of each type?»
Boiled, Filtered, Instant

- almost never,
- 1-3 cups/month
- 1 cup/week,
- 2-4 cups/week,
- 5-6 cups/week
- 1 cup/day, 2-3 cups/day
- 4-5 cups/day
- 6-10 cups/day.

Series 11, 17, 18
Year 1996
«Do you drink coffee?»
Total coffee
Categories: almost never, 1-3 cups/month, 1 cup/week, 2-4 cups/week, 5-6 cups/week, 1 cup/day, 2-3 cups/day,4-5 cups/day,6-10 cups/day.

Series 25, 27, 30
Year 1998, 2000
«Do you drink coffee?»
Total coffee
Categories: almost never, 1-3 cups/month, 1 cup/week, 2-4 cups/week, 5-6 cups/week, 1 cup/day, 2-3 cups/day,4-5 cups/day, 6-10 cups/day.

Series 37
Year 2004
«Do you drink coffee?»
Total coffee
Categories: almost never, 1-3 cups/month, 1 cup/week, 2-4 cups/week, 5-6 cups/week, 1 cup/day, 2-3 cups/day,4-5 cups/day,6-10 cups/day.

Series 12-16, 19-23, 35,36
Year 1996-1997, 2003-2004
«Do you drink those coffee types?»
Boiled, Filtered, Instant
Categories: almost never, 1-6 cups/week, 1 cup/day, 2-3 cups/day, 4-5 cups/dag, 6-7 cup/day, 8+ cups/day.

Series 26, 28,29,32,33
Year 1998, 2002
«Do you drink those coffee types?»
Boiled, Filtered, Instant
Categories: almost never, 1-6 cups/week, 1 cup/day, 2-3 cups/day, 4-5 cups/dag, 6-7 cup/day, 8+ cups/day.

Series 34, 38, 39,42
Year 2002-2005
«Do you drink those coffee types?»
Boiled, Filtered, Instant
Categories: almost never, 1-6 cups/week, 1 cup/day, 2-3 cups/day, 4-5 cups/dag, 6-7 cup/day, 8+ cups/day.

Collapsing of the categories with low consumption from all questionnaires (1 cup/day, 5–6 cups/week, 2–4 cups/week, 1–6 cups/week, 1 cup/week, 1–3 cups /month and almost never) into ≤1 cup/day (reference category)

Those who responded 6–10 cups/day in the total coffee questionnaire were categorized as ≥ 8 cups/day (heavy consumers)

The final categories used in the analysis:
≤1 cup/day (reference category), 2–3 cups/day, 4–7 cups/day and ≥8 cups/day (heavy consumers).