Hormones, Smoking and Mammographic Density in Postmenopausal Norwegian Women

The Tromsø Mammography and Breast Cancer Study

By Yngve Bremnes, M.D.   *Tromsø 2007*

Illustration by László Tabár

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Institute of Community Medicine
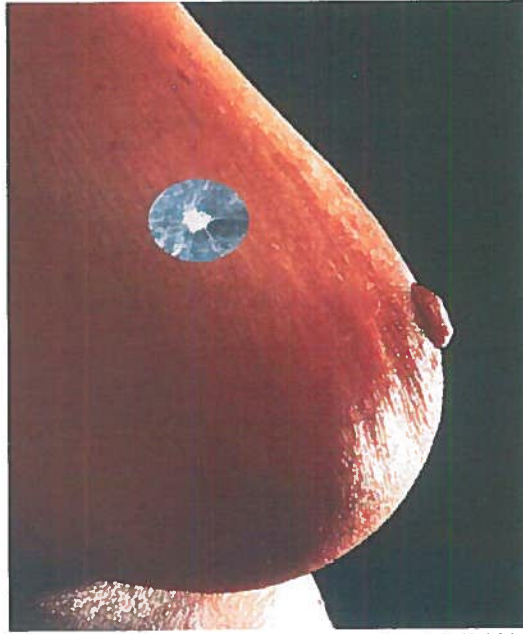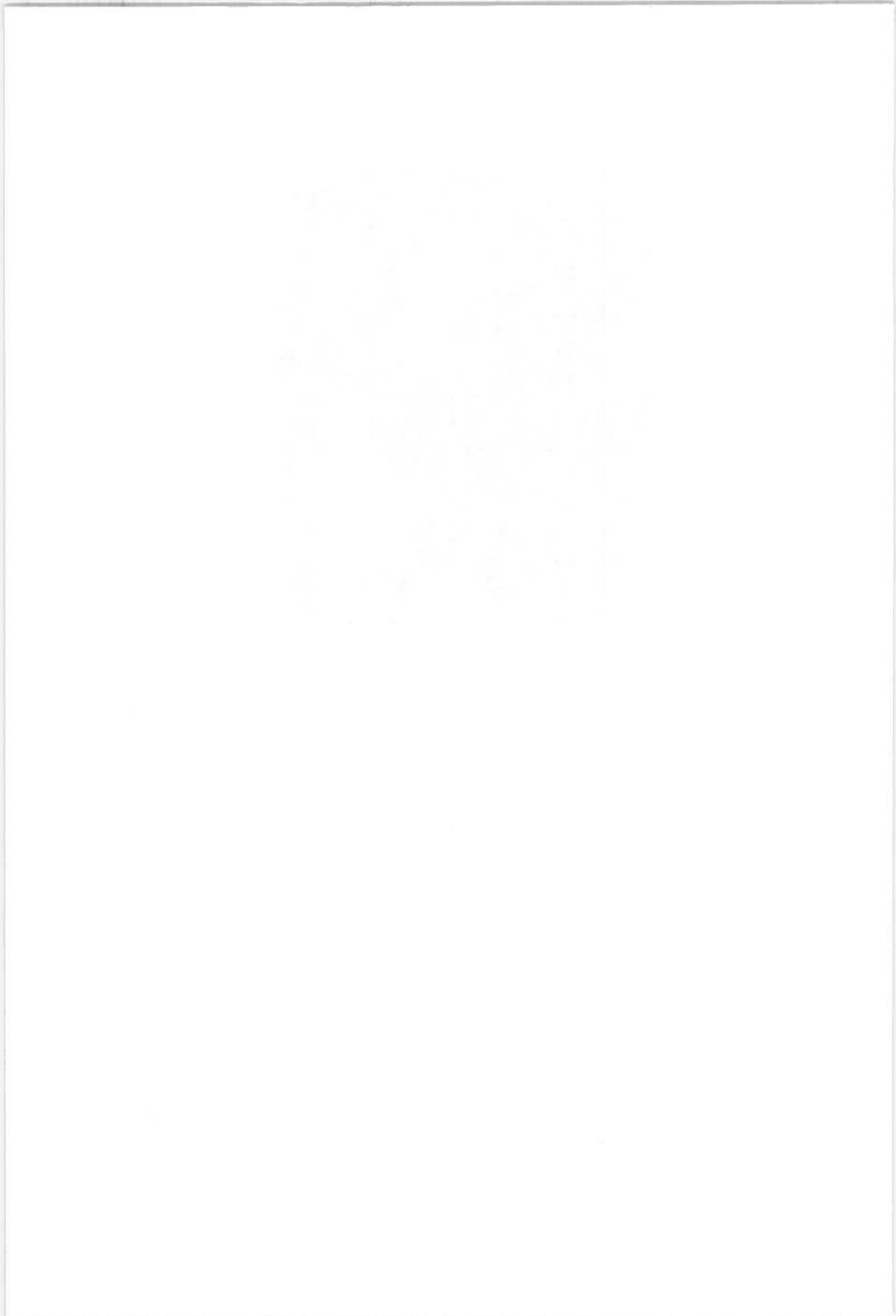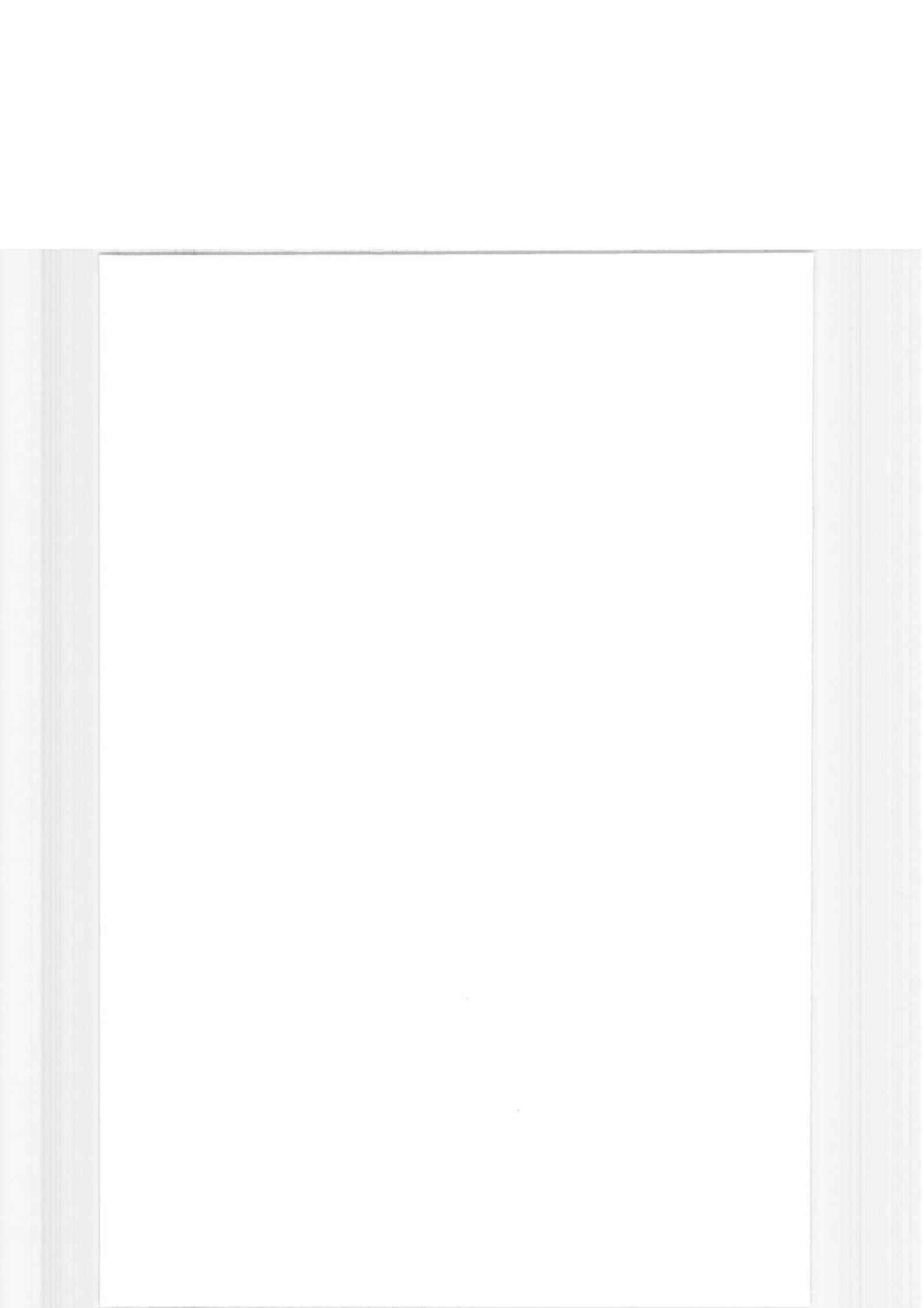University of Tromsø

Norske Kvinners Sanitetsforening

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ACKNOWLEDGEMENTS

LIST OF PAPERS

This thesis is based on the following original papers, which are referred to in the thesis by their Roman numerals:

I. Bremnes Y, Ursin G, Bjurstam N, Lund E, Gram IT. Different types of postmenopausal hormone therapy and mammographic density in Norwegian women. *Int J Cancer* 2007;120:880-4.

II. Bremnes Y, Ursin G, Bjurstam N, Gram IT. Different measures of smoking exposure and mammographic density in postmenopausal Norwegian women: a cross-sectional study. *Submitted.*

III. Bremnes Y, Ursin G, Bjurstam N, Rinaldi S, Kaaks R, Gram IT. Insulin-like growth factor and mammographic density in postmenopausal Norwegian women. *Cancer Epidemiol Biomarkers Prev* 2007;16:57-62.

IV. Bremnes Y, Ursin G, Bjurstam N, Rinaldi S, Kaaks R, Gram IT. Endogenous sex hormones, prolactin and mammographic density in postmenopausal Norwegian women. *Accepted for publication in Int J Cancer.*

LIST OF ABBREVIATIONS

BMI	Body mass index (kg/m^2)
CC	Cranio-caudal
EPT	Estrogen plus progestin therapy
HT	Postmenopausal hormone therapy
IARC	International Agency for Research on Cancer
IGF-I	Insulin-like growth factor-I
IGFBP-3	Insulin-like growth factor binding protein-3
NBCSP	Norwegian Breast Cancer Screening Program
ROI	Region of interest
SHBG	Sex hormone binding globulin
TDLU	Terminal duct lobular units
TEB	Terminal end bud
TMBC	Tromsø Mammography and Breast Cancer
UNN	University Hospital of North Norway

1. INTRODUCTION

Breast cancer is the most commonly occurring malignancy among women, and according to estimates by the International Agency for Research on Cancer, more than one million new cases were diagnosed worldwide in 2002 (1). In Norway, altogether 2,780 women were diagnosed with breast cancer in 2005, corresponding to an age-adjusted (world) incidence rate of 75.7 per 100,000 women per year (2). According to the predictions for the years 2010-2020 by the Cancer Registry of Norway, we can expect a continued increase in breast cancer incidence, resulting in more than 4,000 new cases annually by the year 2020 (estimated to >80 breast cancer cases per 100,000 women per year) (2). About 80% of new breast cancers cases are diagnosed in women 50 years or older. Although breast cancer mainly affects older women, the predicted increase in breast cancer incidence is only partly explained by the change in age distribution to older women (2). Thus, other risk factors than age must be of importance.

The breasts undergo a series of changes throughout a woman's lifetime (3). The growth spurt occurs at puberty with increase in both epithelial tissues, i.e. the lobules (glands) and ducts, and surrounding breast stroma (connective and fat tissue). The epithelial ducts grow and branch out. Within one to two years past menarche, numerous terminal duct lobular units (TDLU) develop from the terminal end buds (TEB). The TDLUs group together and form altogether 15-20 major lobes that are drained by ducts leading to the mammilla (nipple) (Figure 1a).

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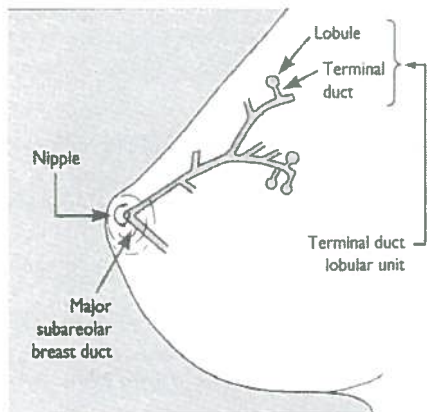
Figure 1a. Anatomy of the female breast.
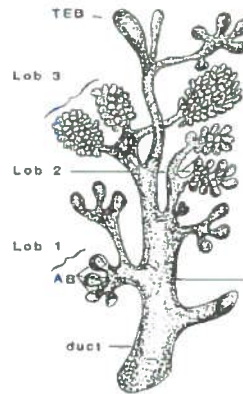
Figure 1b: Lobules of the female breast.

TEB: Terminal end bud. AB: alveolar bud≈TDLU.

The early lobule cells are though to have stem cell capabilities, and thus the ability to differentiate (Figure 1b). With increasing differentiation of the lobular structure the number of ductules per lobule increases. However, the size of the ductules decreases, and the number of cells in a tissue cross-section of a ductule is significantly lower for both Lob 3 and Lob 2 when compared with Lob 1 (4). The differentiation of the glandular tissues into true glands is completed by the end of the first full term pregnancy, and thus, it is considered to never having been attained among nulliparous women. Laboratory studies have suggested that parity also induces a change in the lobule cells that make them more refractory to transforming stimuli (5). In nulliparous women the predominant type of lobules is the more undifferentiated Lob 1 (i.e. alveolar bud ≈ TDLU), while the more differentiated Lob 3 is predominant in parous women until they reach an age of around 40 years. The breasts of both parous and nulliparous women starts to undergo regression towards less differentiated types of lobules as the women approach menopause, and by the age of 50 years the breasts contains mostly Lob 1 like cells (3). Also, with increasing age, epithelial and connective tissues are replaced by fat (6, 7).

Although breast cancer incidence rates increase with age (2, 6), the rate of the increase slows down around the age of 50 years (Figure 3).
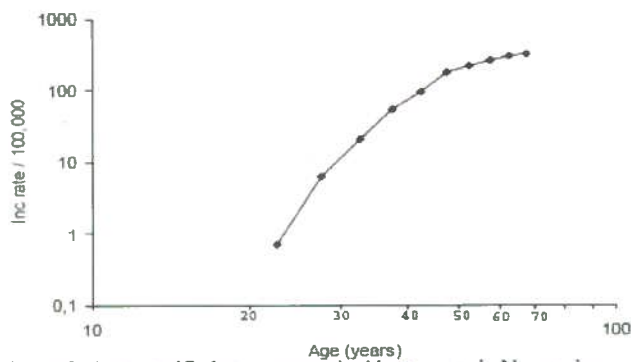
Figure 3: Age-specific breast cancer incidence rates in Norwegian women (2005).

Data source: The Cancer Registry of Norway.

In 1983, Pike and colleagues suggested that the variations in age-specific breast cancer incidence rates were related to the amount of ageing the breast tissue had undergone, and not the women's chronological age (8). They incorporated several established breast cancer risk factors, i.e. age at menarche, age at first birth, and age at menopause, into a model describing the breast tissue ageing rate (Figure 4).
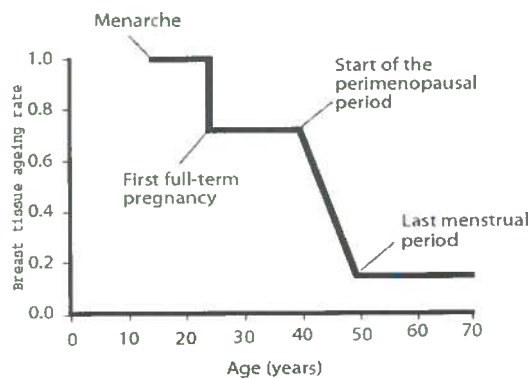
Figure 4: The Pike and colleagues' model of breast tissue ageing rate.

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According to their model, the highest rate of breast tissue ageing occurs between the time of menarche and the first full-term pregnancy. The changed hormonal environment due to the first pregnancy decreases the rate of breast tissue ageing. The next marked change in breast tissue ageing rate comes with the changed hormonal environment in the perimenopausal period. After menopause the breast tissue ageing rate is at its slowest and continues at a constant rate with advancing chronologic age. By fitting numerical values to the different events in their model, they showed that the cumulative ageing of the breast tissue, i.e. the area under the curve, described the curve of the age-specific breast cancer incidence rates in the United States at the time (8). Their model was later extended to also incorporate timing and spacing of pregnancies (9).

Individual differences in the amount and distribution of the different breast tissues will lead to variations in how a breast appears on an x-ray image (mammogram). Mammographic density is a measure of the extent of radiodense tissue in the breast, i.e. how much the x-rays are attenuated by the different tissues in the breast (Figure 2). Epithelial and connective breast tissues are radiodense and will appear light on a mammogram, while fatty breast tissue is radiolucent and will appear dark (10). Higher mammographic density has been found to be associated with greater amounts of epithelial and connective tissues in breast tissue samples collected as autopsies (11, 12).
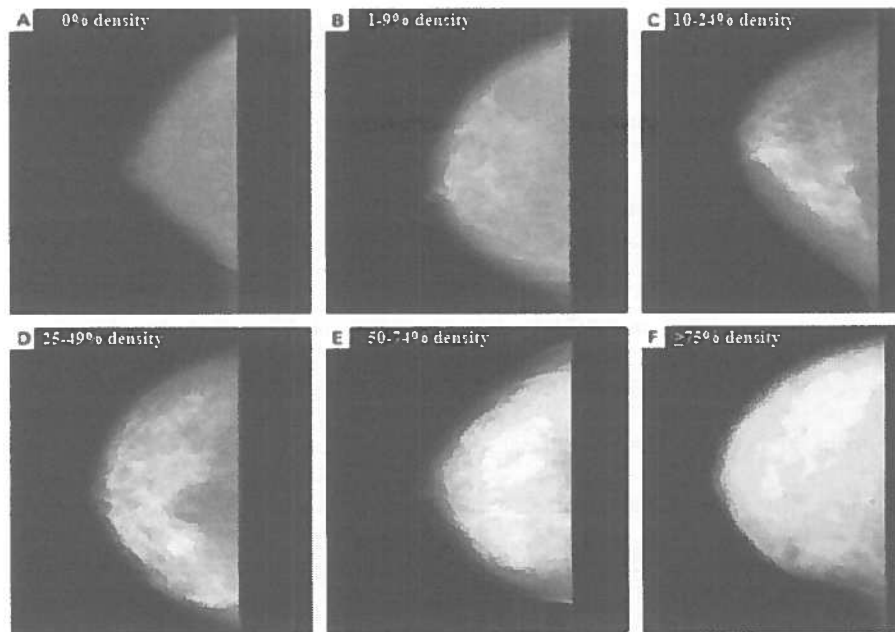
Figure 2. Examples of various percentage categories of mammographic density.

In 1976, Wolfe introduced mammographic density as a risk factor for breast cancer. Wolfe described a classification consisting of four breast parenchymal patterns, and showed that it could be an index of breast cancer risk (13, 14). Over the next decades, other methods of classifying mammographic density emerged. The American College of Radiology adopted a visual four scale method (Breast Imaging Reporting and Data System) of reporting mammographic density for clinical use (15). Tabár described a modification of the Wolfe classification using five, instead of four, categories. Also, in contrast to the simple pattern reading of the Wolfe classification, Tabár based his classification on anatomic-mammographic correlation using a three-dimensional subgross (thick-slice) technique (16). In the last decade, the development has moved in the direction of quantitative mammographic density measurements, with Boyd and colleagues being the first in 1995 (17). Today, mammographic density is commonly measured on a continuous scale using computer-assisted

methods (described in more detail in Chapter 3.5). Although the term mammographic density is used, current mammographic density measurements are based on a projected area, rather than on the volume, of breast tissue. Several research groups are presently working on developing methods for volumetric mammographic density measurements (18-20).

As the woman approaches menopause, mammographic density starts to decline. In a longitudinal Canadian study, percent mammographic density was lower in premenopausal women about to become postmenopausal than in women of similar ages who stayed premenopausal. The lower percent mammographic density was due to both a decrease of the dense area and an increase of the non-dense area. There was no significant difference in the total breast area between the groups (21), and this may be a reflection of fatty replacement. The decrease in mammographic density seemed to be more rapid during the change in menopausal status than the decrease that came with advancing age (21).

In 2001, Boyd and colleagues proposed that the cumulative exposure to dense mammographic breast tissue also could be represented by the area under the curve of the Pike and colleagues model (22). In a similar way as late menarche, early age at first birth, multiple births, and early menopause decrease the cumulative breast tissue ageing, these factors also decrease the cumulative exposure to mammographic density. Consistent with this, a recent longitudinal study from the United States found that the cumulative percent mammographic density increased with age at a rate very similar to the age-specific breast cancer incidence rates (Figure 5) (23).
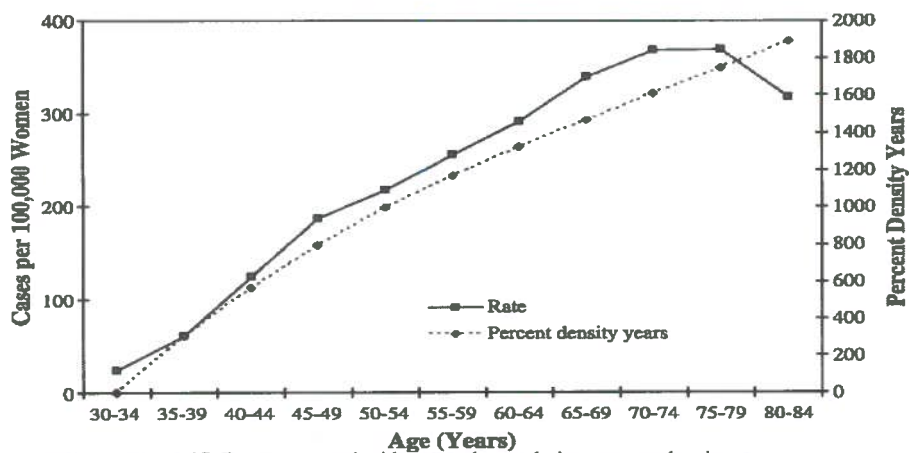
Figure 5. Age-specific breast cancer incidence and cumulative percent density years.

Endogenously mammographic density, i.e. density that is present in absence of pharmacological intervention, is one of the strongest independent risk factors for breast cancer (Table 1) (24-27). Studies on twin pairs have indicated that as much as 65% of the variation in mammographic density between women may be due to heritable factors (28, 29). However, mammographic density is also associated with several of the established breast cancer risk factors (27, 30-35). Many of these breast cancer risk factors, e.g. height, postmenopausal hormone therapy (HT) use and parity, are associated with percent mammographic density as they are associated with breast cancer risk, while age and postmenopausal body mass index (BMI) are associated with percent mammographic density opposite of that with breast cancer risk. The inverse association between postmenopausal BMI and percent mammographic density is inevitable, given how percent mammographic density is measured. Women with a high BMI tend to have higher amounts of fat in the breast (i.e. increased total mammographic area), and fatty tissue is radiolucent (non-dense).

	Relative risk	High-risk group
Age	>10	Elderly individuals
Geographical location	5	Developed countries
Breast density	>5	Extensive dense breast tissue visible on mammogram
Age at menarche	3	Before age 11 years
Age at menopause	2	After age 54 years
Age at first full pregnancy	3	First child after age 40 years
Family history	≥2	Breast cancer in first-degree relative
Previous benign breast disease	4–5	Atypical hyperplasia
Cancer in other breast	>4	Previous breast cancer
Socioeconomic group	2	Groups I and II*
Body-mass index		
Premenopause	0·7	High body-mass index
Postmenopause	2	High body-mass index
Alcohol consumption	1·07	7% increase with every daily drink
Exposure to ionising radiation	3	Abnormal exposure to young girls after age 10 years
Breastfeeding and parity	Relative risk falls by 4·3% for every 12 months of breastfeeding in addition to a 7% reduction for every birth	Women who do not breastfeed
Use of exogenous hormones		
Oral contraceptives	1·2	Current users
Hormone-replacement therapy	1·66	Current users
Diethylstilbestrol	2	Use during pregnancy

Table 1. Risk factors for breast cancer.

Women with high mammographic density have a 4- to 6-fold increase in breast cancer risk compared to women with low mammographic density (25, 26). The magnitude of the association between mammographic density and breast cancer risk, and the fact that mammographic density is present in the organ where the cancer eventually develops, have led to the conclusion that mammographic density probably is a valid surrogate marker for breast cancer risk (36).

The Tromsø Mammography and Breast Cancer (TMBC) study is a cross-sectional study assessing mammographic density as a surrogate marker for breast cancer risk (TMBC homepage:

http://uit.no/med-befolkning/6407/). The idea to design the TMBC study was conceived of by Professor Inger Torhild Gram, based on her previous research in the Third Tromsø Study (37). As a part of the Third Tromsø Study, the first organized mammography screening in Norway was carried out in 1986/87 among women aged 40-56 years. The mammograms from the 3,640 women participating were classified by Dr. László Tabár according to his parenchymal patterns classification (16). These previous studies showed an inverse association between Tabár high-risk mammographic patterns and parity, a positive association with age at first birth (16), and positive associations with early age at menarche and with age at menopause (38). Body height was positively, and BMI inversely, associated with Tabár high-risk mammographic patterns regardless of menopausal status (39). Furthermore, moderately physically active women were less likely to have Tabár high-risk patterns than those inactive (40), and ever oral contraceptive users had an increased risk of having Tabár high-risk patterns (41).

In the TMBC study, the mammographic density has been assessed according to four methods: two categorical methods; i.e. the Wolfe (13, 14) and the Tabár (16) classifications, as well as two computer-assisted methods with mammographic density measured on a continuous scale; i.e. the Madena (42) and the Cumulus (17, 43) methods. We found that the mammographic density readings by the computer-assisted methods conveyed additional information to that of the categorical methods in regards to their associations with breast cancer risk factors (31). For all papers presented in this thesis (Papers I-IV), we have utilized mammographic density measurements using the Madena method. The reason why we chose the mammographic density readings by the Madena method over the readings by the Cumulus method was purely practical: the Madena readings were finished before the Cumulus readings.

2. AIMS OF THE THESIS

The present thesis was aimed at examining the association between hormones, cigarette smoking and mammographic density among postmenopausal Norwegian women.

More specifically the aims were to assess:

- The association between postmenopausal hormone therapy use and mammographic density

- Whether cigarette smoking was associated with mammographic density in this study population

- Whether circulating levels of insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-3, and the IGF-I/IGFBP-3 molar ratio, were associated with mammographic density, overall, and according to HT use

- Whether circulating levels of endogenous sex hormones were associated with mammographic density, overall, and according to IGF-I levels.

3. MATERIALS AND METHODS

3.1 The Tromsø Mammography and Breast Cancer study population

3.1.1 Background population - the Norwegian Breast Cancer Screening Program

The Norwegian Breast Cancer Screening Program (NBCSP) is a governmentally funded screening program administered by the Cancer Registry of Norway. From 1995/1996 to early 2004 the NBCSP was gradually expanded from a project in four counties to a nationwide program (44). The Central Population Register identifies the screening population by use of a unique 11 digit personal identification number assigned to each resident in Norway. In Tromsø, the first screening round started in May 2000 and ended in June 2002. Women aged 50-70 years received a personally addressed letter offering a two-view mammography screening for a fee of approximately 25 Euros. The NBCSP is described in more detail elsewhere (44, 45).

3.1.2 Study population - the Tromsø Mammography and Breast Cancer study

The Tromsø Mammography and Breast Cancer (TMBC) study was planned as a single-center cross-sectional study conducted among postmenopausal women, age 55 and over, residing in the municipality of Tromsø, Norway, and attending the population-based NBCSP at the University Hospital of North Norway (UNN), Tromsø, Norway. By request of the NBCSP steering committee, the TMBC study had to conduct a pilot in spring 2001 to assess whether an invitation to the TMBC study, in addition to the invitation to the NBCSP, would negatively affect the attendance to the NBCSP.

3.1.2.1 The TMBC pilot – spring 2001

The TMBC pilot was conducted among women scheduled to receive their invitation to the NBCSP during the last 15 weeks of the NBCSP screening period in spring 2001. A maximum of 20 women eligible for the TMBC study were randomly selected from the weekly NBCSP invitation list received from the Center for Breast Imaging, UNN. Shortly after the women had received the invitation to the NBCSP, we mailed a letter to the women with a request to enter the TMBC study after they had undergone their screening mammograms. This letter stated the purpose of the TMBC study, and requested a written informed consent (Appendix 1). Furthermore, the letter informed that entering the TMBC study would include a short interview, measurement of anthropometrics, a blood draw, and a questionnaire to be filled in at home and returned in a prepaid envelope. The letter assured the woman that her decision about participating or not in the TMBC study, would not affect her status in the NBCSP. The letter also informed the woman that the TMBC study had been approved by the Regional Committee for Medical Research Ethics and the National Data Inspection Board, and that she could withdraw from the TMBC study at any time. We were able to request 253 women according to this procedure, of which 154 entered the TMBC study. The control group in this pilot study was the remaining 397 eligible women who were invited to the NBCSP during the same period, but who were not mailed a TMBC study request (Figure 6).

A research assistant requested the women in the control group to enter the TMBC study after they had shown up at the NBCSP screening facility. Altogether 270 women were requested this way, of which 229 entered the TMBC study. Another 27 women were eligible, but were not requested due to lack of manpower (Figure 6).

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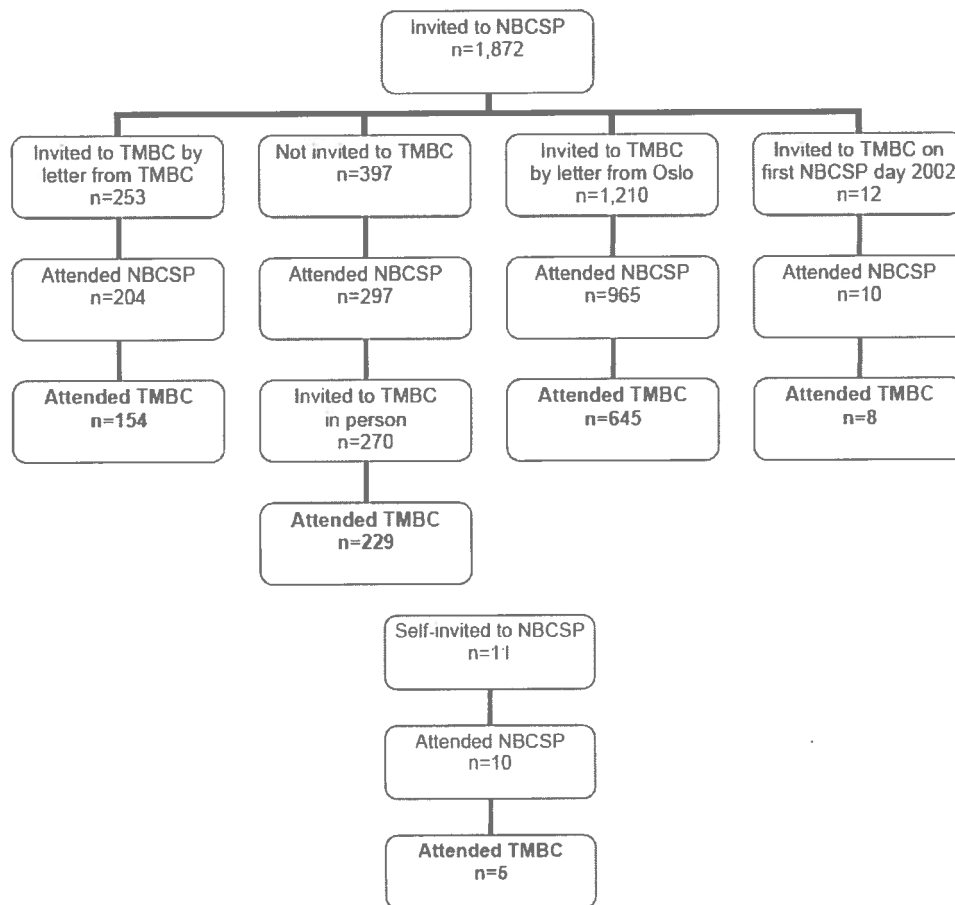
Figure 6: Flow charts of the creation of the Tromsø Mammography and Breast Cancer (TMBC) study population (N=1,041).

The results from the pilot study showed that the additional request to the TMBC study did not negatively influence the attendance rate to the NBCSP (46).

3.1.2.2 **TMBC - spring of 2002**

In spring of 2002, during the last 17 weeks of the NBCSP screening period, eligible women received a request to enter the TMBC study enclosed with the NBCSP invitation mailed from Oslo. Altogether 1,210 women were requested according to this procedure, of which 645

entered the TMBC study. In addition to the above procedures, to start the recruitment in spring of 2002, TMBC study request letters were sent directly from the university to 12 women scheduled to attend the NBCSP. Ten of these women attended the NBCSP, and eight of the women also entered the TMBC study. Also, during spring of 2001 and spring of 2002 a total of 11 women called the NBCSP and asked to be invited (self-invited). Ten of these women attended the NBCSP and five also entered the TMBC study (Figure 6).

3.1.2.3 Participants in analyses Papers I-IV

Altogether, 1,041 eligible women were included in the TMBC study during spring of 2001 and spring of 2002. This accounted to 70% of the 1,486 women attending the NBCSP during these recruitment periods (Figure 6).

For all papers (Papers I-IV), we excluded 23 of the 1,041 women because of previously (n=16) or newly (n=6) diagnosed breast cancer, and one woman because of an ongoing chemotherapy treatment. Among the remaining 1,018 women, we were unable to retrieve mammograms for 11 women, leaving 1,007 women who had mammograms classified according to percent and absolute mammographic density.

Paper I comprises all 1,007 women.

For Paper II, an additional 100 women were excluded because of being equivocal for menopausal status (n=3) or missing smoking history (n=97), leaving 907 women for the analyses.

For Paper III, thirty women were excluded from the 1,007 women because of being equivocal for menopausal status (n=3), not having donated blood samples (n=17), or missing IGF-I measurements (n=10), leaving 977 women for the analyses.

For Paper IV, altogether 285 women were excluded from the 1,007 women because of being equivocal for menopausal status (n=3), current HT use (n=259), past HT use within the last 3 months prior to study inclusion (n=8), or not having donated blood samples (n=15), leaving 722 women for the analyses.

3.2 Interview and questionnaires

After the women had undergone their NBCSP screening mammograms, the women who chose to attend the TMBC study were interviewed by a nurse from the Department of Clinical Research, UNN, about reproductive and menstrual factors, previous history of cancer, smoking status, and use of HT or other medications (Appendix 2). The nurse showed a color photo-leaflet of the altogether 19 HT preparations ever available on the Norwegian market (Appendix 3). This leaflet was originally made for use in the Norwegian Women and Cancer study. The participants had their height measured to the nearest centimeter and weight measured to the nearest half kilogram. The women had blood samples drawn, and each was subsequently given a questionnaire to be completed at home, eliciting information on demographics, additional menstrual and reproductive factors, as well as lifestyle and dietary factors (Appendix 4).

The examination at the screening facility in spring 2002 was the same as in spring 2001, but the questionnaire to be filled in at home was expanded from four to eight pages (Appendix 5).

The additional items consisted of dietary questions previously utilized in the Norwegian Women and Cancer study (47).

A reminder to fill in and return the questionnaire was sent once to all non-responding women in the study. Finally, the response rate to the questionnaire was 92%.

3.3 Exposure classifications

3.3.1 Postmenopausal hormone therapy use

Women indicating use of HT (oral or transdermal administration) at time of enrollment were classified as current HT users. Ever users not indicating current use were classified as past users.

The estrogen plus progestin therapy (EPT) regimens in Norway have all contained one of the three estrogens; estradiol, estriol, or etinylestradiol. The progestins most commonly used are norethisterone/norethisterone acetate and levonorgestrel. The most common HT regime used in Norway is the estradiol plus norethisterone acetate combination. The synthetic steroid tibolone, with a weak estrogenic, progestogenic, and androgenic effect, was introduced in Norway in late 1999. All the HT preparations used by the women could be categorized into the following four groups; 1) estrogen monotherapy, 2) continuous estrogen plus progestin combination, 3) sequential estrogen and progestin combination, and 4) tibolone.

3.3.2 Cigarette smoking

The women were interviewed about current smoking status, and the self-administered questionnaire elicited additional information on lifetime smoking history. We categorized women who had never smoked but had been exposed to tobacco smoke at home or at work as "passive smokers". Women reporting neither having ever smoked nor having been exposed to

passive smoking were categorized as "never active smokers". Never active smokers and passive smokers were also grouped together as "never smokers". Current and past smokers were grouped together as "ever-smokers". Pack-years were calculated as the average number of cigarettes smoked per day divided by 20 and multiplied by the number of years smoked.

We further categorized current smokers according to age at smoking initiation, average number of cigarettes smoked per day, number of years smoked, number of pack-years smoked, and parous women according to smoking initiation before or after first birth.

3.4 Blood samples and plasma analyses

Non-fasting venous blood samples were obtained from the study participants the day of the mammographic screening. The samples were taken from an antecubital vein while the woman was seated. Samples for plasma extraction were collected in two 9mL citrated vials. After centrifugation for 15 minutes at 3000 rpm plasma was extracted and deposited into 2mL cryo-tubes and stored at -70°C.

In September 2003 the plasma samples were retrieved from our storage in Tromsø and shipped frozen to the laboratory for hormone analyses, Nutrition and Cancer Group, International Agency for Research on Cancer (IARC), Lyon France, where all hormone assays were performed. At IARC, the plasma samples were stored at -80°C until the time of analyzing. The laboratory and mathematical methods employed to determine plasma concentrations of IGF and endogenous sex hormones are described in Papers III and IV. The IGF and prolactin measurements were done in spring of 2004, and the remaining plasma was refrozen until the endogenous sex hormones measurements in spring of 2005.

For the analyses the plasma samples were divided into batches. For the IGF and prolactin analyses each batch consisted of 73 plasma samples from our study and two quality controls. For the sex hormone analyses, each batch consisted of 33 plasma samples and two quality controls. The batches were set up without knowledge of the outcome variable, but were grouped according to HT use.

3.5 Mammograms and mammographic density measurements

3.5.1 Mammograms

All mammograms used in this study were obtained at the NBCSP site in Tromsø (Center for Breast Imaging, UNN). In the NBCSP, each woman undergoes two mammograms of each breast, i.e. one cranio-caudal (CC) mammogram of the breast in the transversal plane, and one medio-lateral oblique mammogram of the breast in the axial plane. More details on the imaging technique and quality assurance are described in the NBCSP quality manual (www.kreftregisteret.no/om_kreftregisteret/registrering/masseundersokelser_etc/manual.pdf). The x-ray exposure and processing of the mammograms are standardized in the NBCSP. However, how the woman's breast is positioned and how much it is compressed is controlled by the radiographer. At the NBCSP site in Tromsø, an internal quality control assessment is performed 4-5 times a year, and each radiographer is given a personal feedback on the quality of the mammograms. These assessments are performed to ensure uniform mammogram quality (Kirsten Jensen, personal communication, 2007).

3.5.2 Mammographic density measurements- the Madena method

Previous studies have found almost complete concordance between right and left breast mammographic density readings (48, 49). We chose to study the women's CC mammograms from the left breast. In autumn of 2002, the mammograms were collected from the Center for

Breast Imaging, UNN, and shipped to the University of Southern California, USA. There, percent and absolute mammographic densities were determined by an experienced reader (Giske Ursin) using the previously validated Madena computer-based threshold method developed at the University of Southern California (42). The Madena method works as follows: The women's left CC mammogram is digitized using a Cobrascan CX-812 scanner (Radiographic Digital Imaging, Torrance, CA, USA) at a resolution of 150 pixels per inch (59 pixels per centimeter). This scanner creates an 8-bit image with 256 shades of gray ($8^2=256$). The digitized two-dimensional mammographic image is imported into the Madena computer program and viewed on the screen. A reader defines the total breast area using a special outlining tool (Figure 7a), and the Madena program calculates the breast's total area (total number of pixels within the breast outline).
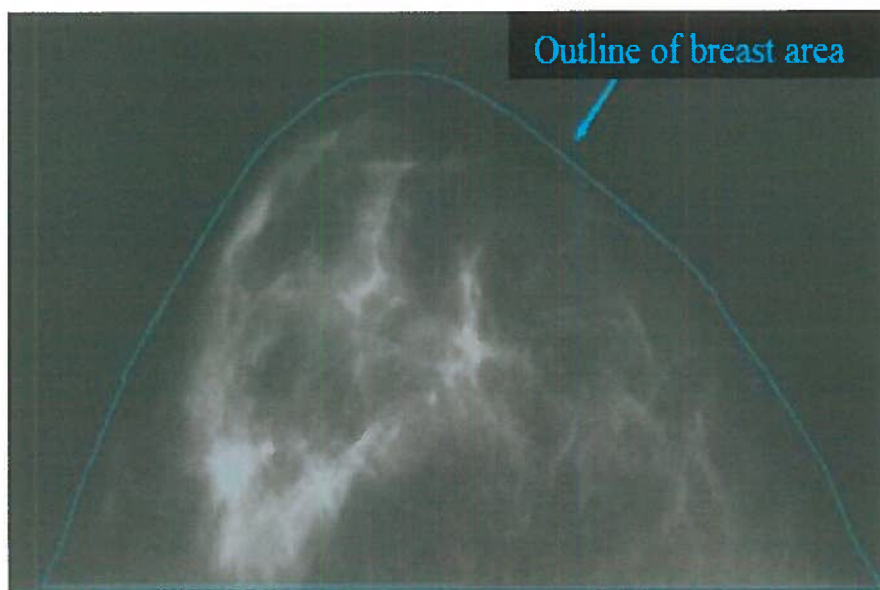
Figure 7a. Definition of the total breast area (Madena method).

Next, the region of interest (ROI) is defined by drawing a red colored outline around the area with mammographic densities; thus excluding radiodense artifacts as the pectoralis muscle, prominent veins, and fibrous strands (Figure 7b).
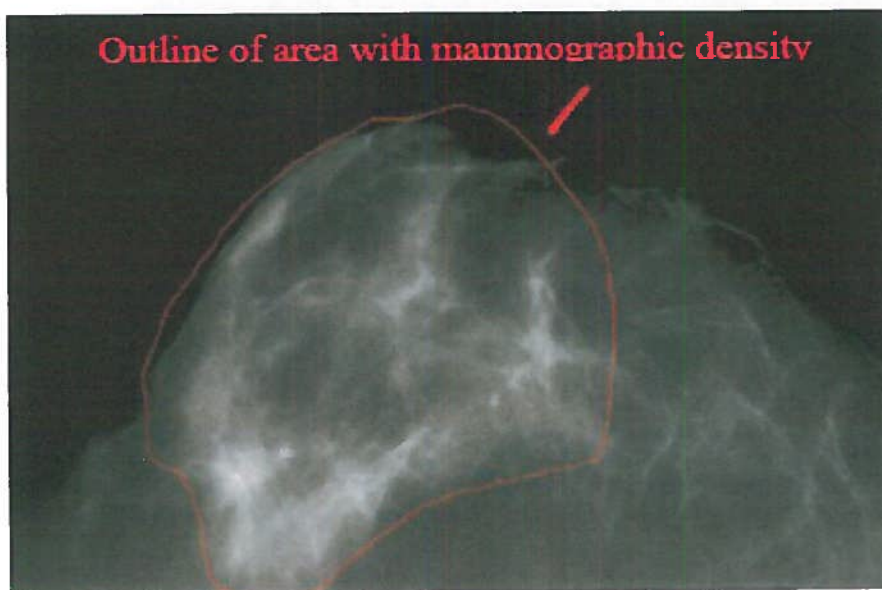
Figure 7b. Definition of the region of interest (Madena method).

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The computer software program assigns a pixel value of 0 to the darkest (black) shade in the image and a value of 255 to the lightest (white) shade, with shades of grey assigned to intermediate values. The reader then uses a tinting tool to apply a yellow tint to dense pixels with grey levels at or above some threshold X. The reader searches for the best threshold where all pixels $\geq$X within the ROI are considered to represent mammographic densities (Figure 7c).
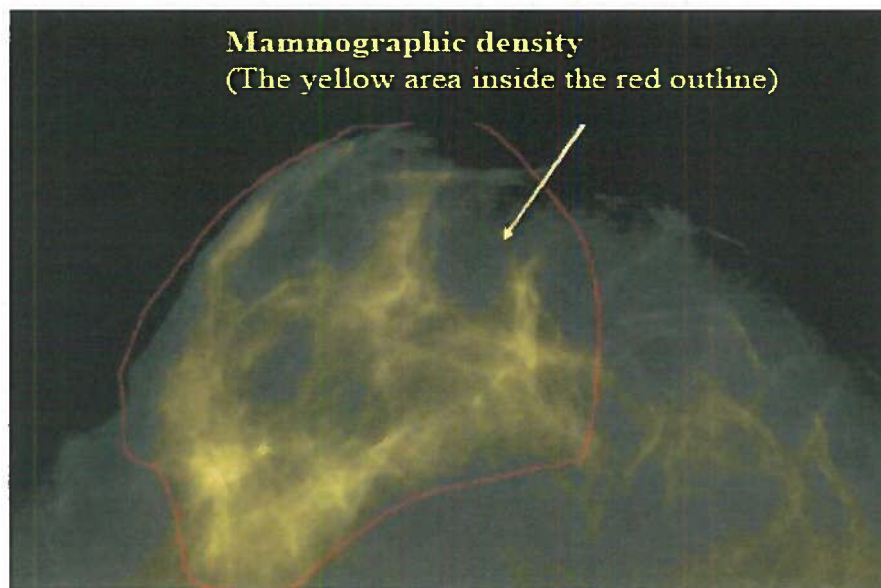
Figure 7c. Yellow tinting of pixels representing mammographic densities (Madena method).

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The software estimates the total number of pixels and the number of tinted pixels within the ROI. Absolute density represents the count of the tinted pixels within the ROI. Absolute density measured in cm^2 is calculated as the number of tinted pixels within the ROI divided by the number of pixels per cm^2 ($59^2=3481$). Percent density (the fraction (%) of the breast with densities) is the ratio of absolute density to the total breast area multiplied by 100.

3.6 Statistical methods

We compared nominal data using two-way tables (chi-square). Interval data from two groups were compared using either a t-test or a non-parametric equivalent. When we compared data from more than two groups, we used analysis of variance (ANOVA, Proc GLM, SAS Institute Inc. Cary, NC). The same assumptions need to be met with ANOVA as with t-tests; independence, normal distributions, and the groups should come from a population with equal

variance. ANOVA has the advantage, over numerous t-tests between all combinations of two groups, of testing if there are any differences between the groups with a single associated probability for that if our finding is occurring by chance.

The following variables had previously been found to be associated with mammographic density in this study population; age at screening, number of children, and BMI (31), and were always adjusted for as confounders in the multivariate analyses. Additionally, we identified confounding variables that were associated with the exposure variable in univariate analyses, and that also were statistically significantly associated with mammographic density in multivariate analyses. This was done to ensure that the effect of these confounders was taken into account when assessing the association between the exposure variable under investigation and mammographic density.

Since all women in our study were postmenopausal, we did not have to adjust for menopausal status. In Paper I we adjusted for age at screening, number of children, and BMI. In Paper II, we additionally adjusted for age at first birth, age at menopause, and HT use. In Papers III and IV, we additionally adjusted for age at menopause and HT use. Further, we also performed analyses with different modeling of BMI, keeping it as a continuous variable, as a categorical variable, excluding the 2.5% lowest and 2.5% highest values, and excluding the 5% highest values. In Paper III we also performed analyses stratified according to HT use.

We checked for effect-measure modification by assessing the analyses stratified by the confounding variables, and by adding multiplicative interaction terms to the ANOVA procedures.

Reported trend test P values correspond to analyses where the categories of the exposure variable under investigation were treated as ordered variables. The use of median values for the category scores did not alter the trend test P values (Paper IV). Correlations between sex hormones, sex hormone binding globulin (SHBG), prolactin, IGF-I, and BMI in Paper IV were tested using the Spearman rank correlation coefficient.

Mammographic densities were not normally distributed. We assessed residual plots after square root and log transformations, and found that log transformation gave the most approximate normal distribution. The crude and adjusted mean mammographic density results were back-transformed, and are presented in the papers (Papers I-IV) with 95% confidence intervals. The significance level was chosen at $P<0.05$. All reported P values are two sided. We conducted data management and all statistical analyses using the SAS® 9.1 for Windows (SAS Institute Inc.). The statistical methods are described in detail in the individual papers (Papers I-IV).

3.7 Ethics

The TMBC study was approved by the Regional Committee for Medical Research Ethics and the National Data Inspection Board. All women signed an informed consent (Appendix 1). All data were stored and handled according to the permission given by the National Data Inspection Board.

4. MAIN RESULTS

4.1 Postmenopausal hormone therapy use and mammographic density (Paper I)

Among the 1,007 women between the ages of 55 to 71 years who were included in this cross-sectional study, 43% were ever users and 26% current users of systemic HT. Median duration of HT use was 72 months among ever users and 48 months among current users. A continuous EPT was the most commonly used HT among current users. After adjustment for potential confounders, current users of HT had a significantly higher mean percent mammographic density compared with never users overall ($P<0.001$). When analyses were stratified by type of HT currently used, the difference in mean percent mammographic density between current users of HT and never users was largest for users of a continuous EPT (6.1% absolute difference) ($P<0.001$). Further, a positive dose-response relationship was found between duration of use and both percent and absolute mammographic densities among current continuous EPT users (both P trends<0.001).

4.2 Cigarette smoking and mammographic density (Paper II)

Among the 907 postmenopausal women included in this cross-sectional study, 65% were ever smokers and 34% were current smokers. Overall, smoking status was inversely associated with mammographic densities after adjustment for potential confounders ($P<0.001$). Current smokers had a 2.4% (absolute difference) lower mean percent mammographic density compared with never smokers ($P<0.001$). Furthermore, we found a modest inverse dose-response relationship with percent mammographic density among current smokers for both numbers of cigarettes smoked as well as pack-years smoked. Current smokers who smoked ≥ 11 cigarettes daily had a 3.3% (absolute difference) lower percent mammographic density

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compared with current smokers who smoked ≤7 cigarettes daily (P=0.007). The results were similar when restricted to current smokers who had smoked for at least 25 years.

4.3 Insulin-like growth factor-I and mammographic density (Paper III)

Among the 977 postmenopausal women included in this cross-sectional study, both plasma IGF-I and the IGF-I/IGFBP-3 molar ratio were positively associated with percent mammographic density (both P trends=0.02). Overall, we found a 1.5% (absolute difference) higher percent mammographic density among women with IGF-I concentrations in the upper quartile compared with women with IGF-I concentrations in the lowest quartile (P=0.04). When analyses were stratified by HT use status, the associations between IGF-I and mammographic densities were statistically significant among women not currently using HT (both P trends<0.05).

4.4 Endogenous sex hormones and mammographic density (Paper IV)

Among the 722 postmenopausal women not currently using HT included in this cross-sectional study, we found positive, but weak, associations between both plasma SHBG and estrone levels and mammographic densities. Women with SHBG concentrations in the upper quartile had a 2.5% (absolute difference) higher mean percent mammographic density compared with women with SHBG in the lower quartile (P=0.007). The corresponding numbers for concentrations of estrone was 1.5% (absolute difference) (P=0.06). These associations were similar when absolute mammographic density was used as the outcome variable. When the analyses were stratified according to median IGF-I concentration, the weak association between estrone and mammographic density was strengthened among women with IGF-I levels below median, while the association disappeared among women with IGF-I levels above median (P for interaction=0.02).

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5. DISCUSSION

5.1 Methodological considerations

5.1.1 Study design

The findings in the papers of this thesis (Papers I-IV) are based on data from a cross-sectional study. Cross-sectional studies provide information on exposure and outcome without information on the temporal relationship between them, since the information on both are collected at the same time. Also, cross-sectional studies are based on prevalence and not incidence of the outcome variable. Thus, cross-sectional studies can not establish or refute a causal relationship, but can suggest presence or absence of an association between exposure and outcome (50).

Most data in the TMBC study were collected from an interview at study entry (Appendix 2) and from self-administered questionnaires (Appendices 4, 5). The TMBC study questionnaires have not been validated. The decision to extend the questionnaire from four pages in 2001 to eight pages in 2002 was based on the benefit of gathering more extensive dietary information. Also, a previous study from the Norwegian Women and Cancer Study showed that the length of a questionnaire did not affect the distribution of risk-factors registered as long as the response rate was good (51). In our study, the response rate to the questionnaire was >90% regardless of the length of the questionnaire.

For epidemiological studies it is important to ensure both internal validity (i.e. that the effect on the outcome variable is due to variation in the exposure variable) and external validity (i.e. that the study sample is representative of the population to which the results will be extrapolated). As there are no absolute criteria for assessing validity of an association (52), it

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is necessary to evaluate if the observed associations are affected by errors (bias) (50), and assess the findings in the context of existing data and biologic plausibility. There are three broad categories of systemic errors; selection bias, information bias, and confounding (53), which may all cause incorrect estimates.

5.1.2 Selection bias

How one recruits study participants, and factors that influence study participation, may lead to selection bias. If selection bias occurs, the associations found in the study population may be different to that in the general population due to different variations of the exposure and outcome variables in the two populations.

When studies recruit from population based screening programs with high attendance rates, as with the TMBC study, a large number of participants can be reached, and the studies ought to be less prone to selection bias. During the recruitment periods of the TMBC study, the attendance rate to the NBCSP among women eligible to our study was high (80%) (46). Further, altogether 70% of the study eligible women attending the NBCSP during the recruitment periods also participated in the TMBC study.

We do not know the associations between the exposure variables and mammographic density among eligible women not attending the TMBC study. However, when we compared the distribution of variables selected from the NBCSP questionnaire, we found that the women participating in the TMBC study did not differ from the eligible women attending the NBCSP in Tromsø according to the following ten selected factors: previous mammography, breast cancer in mother, age at menarche, age at first birth, parity, ever oral contraceptive use, ever HT use, age at menopause, current smoking, and ever consumed alcohol (46).

5.1.3 Information bias

Information bias can occur when measurement or classification of the exposure or outcome variable is invalid. Two main types of misclassification may affect the association between exposure and outcome; non-differential and differential misclassification (53). If the misclassification of either the exposure or outcome variable is independent of the other, the misclassification is non-differential.

With non-differential misclassification all participants in the study have the same chance of being misclassified, and this usually leads to an underestimation of the association, i.e. bias the results toward the null association. When the exposure variable has more than two categories, the non-differential misclassification can lead to an over- or underestimation of the association, depending on which category the participants have been misclassified to (50).

Differential misclassification occurs when either the misclassification of the exposure differs by the outcome status or the misclassification of the outcome differs by the exposure status. Differential misclassification can lead to either an over- or underestimation of the association (50). None of the women participating in our study knew their mammographic density (outcome variable). Thus, how they responded at the interview or in the questionnaire could not have been influenced by their outcome status.

5.1.3.1 Exposure variables

5.1.3.1.1 Postmenopausal hormone therapy use

The information on HT use we utilized for the analyses in Paper I was gathered at the study entry interview. Thus, the registration of HT use among the women in our study was finalized just before the publication of the results from the Women's Health Initiative trial in July 2002

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showing an increased risk of breast cancer with HT use (54). According to information from the Department of Pharmacoepidemiology at the Norwegian Institute of Public Health, the sale of systemic estrogen containing HT has dropped 45% since 2002 (Figure 8) (55).
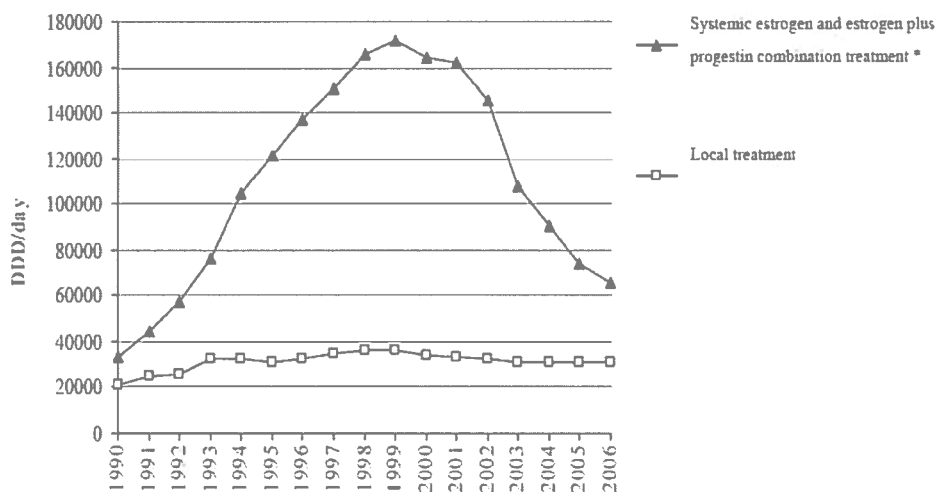
Figure 8. Sales of products with estrogen (ATC group G03C and G03F) in Norway 1990-2006.
DDD = defined daily dose *ATC groups G03CA01+03+04 and G03F.

The prevalence of ever HT use (43%) among TMBC study participants reported in Paper I is similar to the prevalence estimates registered from the NBCSP questionnaires (43.8%) (46). Some misclassification of HT use in our study is to be expected. Recall bias will make it more likely for past HT use to be misclassified than current HT use. The misclassification would presumably be non-differential, and would most likely bias the results toward the null association.

5.1.3.1.2 Cigarette smoking

The information on cigarette smoking used for the analyses in Paper II was collected both from the interview at study entry (current smoking habits) and from the questionnaire

(lifetime smoking history). Smoking prevalence in Norway differs by age groups (Figure 9), as well as by county. The percentage of women in Troms county that are current daily smokers is higher than the national average (Source: Statistics Norway). Although self-reports on smoking usually are accurate (56, 57), some misclassification of smoking status in our study is likely. Recall bias makes it more plausible for past smoking history to be misclassified than current smoking habits. The prevalence estimates for current smoking among the TMBC study participants reported in paper II was 34% and is similar to the prevalence estimates registered at the NBCSP (33.7%) (46). Misclassification of smoking would presumably be non-differential, and thus likely bias our results toward the null association.
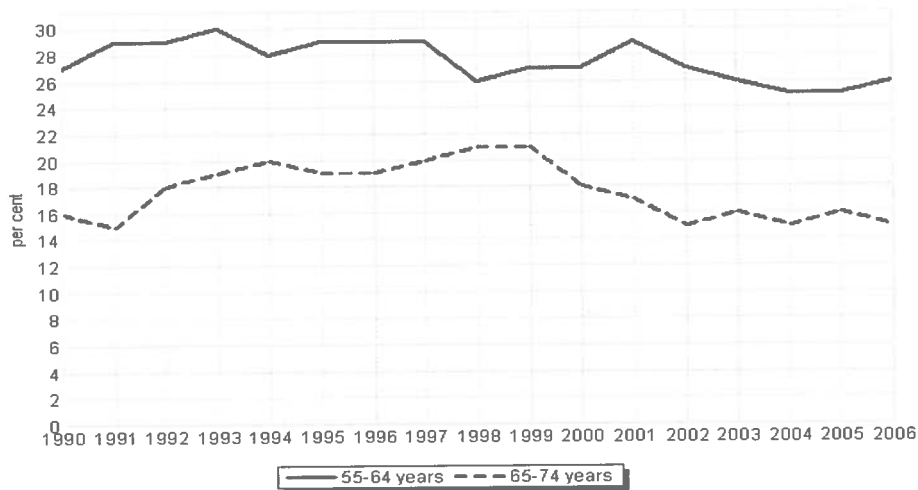
Figure 9. Percentage of Norwegian women aged 55-74 years who are current daily or occasional smokers, by age and time.

Source: Statistics Norway.

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5.1.3.1.3 **Insulin-like growth factors**

To limit errors in measurements of IGF-I and IGFBP-3, the analyses were performed at IARC in a laboratory specialized on hormone analyses. At the time of the IGF analyses, the plasma samples had been stored at -70°C to -80°C for 2-3 years. The intra- and inter-batch coefficients of variation for the quality controls in the IGF-I and IGFBP-3 assays were acceptable (Paper III) (58). Measurements of IGF-I can be erroneous because binding proteins may sequester IGF-I from the assay antibodies. Therefore, in our study the IGF-I assays included an acidification of the plasma samples, followed by an ethanol precipitation, to extract IGF-I from its binding proteins.

We only have IGF measurement from a single blood sample per woman. Both IGF-I and IGFBP-3 concentrations are known to decrease with increasing age. However, previous studies have reported that a single sample estimates average IGF levels fairly well over a period of at least one year (59-61). Also, an imprecision in IGF levels would presumably be non-differential, and would be expected to bias our results toward the null association.

5.1.3.1.4 **Endogenous sex hormones**

To limit errors in measurements of sex-hormone levels, these analyses were performed at IARC. The assays used had previously been validated for use in epidemiological studies comprising postmenopausal women (62). It is important to ensure valid and reproducible hormone measurements within the concentrations of normal physiological postmenopausal range. At the time of the sex hormone analyses, the plasma samples had been stored at -70°C to -80°C for 3-4 years. The samples had also been thawed once for the IGF analyses, and then refrozen. However, steroid hormones are stable during several thawing/freezing cycles (Sabina Rinaldi, personal communication, 2007).

The inter-batch coefficients of variation for the quality controls in the sex hormone assays were acceptable (58), but suggestive of batch differences (Paper IV). However, using batch-specific cut points for the sex hormone categories did not materially change the results of the analyses using the overall hormone quartiles (Paper IV).

As for the IGF analyses, we have only single blood sample sex hormone measurements. It has previously been indicated that one measurement among postmenopausal women is representative for long term levels of estrogens and SHBG, but not so much for androgen and prolactin levels (63). An imprecision in sex hormone levels would presumably be non-differential, and would therefore be expected to bias the results in our study toward the null association.

5.1.3.2 Outcome variable - mammographic density

In all papers of this thesis (Papers I-IV), mammographic densities have been used as the outcome variable. By utilizing mammographic densities measured on a continuous scale, we believe we have been able to detect small effects that may not have been possible with mammographic density as a categorized or dichotomized outcome variable.

The reader of the mammograms in our study was experienced and blinded to characteristics of the women. Determining mammographic density is partly based on a subjective component, but we have previously shown a high intra-rater agreement for the reader in our study (Pearson correlation coefficient=0.86) (31). Further, when the mammograms in our study was read by another reader using the similar, but different, Cumulus computer-assisted mammographic density method, there was a good inter-reader agreement between the external reader and the reader in our study (Pearson correlation coefficient=0.86) (31).

The use of mammographic density measurements from two-dimensional mammograms can be affected by variations in breast positioning and compression (64). The degree of breast compression may vary according to the radiographer, as well as to the woman's discomfort and pain. One could speculate that current HT users might express more discomfort and pain during the mammogram than non HT users because of more glandular tissue, and that this would lead to less breast compression among current HT users. However, adequate breast compression is usually achieved regardless of HT use status (Kirsten Jensen, personal communication, 2007).

We chose to assess both percent and absolute mammographic density as outcome variables in our study (Papers I-IV). Obesity is associated with an increased risk of breast cancer among postmenopausal women, but, as described earlier, obesity is inversely associated with mammographic density (65). Postmenopausal BMI has been found to be strongly and positively correlated with the area of the non-dense region of the breast and the total breast area, thus inversely correlated with percent mammographic density, while a weak inverse correlation has been seen with absolute mammographic density. Therefore, we assessed both relative and absolute measures of mammographic density as outcome variables, since they may be influenced differently by residual confounding by adiposity (66).

Endogenously mammographic density is an independent risk factor for breast cancer, and believed to be on the causal pathway between exposure and breast cancer (17, 67, 68). Mammographic densities are used as surrogate endpoints in research to increase the knowledge of the etiology of breast cancer (49, 69-74). The benefits of using a surrogate endpoint are that studies can be smaller, shorter, and less expensive than studies with cancer as endpoint (75). Studies using a surrogate endpoint can thus propose answers to research

questions within a shorter timeframe (76). The validity of mammographic density as a surrogate endpoint for breast cancer among women using HT is a topic of much debate (25, 32, 36, 77-79), and this will be addressed in ongoing cohort studies, where one can assess whether the breast cancer risk associated with HT use is mediated through a change in mammographic density (78, 79).

5.1.4 Confounding

Rothman describes confounding as a confusion or "mixing of effects" (53), meaning that the effect on the outcome of the exposure variable under investigation is mixed together with the effect of another variable. The confounding variable must be associated with the outcome variable, but the distribution of the confounder also has to differ across the categories of the exposure variable, i.e. be associated with the exposure variable. Further, the confounder must not be an effect of the exposure, i.e. it can not be an intermediate step between exposure and outcome (80).

By using a general linear model that incorporates more than one independent variable at the same time, one can minimize confounding. When several independent variables are fitted into the model at the same time, the effect each of the independent variables has on the dependent variable is not confounded by the other independent variables. How we identified confounding variables is described in more detail above (Chapter 3.6), as well as in the individual papers (Papers I-IV). We can not rule out unknown confounders or some residual confounding in our results.

5.1.5 Effect-measure modification

Rothman describes effect-measure modification as a situation where "a measure of effect changes over values of some other variable" (80). In all papers (Papers I-IV), the overall analyses were also conducted stratified by the confounding variables. We observed a possible effect-measure modification by IGF-I levels on the association between estrone and percent mammographic density (Paper IV).

5.2 Discussion of results

The research described in this thesis (Papers I-IV) has contributed to more insight into mechanisms that may contribute to the etiology of breast cancer. We have added to the insight into the association between HT use and mammographic density (Paper I), as well as the association between cigarette smoking and mammographic density (Paper II). We are the first to suggest an association between IGF-I and mammographic density among postmenopausal women (Paper III). Also, the possible effect-measure modification by IGF-I on the association between estrone and mammographic density is new (Paper IV).

5.2.1 Postmenopausal hormone therapy and mammographic density

In Paper I we show that current use of HT was associated with a higher mammographic density compared with never HT use, and that women with prolonged current use of a continuous EPT had the highest mean mammographic density. Our findings are in overall agreement with the previous studies on the subject (Paper I). Recent results from two longitudinal studies, with long intervals between mammographic density measures and fairly short HT use duration, have indicated that the use of HT, and especially the use of an EPT, diminish the decrease in mammographic density seen with increasing age (23, 81). Based on the hypothesis by Boyd and colleagues (22), a delay in the age related decrease in

mammographic density due to HT use would increase the cumulative exposure to mammographic density and thus suggest a higher risk of breast cancer compared with those not using HT. Whether an increase, or slowing of the age related decrease, in mammographic density resulting from EPT use is an index of increased breast cancer risk remains unknown. Results from ongoing cohort studies will shed more light on this topic (78).

In the NBCSP population, interval cancers are more frequently diagnosed among HT users than among nonusers. One of the reasons for this is believed to be masking of tumors due to the higher mammographic density caused by HT use (82, 83). Gathering information in the NBCSP on type of HT used, as well as longitudinal mammographic density measurements, will give more insight into the HT and breast cancer association, as well as the validity of mammographic density as a surrogate marker for breast cancer risk.

5.2.2 Cigarette smoking and mammographic density

In Paper II we show that cigarette smoking was inversely associated with mammographic density. Further, an inverse dose-response relationship was seen among current smokers and mammographic density for amount smoked. Our results are consistent with an antiestrogenic effect of smoking (Paper II). An antiestrogenic effect of smoking is also believed to contribute to the earlier age at menopause among smokers when compared with non-smokers (84). In a recent study among postmenopausal women from the Women's Health Initiative trial, no association was observed between cigarette smoking and proliferative epithelial disorders, either with or without atypia, in the breast (85). Thus, it seems that if there is a carcinogenic effect from smoking on the breast tissue, it is not mediated through increased mammographic density.

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5.2.3 Insulin-like growth factor-I and mammographic density

In Paper III we show that plasma IGF-I concentration was positively associated with mammographic density among postmenopausal women not currently using HT, and that the women with IGF-I concentrations in the highest quartile had the highest mean mammographic density. Our findings are not in agreement with the previous studies among postmenopausal women, all reporting no association between IGF-I concentration and mammographic density (Paper III). In a recent case-control study nested within the Melbourne Collaborative Cohort (mean follow up 9 years), both baseline plasma IGF-I and IGFBP-3 concentrations were positively associated with risk of breast cancer after the age of 60 years (86). In a recent study from the Dutch Prospect - European Prospective Investigation into Cancer cohort, mammographic density and IGF-I levels were measured among 684 premenopausal women. The mammographic density measurements were repeated on average 5.5 years later as the women had become postmenopausal. In this Dutch study, premenopausal IGF-I levels were not associated with premenopausal mammographic density, but were positively associated with postmenopausal percent mammographic density (87). Although more studies are needed, these findings support our assumption that mammographic density may be used as a surrogate endpoint in studies on the IGF-I - breast cancer association among postmenopausal women.

5.2.4 Endogenous sex hormones and mammographic density

In Paper IV we show that there was a positive but weak association between SHBG levels and mammographic density, and a positive but weak association between estrone levels and mammographic density. The latter association was possibly effect-measure modified by IGF-I levels. Our findings in regards to SHBG were in support of most previous studies, while the findings in regards to estrone were not (Paper IV). However, the dominant estrogen after menopause is estrone. This is due to the aromatization of androstenedione to estrone in

adipose tissue (88). Thus, even though estrone has less potency than estradiol, estrone may be the hormone that quantitatively exerts the most estrogen-related activity in regards to mammographic density among postmenopausal women.

We expected to find a synergism between estrogens and IGF-I levels in regards to mammographic density, and we have no explanation for the possible effect-measure modification by IGF-I levels.

6. CONCLUSIONS AND IMPLICATIONS FOR FURTHER RESEARCH

In this thesis we have studied mammographic density as a surrogate marker, to suggest how different exposure factors may be associated with breast cancer. Some of the associations we have presented have been associated with mammographic density in a similar way as to that with breast cancer, i.e. the associations with HT use (Paper I) and IGF-I (Paper III). However, we have also presented associations that suggest that there are alternate pathways between breast cancer risk factors and breast cancer that do not involve mammographic density (Papers II and IV).

In future research we will assess if other exposure factors are associated with mammographic density in this study population. Examples of such factors are alcohol consumption, diet, and physical activity. The TMBC study is also a part of an international collaboration studying mammographic density among women from four geographically different populations (Gifu, Japan, Arizona and Hawaii, USA, and Tromsø) with different risks of breast cancer (89). We have also been invited to participate in the planning of an international consortium on mammographic density. One of the purposes of such a consortium would be to pool the data from different studies.

Since women between 50 and 70 years of age are advised to attend the NBCSP biannually, longitudinal mammographic density measurements can be obtained without additional x-ray exposure to the women, and at a very low additional cost. Thus, the NBCSP is an ideal setting for continued research on mammographic density. More studies are needed to determine if mammographic density is a valid surrogate marker for breast cancer risk.

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PAPER I

Different types of postmenopausal hormone therapy and mammographic density in Norwegian women

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Postmenopausal hormone therapy (HT) is associated with increased risk of breast cancer. The HTs used in Scandinavia is associated with higher risk estimates than those used in most other countries. Mammographic density is one of the strongest risk factors for breast cancer, and possibly an intermediate marker for breast cancer. We decided to examine the relationship between use of different types of HT and mammographic density in Norwegian women. Altogether, 1,007 postmenopausal participants in the governmental mammographic screening program were asked about current and previous HT use. Mammograms were classified according to percent and absolute mammographic density. Overall, current users of HT had on average 3.6% higher mean percent mammographic density when compared with never users ($p < 0.001$). After adjustment for age at screening, number of children and BMI in a multivariate model, women using the continuous estradiol (E_2) plus norethisterone acetate (NETA) combination had a mean percent mammographic density significantly higher than never users (6.1% absolute difference). Those using the continuous E_2 plus NETA combination had an 4.8% (absolute difference) higher mean percent mammographic density after <5 years of use when compared with never users, while the corresponding number for ≥5 years of use was 7% (p-trend < 0.001). We found similar associations when absolute mammographic density was used as the outcome variable. In summary, our study shows a statistical significant positive dose–response association between current use of the continuous E_2 plus NETA combination and both measures of mammographic density.
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Key words: postmenopausal hormone replacement therapy; mammography; breast density

Current and recent use of postmenopausal combined estrogen and progestin therapy (EPT) have been shown to increase the risk of breast cancer, both in Randomized Controlled Trials and in observational studies.[1–3] The dose and type of the progestin constituent of EPT seems to influence risk of postmenopausal breast cancer more than the estrogen constituent.[3] The risk estimates for breast cancer with current EPT use found in recent Scandinavian cohort studies were higher than those found in both the Women's Health Initiative (WHI) Study and the Million Women Study (MWS).[1,2,4,5] This may be attributed to the more potent testosterone-derived norethisterone acetate (NETA) progestin used in EPT's in Scandinavia compared to the less potent medroxyprogesterone acetate (MPA) progestin used in most other countries.[3]

Mammographic density is one of the strongest independent risk factors for breast cancer, and possibly an intermediate marker for breast cancer.[6] Percent mammographic density has consistently been shown to be strongly associated with breast cancer risk in different populations,[6] as well as associated with several breast cancer risk factors.[7–10]

There is substantial evidence that hormones are associated with mammographic density, both from cross-sectional and clinical trials.[11–14] However, most of these studies have been from the US, where the predominant preparation until recently has been EPT with conjugated equine estrogen (CEE) and MPA.

In a study from the Norwegian Breast Cancer Screening Program (NBCSP) cohort, mammographic density was assessed among 728 women using a coarse 3-point scale mammographic density classification. Wang *et al.* found a significant relationship between ever-use of postmenopausal hormone therapy (HT) and mammographic density, but no information on the type of HT was available.[15]

On the basis of the differences in HT formulas described, more knowledge about how different types of HT effect mammographic density could increase the understanding of the etiology of breast cancer.

The objective with this paper was to examine the relationship between use of different types of HT and quantitative measures of mammographic density among postmenopausal women attending the NBCSP in Tromsø, Norway.

We especially wanted to explore how the EPTs used in Norway are related to mammographic density, since this is not previously examined.

Material and methods

Study population

The Mammography and Breast Cancer Study is a cross-sectional study among postmenopausal women residing in the municipality of Tromsø, Norway, aged 55–71 years, and attending the NBCSP at the University Hospital of North Norway. The women were recruited in spring of 2001 and 2002. After the women had undergone their screening mammograms, they were interviewed by a trained research nurse about their current and previous HT use. The nurse showed a color photo-leaflet of the altogether 19 HT preparations ever available on the Norwegian market. The different estrogen therapy (ET), EPT and Tibolone formulas were also listed with the available strengths of the preparations. The women were asked about reproductive and menstrual factors, previous history of cancer, smoking status and use of other medications. The participants had their height measured to the nearest centimeter and weight measured to the nearest half kilogram. The women had a blood sample drawn, and were subsequently given a questionnaire to be completed at home, eliciting information on demographics, additional menstrual and reproductive factors, as

TABLE I – CHARACTERISTICS OF THE STUDY POPULATION (N = 1,007), OVERALL AND STRATIFIED BY POSTMENOPAUSAL HORMONE THERAPY USE, GIVEN AS MEAN ($\pm$SD) OR PERCENTAGE. MAMMOGRAPHY AND BREAST CANCER STUDY, TROMSØ (2001/2002)

| | All (N = 1,007) | Postmenopausal hormone therapy use | | | p-value (past vs. never use) | p-value (current vs. never use) |
		Never used (n = 573)	Past use (n = 175)	Current use (n = 259)		
Mean						
Age at screening (y)	61.4 ($\pm$4.6)	62.3 ($\pm$4.7)	60.6 ($\pm$4.0)	60.0 ($\pm$4.2)	<0.001	<0.001
Age at menarche (y)	13.3 ($\pm$1.4)	13.4 ($\pm$1.4)	13.3 ($\pm$1.3)	13.1 ($\pm$1.3)	0.98	0.07
Age at first birth (y)	22.9 ($\pm$3.7)	23.0 ($\pm$3.8)	22.5 ($\pm$3.1)	22.7 ($\pm$3.6)	0.11	0.36
Number of children[1]	2.7 ($\pm$1.4)	2.8 ($\pm$1.5)	2.6 ($\pm$1.3)	2.5 ($\pm$1.2)	0.14	0.01
Education (y)	9.8 ($\pm$3.4)	9.4 ($\pm$3.3)	10.0 ($\pm$3.3)	10.4 ($\pm$3.7)	0.05	<0.001
Age at menopause (y)	48.5 ($\pm$5.1)	48.5 ($\pm$4.9)	49.1 ($\pm$5.4)	48.3 ($\pm$5.1)	0.16	0.62
BMI (kg/m^2)	27.4 ($\pm$4.8)	27.6 ($\pm$5.1)	27.5 ($\pm$4.3)	26.7 ($\pm$4.4)	0.88	0.01
Alcohol consumption[2] (g/day)	3.7 ($\pm$3.9)	3.5 ($\pm$4.0)	3.7 ($\pm$3.7)	4.2 ($+$3.8)	0.66	0.07
Frequency (%)						
Ever oral contraceptive use	51.1	46.8	60.0	54.8	<0.01	0.03
Parous	92.8	92.7	92.0	93.4	0.77	0.69
Daily smokers	27.7	26.9	30.3	27.8	0.66	0.64
Family history of breast cancer	18.2	17.6	18.3	19.3	0.84	0.56

[1]Among parous women only.–[2]Among alcohol drinkers only.

well as lifestyle and dietary factors. All women signed an informed consent. The National Data Inspection Board and the Regional Committee for Medical Research Ethics approved the study. Altogether, 1,041 women were included in the study. This accounted for 70.1% of the women attending the breast cancer screening program during the recruitment period.

We excluded 22 women because of a previously (n = 16) or newly (n = 6) diagnosed breast cancer, and 1 woman because of ongoing chemotherapy treatment. Among the remaining 1,018 women, we were unable to retrieve 11 mammograms. Thus, 1,007 women had mammograms classified according to percent and absolute mammographic density. More details are described elsewhere.[7]

Mammographic classifications

The left cranio-caudal mammogram was digitized using a Cobrascan CX-812 scanner (Radiographic Digital Imaging, Torrance, CA) at a resolution of 150 pixels per inch. Percent and absolute mammographic density were determined using the University of Southern California Madena computer-based threshold method; this method has been described and validated elsewhere.[16] Briefly, the method works as follows: The digitized mammographic image is viewed on a computer screen, and a reader defines the total breast area using a special outlining tool. Next, the region of interest (ROI), excluding the pectoralis muscle, prominent veins and fibrous strands, is defined. The reader then uses a tinting tool to apply a yellow tint to dense pixels with grey levels at or above some threshold X and a pixel value of $\leq$255. The reader searches for the best threshold where all pixels $\geq$X within the ROI are considered to represent mammographic densities. The software estimates the total number of pixels and the number of tinted pixels within the ROI. Absolute density represents the count of the tinted pixels within the ROI. Percent density, or the fraction (%) of the breast with densities, is the ratio of absolute density to the total breast area multiplied by 100.

The reader of the mammograms was blinded to the characteristics of the study participants.

Menopausal status

Women were classified as postmenopausal if they were 56 years or older, or reported having no natural menses during the last 12 months, or if the serum follicle-stimulating-hormone level was above 20 IU/l. According to these criteria, 3 of the 1,007 women were equivocal for menopausal status. Excluding these, 3 women did not alter the results, and they were included as postmenopausal.

Classification of HT use

Women indicating use of HT (oral or transdermal administration) at the time of enrollment were classified as current users. Ever users not indicating current use were classified as past users. The EPT regimens in Norway have all contained 1 of the 3 available estrogens: estradiol (E_2), estriol or etinylestradiol. The progestins most commonly used are the testosterone-derived norethisterone/norethisterone acetate (NETA) and levonorgestrel. The most common HT regime used in Norway is the E_2 and NETA combination. The synthetic steroid tibolone, with a weak estrogenic, progestogenic and androgenic effect, was introduced in Norway in the late 1999. All the HT preparations reported used by the women could be categorized into the following 4 groups: (i) estrogen monotherapy, (ii) continuous estrogen plus progestin combination, (iii) sequential estrogen and progestin combination and (iv) tibolone.

Statistical analysis

We used analysis of variance for unbalanced design to study the association between use of HT and mammographic density (Proc GLM, SAS). Percent and absolute mammographic density were log transformed to obtain an approximate normal distribution. The unadjusted and adjusted mean mammographic density results were back-transformed and are presented with 95% confidence intervals (CI). Trend tests across the categories of HT use were performed by treating the categories as continuous variables in the analyses.

Analyses were performed for HT use overall, by type of HT, and also separately for transdermal and oral administration.

Each of the following factors was evaluated as a potential confounder of the relation between HT use and mammographic density: age at screening (continuous), age at menarche (continuous), age at menopause (continuous), number of children (continuous), age at first birth (continuous), years of education (continuous), family history of breast cancer in first degree relatives (yes, no), smoking (daily, sometimes, no), alcohol intake (grams/day) and body mass index (BMI: weight in kilogram divided by height in meters squared) (continuous).

We performed univariate and multivariate analyses with models that included the above listed variables as independent variables and mammographic density as the dependent variable. Since all the above factors were presumed to be associated with HT use, we used the following criteria to include them in the model as a confounder: the factor had to either have been associated with the outcome variable in this study population previously,[7] or it changed the estimate by 10% or more when included in the multivariate

TABLE II – ADJUSTED[1] MEAN[2] (95% CONFIDENCE INTERVAL) PERCENT MAMMOGRAPHIC DENSITY ACCORDING TO POSTMENOPAUSAL HORMONE THERAPY USER STATUS AND DURATION OF USE AMONG 1,007 NORWEGIAN WOMEN, MAMMOGRAPHY AND BREAST CANCER STUDY, TROMSØ

	Percent mammographic density	*p*-value trend
	Adjusted[1] mean[2]	
PHT use		
Never used (*n* = 573)	7.2 (6.6–7.8)	
Past use ≥5 years ago (*n* = 45)	6.8 (5.1–9.1)	
Past use <5 years ago (*n* = 130)	9.1 (7.4–11.3)	
Current use for <5 years (*n* = 135)	10.3 (8.7–12.2)	
Current use for ≥5 years (*n* = 124)	11.5 (9.7–13.7)	<0.001

[1]Analyses are adjusted for age at screening, number of children and BMI.–[2]Reported mean is back-transformed from log-transformed estimated mean.

TABLE III – ADJUSTED[1] MEAN[2] (95% CONFIDENCE INTERVAL) PERCENT MAMMOGRAPHIC DENSITY BY TYPE OF CURRENT POSTMENOPAUSAL HORMONE THERAPY USED AMONG 832 NORWEGIAN WOMEN, MAMMOGRAPHY AND BREAST CANCER STUDY, TROMSØ

Type of current postmenopausal hormone therapy used	Percent mammographic density	
	Adjusted[1] mean[2]	*p*-value[1]
Never used (*n* = 573)	7.2 (6.6–7.8)	
Tibolone (*n* = 52)	8.8 (6.7–11.5)	0.17
Estrogen monotherapy (*n* = 70)	9.0 (7.2–11.4)	0.07
Sequential estrogen and progestin combination (*n* = 19)	10.2 (6.5–15.9)	0.14
Continuous estrogen plus progestin combination (*n* = 118)	13.3 (11.1–15.8)	<0.001
All estrogen plus progestin combinations (*n* = 137)	12.8 (10.8–15.1)	<0.001

[1]Analyses are adjusted for age at screening, number of children and BMI.–[2]Reported mean is back-transformed from log-transformed estimated mean.–[3]Current versus never use.

TABLE IV – ADJUSTED[1] MEAN[2] (95% CONFIDENCE INTERVAL) PERCENT AND ABSOLUTE MAMMOGRAPHIC DENSITY BY DURATION OF CURRENT CONTINUOUS ESTROGEN PLUS PROGESTIN COMBINATION USE AMONG 691 NORWEGIAN WOMEN, MAMMOGRAPHY AND BREAST CANCER STUDY, TROMSØ

Duration of current continuous estrogen plus progestin combination use	Percent mammographic density	*p*-value trend	Absolute mammographic density (cm²)	*p*-value trend
	Adjusted[1] mean[2]		Adjusted[1] mean[2]	
Never used (*n* = 573)	7.0 (6.5–7.6)		9.9 (9.0–10.8)	
<5 years (*n* = 52)	11.8 (9.0–15.5)		18.6 (13.4–23.4)	
≥5 years (*n* = 66)	14.0 (11.1–17.8)	<0.001	20.8 (15.8–27.2)	<0.001

[1]Analyses are adjusted for age at screening, number of children and BMI.–[2]Reported mean is back-transformed from log-transformed estimated mean.

model. This procedure left the following factors in the final model: age at screening, number of children and BMI.

Results were considered statistically significant if the two-sided *p*-value was <0.05. We performed data management and statistical analyses using the SAS statistical software package, version 9.1 (SAS Institute Inc., Cary, NC).

Results

Study population characteristics

Among the women, 26% were current and 43% ever HT users. Table 1 shows selected characteristics of the study population overall, and according to HT use. Current users of HT were younger, had fewer children, were more educated, had lower BMI and were more likely to be ever oral contraceptive users, than never users. Altogether, 228 of the 259 current users used an orally administered HT. The remaining 31 (12%) women used an ET administered transdermally.

HT use and mammographic density

Overall, current users of HT had a significant higher mean percent mammographic density (10.8%; 95% CI 9.6–12.2) when compared with never users (7.2%; 95% CI 6.6–7.8) after adjustment for age at screening, number of children and BMI (*p* < 0.001). Trend tests across never-, past- and current use of HT were significant (*p*-trend < 0.001). We found similar associations when absolute mammographic density was used as the outcome variable (data not shown).

The median duration of HT use was 72 months among ever and 48 months among current users. Women who had used HT for 1 year or more had a significantly higher mean percent mammographic density (10.8%; 95% CI, 9.5–12.3) when compared with never users (7.2%; 95% CI 6.6–7.8, *p* for comparison <0.001). When we stratified according to status and duration of HT use, a positive trend was shown for percent mammographic density

(Table II). Women with current use of HT for 5 years or more had the highest mean percent mammographic density. We also found a significant trend test for the different levels of HT use and absolute mammographic density (data not shown).

Type and duration of current HT use and mammographic density

The most commonly used HT among current users was a continuous EPT with E_2 plus NETA (46%), where 85% used 2 mg E_2 plus 1 mg NETA (Kliogest®) and the remaining 15% used the lower dose 1 mg E_2 plus 0.5 mg NETA (Activelle®).

Table III shows that when current HT use was stratified by type, users of the continuous EPT had a mean percent mammographic density that was 6.1% (absolute difference) higher when compared with never users (*p* < 0.001). This equals an 85% relative difference. Stratifying the women on ET according to the type of administration did not change the results (data not shown). Current use of tibolone gave an absolute difference in mean percent mammographic density of 1.6% when compared with never users (*p* = 0.17). Similar associations were found when we evaluated the relationship between the different types of current HT use with absolute mammographic density as the outcome variable (data not shown).

Table IV shows that mean percent mammographic density increased with longer duration of current continuous EPT use (*p*-trend < 0.001). Current users of continuous EPT had a 7.0% (absolute difference) higher mean percent mammographic density after >5 years of use when compared with never users (*p* < 0.001). The association was similar when we evaluated duration of continuous EPT with absolute mammographic density as the outcome variable (Table IV).

Discussion

Our study is, to our knowledge, the first to examine the relationship between use of different types of HT and quantitative mammographic density among Norwegian women. We find that cur-

rent users of systemic HT have a significant higher mean percent mammographic density when compared with never users. Furthermore, we find a dose–response relationship between HT user status and duration of use and mammographic density. When the association between the 4 types of HT and mammographic density was examined in detail, only the association with current use of continuous E_2 plus NETA EPT was statistically significantly different from that with never users. Also, our study finds a dose-response relationship between the duration of continuous E_2 plus NETA EPT and mammographic density. We found similar associations when absolute mammographic density was the outcome variable.

Strengths of our study are that it was a part of a population-based screening project with a high attendance rate, and that our study has a large sample size. The reader of the mammograms was experienced and blinded to the characteristics of the women. The limited number of HT preparations ever available in Norway, and the use of a photo-leaflet to aid in the recall of HT use, limits the misclassification of exposure. Even so, there will be some misclassification of HT use. However, this will most likely be nondifferential, and thus bias the results toward the null association.

One limitation with our study is that it is cross-sectional and we therefore do not have information on the temporal relationship between the HT use and mammographic density. Also, assessing mammographic density is partly based on a subjective component. We have previously shown that the reader of the mammograms had a good correlation (Pearson correlation coefficient 0.86) for a independent reread of percent mammographic density of 37 mammograms performed as long as 18 months after the first reading.[7]

In our study of postmenopausal women, the mean percent mammographic density is relatively low. However, it is similar to the baseline mean mammographic density found among non-Hispanic white in the WHI Study.[14]

Several studies, mostly from the US, have looked at the relationship between different types of postmenopausal HT and mammographic density.[11,13,14,17–19] The magnitudes of the differences in mammographic density in our study are comparable to those of other studies. In a subset of the Postmenopausal Estrogen/Progestin Interventions Trial, Greendale et al. found an increase in mean percent mammographic density of close to 5% in women treated with CEE plus MPA EPT for 12 months compared with baseline. This increase was significant, while the increase among ET users was not.[13] In the WHI Study, McTieman et al. found that the 202 women in the continuous CEE plus MPA EPT group had a 6% higher mean percent mammographic density after 12 months when compared with what they had at baseline. This absolute difference decreased to 5% after 24 months.[14] In a Swedish Randomized Controlled Trial comprising 154 postmenopausal women, Lundström et al. found a significant increase in qualitative percentage mammographic density (5 class scale) among 48 women taking continuous E_2 plus NETA EPT for 6 months when compared with 55 women in the placebo group ($p < 0.001$). In contrast, the 51 women treated with tibolone did not differ from those in the placebo group according to percentage mammographic density.[17] Lundström has previously shown that current use of EPT was more likely to give an increase in qualitative mammographic density than the use of other HTs. Furthermore, they found that among EPT users, the use of a continuous E_2 plus NETA EPT was more likely to give an increase than the use of a continuous CEE plus MPA EPT.[18,19]

The dose–response associations between duration of HT use with mammographic density in our study are in agreement with a review on hormones and mammographic breast density that concluded that the effect is more likely to follow prolonged HT use.[11] This was based on the finding in a case-control study nested in the European Prospective Investigation on Cancer in Norfolk, where it was shown that the odds of having high-risk mammographic patterns increased significantly with increasing duration of current HT use.[20] Also, in a Observational Cohort Study of 5,212 postmenopausal women it was shown that women who were current users at first mammogram and continued to use HT were more

likely to show an increase in mammographic density at the next mammographic screening when compared to nonusers.[21]

The higher mammographic density we find for current E_2 plus NETA EPT use compared to never users is in agreement with the more pronounced risk of breast cancer among HT users in the Norwegian when compared to the US population.[1,2,4,5,22] Although the differences in our study may seem small, the 7% absolute difference in mammographic density between never use and current EPT use for 5 or more years translate to a 100% relative difference.

Our results are also in support of the numerous studies having found that use of EPT is associated with a higher risk of breast cancer when compared with use of ET or tibolone.[2,23] In the MWS, those using the continuous CEE plus MPA EPT for 5 years or more had the highest risk estimates for breast cancer.[2] In the Norwegian Women and Cancer Study, comprising 31,451 postmenopausal women, Bakken et al. found that current use of E_2 plus NETA EPT conferred a higher relative risk for breast cancer than ET alone. However, this difference did not achieve statistical significance. Also, women who were current users of the continuous E_2 plus NETA EPT had a significantly higher risk of breast cancer than current users of the sequential E_2 plus NETA EPT. Women who had used the continuous E_2 plus NETA EPT for 5 years or more had the highest risk of breast cancer.[4] This increased risk of breast cancer with EPT use is supported by the findings from the Danish Nurse Cohort Study,[5] and from a Swedish Case-Control Study.[24] In an overview on EPT use and breast cancer risk, Lee et al. found a significantly higher risk of breast cancer with EPT use in European studies when compared to studies from the US, with Scandinavian studies finding the highest risk in Europe. The authors suggests that this might be due to the higher total dose of progestin used in sequential EPT regimens used in Europe, and also the use of the more potent NETA in Europe when compared with the progestins used in the US.[3]

Even though the estrogen constituent of the EPTs used in the US and in Europe also differs, CEE and E_2 are both considered to be of medium estrogen potency and have similar breast cancer risk estimates.[25] The hypothesis that the progestin EPT component confers most of the increased risk of breast cancer seen with EPT use is supported by studies on the proliferative effect of HT on postmenopausal breast tissue. HT use has been shown to increase proliferation and density of epithelial cells in the parenchyma of postmenopausal breast tissue,[26] and the use of EPT has a significantly greater proliferative effect when compared with the use of ET or tibolone.[26,27] In a study of benign breast biopsies from 86 postmenopausal women, the proliferative effect of progestins was localized to the terminal ductules and lobules in the breast, which is the site where most breast cancers origin.[28] An increase in the number and density of parenchymal epithelial cells in the human breast may be reflected in increased mammographic density.[29] Ursin et al. have shown that the change in percent mammographic density with EPT was primarily due to changes in the dense area of the breast, rather than a decrease in the nondense area.[30] This supports the hypothesis that epithelial cell proliferation related to EPT use is reflected in percent mammographic density changes.

The effects of the different HTs on mammographic density may give us more insight in the etiology of breast cancer.

In conclusion, our study shows a positive dose–response association between the use of the continuous E_2 plus NETA combination and percent mammographic density measured on a continuous scale. The associations are similar when absolute mammographic density is used as the outcome variable.

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PAPER II

Different measures of smoking exposure and mammographic density in postmenopausal Norwegian women: a cross-sectional study

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Running title: Smoking and mammographic density

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Abstract

Background Results from epidemiologic studies on the association between smoking and breast cancer risk have been conflicting. However, recent cohort studies have suggested an increased risk of breast cancer with long duration of smoking, and with smoking initiation before first birth. Thus, cigarette smoking may have both carcinogenic and antiestrogenic effects on the breast tissue. Mammographic density is one of the strongest independent risk factors for breast cancer, and possibly an intermediate marker for breast cancer. We examined the relationship between different measures of smoking exposure and mammographic density among 907 postmenopausal participants in the governmental Norwegian breast cancer screening program.

Methods Lifetime smoking history was collected through interview and questionnaires, and mammograms were classified according to percent and absolute mammographic densities using a previously validated computer-assisted method.

Results Sixty-five percent of the women reported having ever smoked cigarettes, while 34% were current smokers. After adjustment for age, age at first birth, parity, age at menopause, postmenopausal hormone therapy use, and body mass index, smoking was inversely associated with both measures of mammographic density (Both P trends <0.01). The magnitude of the difference in mean percent mammographic density between current and never smokers was 2.2% (absolute, 23% relative difference). When the analyses were restricted to current smokers only, an inverse dose-response relationship was found between both numbers of cigarettes smoked as well as pack-years smoked and mammographic density. Current smokers who smoked eleven or more cigarettes daily had a 3.2% (absolute, 34% relative difference) lower percent mammographic density compared with current smokers who smoked seven or less cigarettes daily (P value 0.009).

Conclusions We found a modest inverse dose-response association between current smoking and both percent and absolute mammographic densities. These findings are consistent with an antiestrogenic effect of cigarette smoking on the breast tissue. Our results also suggest that the possible carcinogenic effects of smoking on breast tissue are not mediated through increased mammographic density.

Introduction

Constituents in tobacco smoke may have carcinogenic effects on the breast tissue [1-3]. However, tobacco smoking may also have antiestrogenic effects that can reduce breast cancer risk [4;5]. These conflicting effects may explain the overall inconsistent results from epidemiologic studies on the association between smoking and breast cancer risk [2;6-9]. However, although most case-control studies do not find any positive associations [2;10], several recent cohort studies have indicated an increased breast cancer risk among women who are long term smokers [11-14], and also among those who start to smoke before their first birth [12-17].

Mammographic density is one of the strongest independent risk factors for breast cancer [18;19], and possibly an intermediate marker for breast cancer [20]. Women with high mammographic density have a 4- to 6-fold increase in breast cancer risk compared with those with low mammographic density [18;19].

So far, the published results on the association between smoking and mammographic density have also been conflicting [21-27], and most studies have used crude measures of smoking exposure.

The objective of this cross-sectional study was to examine the relationship between cigarette smoking and mammographic density among postmenopausal women with a high smoking prevalence, according to different measures of smoking exposure.

Materials and methods

Study population The Tromsø Mammography and Breast Cancer Study was conducted among postmenopausal women, aged 55 to 71 years, residing in the municipality of Tromsø, Norway, and attending the population-based Norwegian Breast Cancer Screening Program (NBCSP) at the University Hospital of North Norway [28]. Women were recruited in the spring of 2001 and 2002. After the women had undergone their screening mammograms, they were interviewed by a trained research nurse about reproductive and menstrual factors, previous history of cancer, current smoking status, and use of postmenopausal hormone therapy (HT) or other medications. The participants had their height measured to the nearest centimeter and weight measured to the nearest half kilogram. Women had blood samples drawn, and each was subsequently given a questionnaire to be completed at home, eliciting information on demographics, additional menstrual and reproductive factors, lifetime smoking history, as well as lifestyle and dietary factors. All women signed an informed consent. The National Data Inspection Board and the Regional Committee for Medical Research Ethics approved the study. Altogether, 1041 women were included in this cross-sectional study. This accounted for 70% of the women attending the NBCSP during the recruitment period.

We excluded 22 women because of a previously (n=16) or newly (n=6) diagnosed breast cancer, and one woman because of an ongoing chemotherapy treatment. Among the remaining 1018 women, we were unable to retrieve mammograms on 11 women. Thus, we obtained mammographic density readings on 1007 women. More details are described elsewhere [28]. We further excluded three women because they were equivocal for menopausal status, and 97 women because of missing smoking history, leaving 907 women for the analyses.

Mammographic classifications The women's left cranio-caudal mammogram was digitized using a Cobrascan CX-812 scanner (Radiographic Digital Imaging, Torrance, CA, USA) at a resolution of 150 pixels per inch. Percent and absolute mammographic densities were determined by an experienced reader (G.U.) using the University of Southern California Madena computer-based threshold method which has been described in detail and validated elsewhere [29]. Briefly, the method works as follows: The digitized mammographic image is viewed on a computer screen. A reader defines the total breast area using a special outlining tool. Next, the region of interest (ROI), excluding the pectoralis muscle, prominent veins, and fibrous strands, is defined. The computer software program assigns a pixel value of 0 to the darkest (black) shade in the image and a value of 255 to the lightest (white) shade with shades of grey assigned to intermediate values. The reader then uses a tinting tool to apply a yellow tint to dense pixels with grey levels at or above some threshold X and a pixel value of ≤ 255. The reader searches for the best threshold where all pixels $\geq X$ within the ROI are considered to represent mammographic densities. The software estimates the total number of pixels and the number of tinted pixels within the ROI. Absolute density represents the count of the tinted pixels within the ROI. Percent density, or the fraction (%) of the breast with densities, is the ratio of absolute density to the total breast area multiplied by 100. Absolute density measured in cm^2 was calculated as the number of tinted pixels within the ROI divided by the number of pixels per cm^2.

The reader of the mammograms was blinded to characteristics of the study participants.

Smoking assessments The women were interviewed about current smoking status. The self-administered questionnaire elicited additional information on lifetime smoking history. Women reporting to never having smoked or having been exposed to passive smoking were categorized as "never active smokers". We further categorized women who had never actively smoked but had been exposed to passive smoking at home or at the workplace as "passive

smokers". Never active and passive smokers were also grouped together as "never smokers". This group serves as the reference group in all analyses, if not specified otherwise. Current and former smokers were grouped together as "ever-smokers". Pack-years were calculated as the number of cigarettes smoked daily divided by 20 and multiplied by the number of years smoked.

We categorized current smokers according to age at smoking initiation (tertiles), average number of cigarettes smoked per day (tertiles), number of years smoked (≤25, 26-40, 41+), number of pack-years smoked (tertiles), and parous women according to smoking initiation before or after first birth.

Statistical analyses Mammographic density was not normally distributed. Both percent and absolute mammographic densities were log transformed to obtain approximate normal distributions. We used ANOVA for an unbalanced design to study the associations between cigarette smoking and mammographic densities (Proc GLM, SAS Institute Inc. Cary, NC). Each of the following factors was evaluated as a potential confounder of the association between smoking and mammographic density: age at screening (continuous), age at menarche (continuous), age at menopause (continuous), number of children (continuous), age at first birth (continuous), years of education (continuous), family history of breast cancer in first degree relatives (yes, no), alcohol intake (grams/day, continuous), HT use (never used, past use, current use), and body mass index (BMI, weight in kilogram divided by height in meters squared; continuous).

We identified the above listed variables that were associated with cigarette smoking in univariate analyses, and that also were significantly associated with mammographic density. We kept these variables in the multivariate model along with the variables that previously have been found to be associated with mammographic density in this study population

[28;30]. This procedure left the following factors in the final model: age at screening, age at first birth, number of children, age at menopause, HT use and BMI.

Trend tests across the categories of cigarette smoking exposure were performed by treating the categories as ordinal variables in the analyses. We tested for possible effect modification by analyzing the association between smoking and mammographic density stratified by the confounders, and by adding multiplicative interaction terms to the ANOVA procedure.

The crude and adjusted mean mammographic density results were back-transformed, and are presented with 95% confidence intervals (95% CI). Results were considered statistically significant if the two-sided P value was <0.05. We conducted all statistical analyses using SAS® 9.1 for Windows (SAS Institute Inc.).

Results

Altogether, 65% of the women were ever smokers. Among the 310 (34%) women who reported current smoking, 82% smoked daily. Among those reporting to be former smokers (n=279), more than 70% had stopped smoking ten or more years ago. Altogether 318 women reported to have never been smokers, among whom 73% reported ever exposure to passive smoking at home or at the workplace.

Table 1 shows the distribution of selected characteristics according to smoking status. Current smokers were younger at screening (P value <0.001), younger at time of first birth (P value <0.001), had less formal education (P value 0.02), reached menopause at an earlier age (P value <0.001), were leaner (P value <0.001), and were more likely to have ever used oral contraceptives (P value <0.001), when compared to never smokers. Current smokers also had lower crude mean mammographic density compared with never smokers, according to both percent and absolute mammographic density (P values 0.03 and 0.003, respectively). Former smokers also differed from never smokers, with values in-between current smokers and never smokers, in regards to age at first birth (P value 0.007) and ever oral contraceptive use (P value 0.004). Former smokers had significantly higher BMI (P value 0.04), and lower crude mean mammographic densities (both P values <0.001), when compared to never smokers. Passive smokers were more likely to have ever used HT (P value <0.05), but were otherwise similar to never smokers in the other listed characteristics (results not shown).

Table 2 shows the mean mammographic density across smoking status, adjusted for age at screening, age at first birth, number of children, HT use and BMI. Both current and former smokers had significantly lower adjusted mean percent mammographic density compared to never smokers (P values 0.003 and 0.006, respectively). The magnitude of the difference in

mean percent mammographic density between current and never smokers was 2.2% (absolute, 23% relative difference). A similar association was found between smoking status and absolute mammographic density (Table 2). These associations did not change materially when we excluded passive smokers from never smokers (results not shown), or when we excluded occasional smokers from current smokers (results not shown).

Current smokers smoked on average 10 cigarettes per day. More than half the current smokers had initiated smoking by the age of 20 years, and had been smoking for 39 years or more. Table 3 shows the association between different measures of smoking exposure and percent mammographic density among current smokers, overall, and restricted to women that had been smoking 25 years or more. There was a statistically significant inverse association between number of cigarettes smoked daily (never smokers, 0-7, 8-10, 11+), number of years smoked (never smokers, ≤25, 26-40, 41+), number of pack-years smoked (never smokers, 0-11, 12-20, 21+), age at smoking initiation (never smokers, 21+ years, 18-20 years, 13-17 years), smoking initiation before first birth (parous never smokers, no, yes), and percent mammographic density. These trends kept when analyses were restricted to long term smokers. When analyses were restricted to current smokers, the inverse dose response relationship between number of cigarettes smoked daily and percent mammographic density remained statistically significant (P trend 0.008), as did the trend when the analysis were further restricted to long term smokers (P trend 0.01). Current smokers who smoked eleven or more cigarettes daily had a 3.2% (absolute, 34% relative difference) lower mean percent mammographic density compared with current smokers who smoked seven or less cigarettes daily (P value 0.009). Furthermore, inverse dose-response relationships were found between number of pack-years and percent mammographic density among current smokers overall and among long term smokers (P trends 0.09 and 0.08, respectively) (Table 3). These associations

were similar when absolute mammographic density was used as the outcome variable (results not shown).

There was no interaction on the overall association between the three smoking status groups (current, former and never smokers) and percent mammographic density by age at screening (P interaction 0.63), age at first birth (P interaction 0.95), number of children (P interaction 0.57), age at menopause (P interaction 0.97), HT-use (P interaction 0.76), or by tertiles of BMI (P interaction 0.89) (Table 4).

Discussion

This population-based cross-sectional study found an inverse association between smoking and percent mammographic density among postmenopausal women, after adjustment for potential confounders. We also observed an inverse dose-response relationship among current smokers between both numbers of cigarettes smoked as well as pack-years smoked and percent mammographic density. We did not find any overall effect modification by age at screening, age at first birth, number of children, age at menopause, HT use, or tertiles of BMI. These associations were similar when absolute mammographic density was used as the outcome variable.

The strengths of our study are the large sample size and that it was a part of a population-based screening project with a high attendance rate [31]. The reader of the mammograms was experienced and blinded to the characteristics of the women. Further, we found similar associations between smoking and mammographic density when we performed the analyses using absolute mammographic density as the outcome variable. Also, we have a large proportion of current smokers (34%) among the women in our study compared to that among postmenopausal women in the previous studies on these associations (all less than 14% current smokers) [21-24].

One limitation of our study is the possible misclassification of smoking exposure. Any misclassification of smoking would presumably be non-differential with respect to mammographic density, and would therefore be expected to bias the results toward the null association. It further seems plausible that past smoking history would more likely be misclassified than current smoking habits, and it is therefore possible that this is why we found stronger associations for current exposure than for past smoking history. Another

limitation is that the mean mammographic density in our study is low. However, we have previously shown a high intra-rater agreement for the reader in our study (Pearson correlation coefficient=0.86) [28]. Also, in another study with a different reader, women from our study had significantly lower percent mammographic density compared with Caucasians from Hawaii and Arizona [32].

Our finding of an inverse association between smoking and mammographic density is in agreement with the finding of a recent study from the United States [21]. In this study, comprising 239 women aged 70 years or more, Modugno and colleagues found that current smokers had a significantly lower percent mammographic density compared with non-current smokers. This study only had information about current smoking habits and could therefore not analyze the association in more detail [21].

Our results are in contrast to that of three other studies that found no association between smoking and mammographic density among postmenopausal women [22-24]. In the British study comprising 406 women from the European Prospective Investigation on Cancer - Norfolk cohort, Sala and colleagues found no significant association between smoking and high-risk Wolfe's parenchymal patterns among the 313 postmenopausal women in their study, even though current smokers had a significantly reduced odds ratio of having high-risk Wolfe's parenchymal patterns compared to never smokers when the analyses also included premenopausal women [22]. In another American study, Gapstur and colleagues found no association between smoking and mammographic density among 191 postmenopausal Hispanic women [23]. Also, Vachon and colleagues found no association between smoking duration and intensity and percent mammographic density among 1554 postmenopausal women, but a inverse association was found among the 346 premenopausal women in their study [24]. These women were recruited from American breast cancer families [24].

Mammographic density is an independent risk factor for breast cancer, and lower mammographic density could suggest lower risk for breast cancer [18;19]. One of the suggested mechanisms for how smoking might increases the risk of breast cancer is carcinogenic effects of constituents in tobacco smoke on the breast tissue [1-3]. It is possible that this suggested mechanism does not affect mammographic density, and thus would not influence mammographic density as measured in our study.

Conversely, the other suggested effect of smoking is believed to be an antiestrogenic effect [4;5;33]. Smoking has been shown to enhance the metabolism of estradiol to metabolites believed to have minimal peripheral estrogen activity, to increase estrogen binding by sex-hormone binding globulin, and because smokers tend to be leaner than non-smokers, lower the amount of estrogens derived from adipose tissue [4;5]. Mammographic density is influenced by hormonal manipulation [34;35], and change in serum estrogen levels have been shown to influence mammographic density [36]. The findings in our study are consistent with an antiestrogenic effect of cigarette smoking on breast tissue, reflected in lower mammographic density among smokers.

However, we do not believe that our finding, that smokers have lower mean mammographic density when compared with never smokers, will be reflected in a lower risk of breast cancer among smokers. Our findings in regards to smoking and mammographic density may be similar to the association between BMI and mammographic density. Although an inverse relationship exists between BMI and percent mammographic density [28;37], BMI is positively associated with breast cancer risk among postmenopausal women [37].

Further, the magnitudes of the difference in mean percent mammographic density between current and never smokers were modest and may be due to residual confounding. We have previously shown, in this study population, that women using a continuous estrogen and progestin combination HT for five years or more had a significantly higher percent mammographic density compared to never HT users. The magnitude of that difference was substantially higher (7% absolute difference, 100% relative difference) than what we observed in the current study [30], suggesting that the effect of cigarette smoking may not be clinically relevant.

Conclusions

We found inverse associations between cigarette smoking and both percent and absolute mammographic density among the postmenopausal women in our cross-sectional study. We also observed a modest inverse dose-response association among current smokers between the amount smoked and mammographic density. These findings are consistent with an antiestrogenic effect of cigarette smoking on the breast tissue as measured by mammographic density. Our findings further suggest that the possible carcinogenic effects of smoking on breast tissue may act through mechanisms that do not involve mammographic density.

List of abbreviations

NBCSP= Norwegian Breast Cancer Screening Program; HT= postmenopausal hormone therapy; ROI= region of interest; BMI = body mass index; CI = confidence interval;

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

YB had full access to all data in the study, and takes responsibility for the integrity of the data and the accuracy of the data analyses.

ITG conceived of the study, and had full access to all data.

Classification of mammograms was performed by GU.

YB, GU, NB, and ITG performed the analysis and interpretation of data.

YB and ITG drafted the manuscript.

Critical revision of the manuscript for important intellectual content was performed by YB, ITG, GU, and NB.

All authors read and approved the final manuscript.

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Table 1

Characteristics of the study population (N=907) by smoking status, given as mean (±SD), frequency, and median (range).

	Smoking status			
Mean	Current smokers (n=310)	Former smokers (n=279)	Never smokers (n=318)	P value[1]
Age at screening, y	60.7 (4.4)	61.6 (4.5)	61.9 (4.6)	<0.001
Age at menarche[*], y	13.3 (1.4)	13.3 (1.4)	13.3 (1.4)	0.47
Age at first birth[*], y	22.3 (3.6)	22.7 (3.3)	23.5 (3.9)	<0.001
Number of children[*]	2.9 (1.2)	2.8 (1.1)	2.9 (1.4)	0.82
Education, y	9.4 (3.2)	9.7 (3.4)	10.2 (3.6)	0.02
Age at menopause, y	47.5 (5.5)	48.8 (4.8)	49.1 (4.8)	<0.001
BMI, kg/m²	26.1 (4.4)	28.4 (5.1)	27.6 (4.7)	<0.001
Alcohol consumption[§], g/day	3.8 (3.8)	4.0 (3.9)	3.4 (4.0)	0.36
Frequency (%)				
Ever Oral Contraceptive use	54.8	49.5	37.4	<0.001
Parous	93.6	93.6	90.3	0.13
Ever postmenopausal hormone therapy use	43.2	45.2	41.8	0.72
Breast Cancer in 1st degree relative	6.8	9.3	9.8	0.18
Median				
Percent mammographic density, %	9.4 (0-59.4)	7.7 (0-51.1)	11.9 (0-69.2)	0.03
Absolute mammographic density, cm²	13.7 (0-110.1)	11.9 (0-155.2)	17.1 (0-152.3)	0.003

§ Among alcohol drinkers only. * Among parous women only. [1] Differences between current and never smoking groups.

Table 2
Adjusted* mean§ (95% CI) percent and absolute mammographic density by smoking status.

Adjusted* mean§ (95% CI)	Current smokers	Former smokers	Never smokers	P trend
	n=310	n=279	n=318	
Percent mammographic density (%)	7.3 (6.5-8.3)	7.5 (6.6-8.5)	9.5 (8.4-10.7)	0.003
Absolute mammographic density (cm²)	10.4 (9.0-12.0)	10.6 (9.2-12.2)	13.7 (11.9-15.7)	0.005

*Analyses are adjusted for age at screening, age at first birth, number of children, age at menopause, postmenopausal hormone therapy use, and BMI.
§Reported means are back-transformed from log-transformed estimated means.

Table 3
Adjusted* mean§ (95% CI) percent mammographic density among current smokers according to smoking exposure, overall, and by long-term smokers.

	Adjusted* mean§ (95% CI) percent mammographic density (%)			
	Current smokers			
	All		**Smoked ≥25 years**	
Smoking exposure	n		n	
Never smokers	318	10.1 (8.9-11.5)	318	10.0 (8.8-11.4)
Number of cigarettes smoked per day				
0-7	87	9.4 (7.5-11.7)	65	9.7 (7.5-12.6)
8-10	79	8.4 (6.6-10.7)	62	8.7 (6.6-11.4)
11+	77	6.2 (4.9-7.8)	61	5.7 (4.4-7.4)
	P trend¹	*<0.001*	*P trend¹*	*<0.001*
	P trend²	*0.008*	*P trend²*	*0.01*
Never smokers	318	10.1 (8.9-11.5)		NA
Number of years smoked				
≤25	56	8.1 (6.2-10.7)		NA
26-40	92	8.4 (6.7-10.6)		NA
41+	95	7.4 (6.0-9.2)		NA
	P trend¹	*0.01*		
	P trend²	*0.57*		

Never smokers	318	10.1 (8.9-11.5)	318	9.9 (8.7-11.3)
Number of pack-years smoked				
0-11	82	9.3 (7.4-11.7)	54	9.8 (7.3-13.1)
12-20	85	7.9 (6.3-10.0)	68	7.9 (6.1-10.3)
21+	75	6.9 (5.4-8.7)	62	6.6 (5.0-8.5)
	P trend[1]	*0.002*	*P trend[1]*	*0.003*
	P trend[2]	*0.09*	*P trend[2]*	*0.08*
Never smokers	318	10.0 (8.8-11.3)	318	9.9 (8.7-11.3)
Age at smoking initiation (y)				
21+	94	8.4 (6.8-10.3)	53	8.4 (6.3-11.2)
18-20	114	8.0 (6.6-9.8)	75	8.0 (6.2-10.3)
13-17	78	7.0 (5.5-8.8)	54	7.1 (5.3-9.4)
	P trend[1]	*0.004*	*P trend[1]*	*0.02*
	P trend[2]	*0.26*	*P trend[2]*	*0.47*
Parous never smokers	287	9.9 (8.8-11.3)	287	9.9 (8.7-11.3)
Smoking initiation before first birth[3]				
No	79	8.8 (6.9-11.0)	43	10.0 (7.3-13.7)
Yes	186	7.5 (6.5-8.7)	125	7.2 (6.0-8.7)
	P trend[1]	*0.004*	*P trend[1]*	*0.007*
	P value[2]	*0.24*	*P value[2]*	*0.16*

*Analyses are adjusted for age at screening, age at first birth, number of children, age at menopause, postmenopausal hormone therapy use, and BMI.

[§]Reported means are back-transformed from log-transformed estimated means.

[1]Trend test between levels of smoking exposure including never smokers.

[2]Trend test/t-test between levels of current smoking excluding never smokers.

[3]Among parous women only.

Table 4
Adjusted* mean[§] (95% CI) percent mammographic density by smoking status, stratified by confounders.

	Adjusted* mean[§] (95% CI) percent mammographic density (%)		
	Current smokers	Former smokers	Never smokers
				P trend
Age at screening (y)				
	n=151	n=114	n=110	
<60	8.1 (6.8-9.7)	7.8 (6.4-9.6)	9.5 (7.7-11.7)	0.29
	n=90	n=88	n=111	
60-64	6.6 (5.3-8.2)	8.1 (6.6-10.1)	9.7 (7.9-11.9)	0.009
	n=69	n=77	n=97	
65+	7.3 (5.4-9.8)	6.3 (4.9-8.2)	9.2 (7.2-11.9)	0.11
Age at first birth (y)				
	n=100	n=72	n=66	
≤20	5.7 (4.6-7.1)	5.8 (4.5-7.5)	7.9 (6.1-10.4)	0.06
	n=98	n=97	n=95	
21-23	7.5 (6.1-9.2)	7.2 (5.9-8.8)	9.9 (8.1-12.1)	<0.05
	n=88	n=87	n=122	
24+	8.6 (6.8-10.9)	9.3 (7.4-11.7)	10.6 (8.7-13.0)	0.14
Number of children				
	n=20	n=18	n=31	
0	13.2 (8.7-19.9)	12.4 (8.3-18.7)	16.8 (11.7-24.1)	0.33
	n=112	n=112	n=132	
1-2	9.3 (7.7-11.4)	9.9 (8.2-12.0)	10.8 (9.0-12.9)	0.28
	n=109	n=94	n=86	
3	7.9 (6.5-9.6)	7.9 (6.4-9.7)	9.9 (8.0-12.4)	0.11

	n=69	n=55	n=69	
4+	4.0 (3.0-5.4)	4.0 (3.0-5.5)	6.7 (5.0-8.9)	0.009
Age at menopause (y)				
	n=121	n=84	n=83	
<48	7.0 (5.7-8.7)	7.0 (5.6-8.9)	11.1 (8.8-14.0)	0.005
	n=97	n=91	n=107	
48-50	6.7 (5.4-8.4)	7.0 (5.6-8.8)	8.0 (6.4-10.0)	0.23
	n=88	n=98	n=114	
51+	8.1 (6.4-10.2)	8.3 (6.7-10.2)	9.9 (8.1-12.2)	0.15
Postmenopausal hormone therapy (HT) use				
	n=76	n=73	n=88	
Current HT use	10.0 (7.9-12.8)	9.8 (7.7-12.6)	14.3 (11.4-18.0)	0.03
	n=58	n=53	n=45	
Past HT use	7.6 (5.7-10.1)	7.6 (5.7-10.1)	8.0 (5.9-11.0)	0.81
	n=176	n=153	n=185	
Never HT use	5.8 (5.0-6.8)	5.9 (5.0-6.9)	7.4 (6.3-8.7)	0.03
Tertiles of BMI (kg/m^2)				
	n=130	n=65	n=99	
13.1-24.9	12.6 (10.6-15.1)	11.7 (9.3-14.9)	15.7 (12.9-19.2)	0.11
	n=98	n=111	n=102	
25.0-28.8	7.5 (6.0-9.5)	8.2 (6.6-10.2)	9.6 (7.6-12.7)	0.14
	n=82	n=103	n=117	
>28.8	4.5 (3.5-5.8)	4.4 (3.5-5.4)	5.9 (4.8-7.4)	0.06

*Analyses are adjusted for age at screening, age at first birth, number of children, age at menopause, postmenopausal hormone therapy use, and BMI – where applicable.
§Reported means are back-transformed from log-transformed estimated means.

PAPER III

Insulin-like Growth Factor and Mammographic Density in Postmenopausal Norwegian Women

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Abstract

Insulin-like growth factor-I (IGF-I) is associated with breast cancer risk among premenopausal women but rarely among postmenopausal women. Recent data from two European studies suggested an increased risk of breast cancer with increasing levels of IGF-I among women >50 years old or among postmenopausal hormone therapy users ≥55 years old. Mammographic density is one of the strongest risk factors, and possibly an intermediate marker, for breast cancer. We examined the relationship between IGF and mammographic density among postmenopausal women overall and according to hormone therapy use. Altogether, 977 postmenopausal participants in the Norwegian governmental mammographic screening program had IGF concentrations measured by ELISA. Mammograms were classified according to percent and absolute mammographic densities using a previously validated computer-assisted method. After adjustment for age, number of children, age at menopause, body mass index, and hormone therapy use, both plasma IGF-I concentration (P_{trend} = 0.02) and IGF-I/IGF binding protein 3 ratio (P_{trend} = 0.02) were positively associated with percent mammographic density. The magnitudes of differences in percent mammographic density between women in the lowest and highest quartiles of IGF-I concentrations were 1.5% absolute difference and 21% relative difference. These associations were similar with absolute mammographic density as the outcome variable. When the analyses were stratified according to hormone therapy use, the associations between IGF-I and mammographic density were significant among noncurrent users (P_{trend} = 0.03). In conclusion, we found a positive but weak association between plasma IGF-I concentrations and both percent and absolute mammographic densities among postmenopausal women. These associations were found among noncurrent hormone therapy users but not among current users. (Cancer Epidemiol Biomarkers Prev 2007;16(1):57–62)

Introduction

Mammographic density is one of the strongest independent risk factors for breast cancer and possibly an intermediate marker for breast cancer (1). Women with high mammographic density have a 5- to 6-fold increase in breast cancer risk (2, 3).

Insulin-like growth factor I (IGF-I) has almost consistently been shown to be associated with breast cancer risk in young women (4-10), but more rarely so in older women (11-14). Meta-analyses of IGF-I and breast cancer association have shown that the effect differs by menopausal status rather than age (4-7, 10). However, the mechanism for this effect modification is not understood. Recently, a European study found an increased risk of breast cancer with increasing levels of IGF-I among women >50 years old (13). Another European study found an association of IGF-I with breast cancer among women ≥55 years old, especially among postmenopausal hormone therapy users (15).

Among premenopausal women, the association between IGF-I and mammographic density seems to mirror that between IGF-I and breast cancer (16-21). All studies of IGF-I and mammographic density association among postmenopausal women have thus far been restricted to noncurrent users of hormone therapy from North America (16, 17, 19, 20, 22) and United Kingdom (21). The only statistically significant finding among postmenopausal women thus far has been an inverse relationship between IGF-I/IGF binding protein 3 (IGFBP-3) ratio and percent mammographic density among 43 overweight former hormone therapy users (22).

The objective of this cross-sectional study was to examine the relationship between circulating concentrations of IGF-I and IGFBP-3, or IGF-I/IGFBP-3 molar ratio, and quantitative mammographic density among Norwegian postmenopausal women overall and according to hormone therapy use.

Materials and Methods

Study Population. The Mammography and Breast Cancer Study was conducted among postmenopausal women, ages 55 to 71 years, residing in the municipality of Tromso, Norway, and attending the population-based Norwegian Breast Cancer Screening Program at the University Hospital of North Norway. The women were recruited in the spring of 2001 and 2002. After the women had undergone their screening mammograms, they were interviewed by a trained research nurse about reproductive and menstrual factors, previous history of cancer, smoking status, and use of postmenopausal hormone therapy or other medications. The participants had their height measured to the nearest centimeter and their weight measured to the nearest half kilogram. The women had blood samples drawn and each was subsequently given a questionnaire to be completed at home, eliciting information

on demographics, additional menstrual and reproductive factors, as well as lifestyle and dietary factors. All women signed an informed consent. The National Data Inspection Board and the Regional Committee for Medical Research Ethics approved the study. Altogether, 1,041 women were included in the study. This accounted for 70.1% of the women attending the Norwegian Breast Cancer Screening Program during the recruitment period.

We excluded 22 women because of a previously ($n = 16$) or newly ($n = 6$) diagnosed breast cancer and one woman because of an ongoing chemotherapy treatment. Among the remaining 1,018 women, we were unable to retrieve mammograms on 11 women. Thus, 1,007 women had mammograms classified according to percent and absolute mammographic densities. More details are described elsewhere (23). We further excluded three women because they were equivocal for menopausal status and 17 women because they had not donated blood samples. In addition, 10 women were excluded due to missing IGF-I measurements, leaving 977 women for the analyses.

Mammographic Classifications. The women's left cranio-caudal mammogram was digitized using a Cobrascan CX-812 scanner (Radiographic Digital Imaging, Torrance, CA) at a resolution of 150 pixels/in. Percent and absolute mammographic densities were determined using the University of Southern California Madena computer-based threshold method, which has been described in detail and validated elsewhere (24). Briefly, the method works as follows: The digitized mammographic image is viewed on a computer screen. A reader defines the total breast area using a special outlining tool. Next, the region of interest, excluding the pectoralis muscle, prominent veins, and fibrous strands, is defined. The computer software program assigns a pixel value of 0 to the darkest (black) shade in the image and a value of 255 to the lightest (white) shade, with shades of gray assigned to intermediate values. The reader then uses a tinting tool to apply a yellow tint to dense pixels with gray levels at or above some threshold X and a pixel value of ≤ 255. The reader searches for the best threshold where all pixels $\geq X$ within the region of interest are considered to represent mammographic densities. The software estimates the total number of pixels and the number of tinted pixels within the region of interest. Absolute density represents the count of the tinted pixels within the region of interest. Percent density, or the fraction (%) of the breast with densities, is the ratio of absolute density to the total breast area multiplied by 100.

The reader of the mammograms was blinded to the characteristics of the study participants.

Peptide Assays. Nonfasting venous samples were obtained from study participants at the day of mammographic screening. After centrifugation, plasma samples were stored at −70°C.

IGF-I and IGFBP-3 were measured by ELISA from Diagnostic Systems Laboratories, Inc. (Webster, TX). The IGF-I assays included an acid-ethanol precipitation to extract IGF-I from its binding proteins. Measurements were done on previously never-thawed plasma samples.

All IGF-I and IGFBP-3 assays were done at the laboratory for hormone analyses (Nutrition and Cancer Group, IARC, Lyon, France), under supervision by one of the authors (S.R.). The mean intrabatch coefficients of variation were 5.1% for IGF-I and 6.1% for IGFBP-3. The interbatch coefficients of variation were 10.6% for IGF-I and 9% for IGFBP-3.

The IGF-I/IGFBP-3 molar ratio was calculated as a possible indicator of IGF-I bioavailability.

Statistical Analysis. We used ANOVA for an unbalanced design to study the association between plasma levels of IGF-I and IGFBP-3, or IGF-I/IGFBP-3 molar ratio, and mammographic density (Proc GLM, SAS Institute Inc., Cary, NC).

Percent and absolute mammographic densities were log-transformed to obtain an approximate normal distribution. The crude and adjusted mean mammographic density results were back-transformed and are presented with 95% confidence intervals (95% CI). Trend tests across the quartiles of IGF concentrations were done by treating the quartile categories (scored 1, 2, 3, and 4) as continuous variables in the analyses.

Each of the following factors was evaluated as a potential confounder of the relationship between IGF and mammographic density: age at screening (continuous), age at menarche (continuous), age at menopause (continuous), number of children (continuous), age at first birth (continuous), years of education (continuous), family history of breast cancer in first-degree relatives (yes, no), smoking (daily, sometimes, no), alcohol intake (g/d), hormone therapy use (ever, never, past, current; never and past hormone therapy users were also grouped as noncurrent users, whereas past and current hormone therapy users were grouped as ever users), and body mass index (BMI, weight in kilogram divided by height in meters squared; continuous).

We did univariate and multivariate analyses with models that included the above-listed variables as independent variables and mammographic density as the dependent variable. Because all the above factors were presumed to be associated with IGF concentrations, we used the following criteria to include them in the model as a confounder: The factor had to either have been previously associated with the outcome variable in this study population (23) or it changed the estimate by 10% or more when included in the multivariate model. This procedure left the following factors for inclusion in the final model as confounders: age at screening, number of children, age at menopause, BMI, and hormone therapy use.

We did stratified multivariate analyses to examine the association between IGF and mammographic density according to hormone therapy use.

Results were considered statistically significant if the two-sided P value was <0.05. We did data management and statistical analyses using the SAS statistical software package, version 9.1 (SAS Institute).

Results

Among the 977 postmenopausal women in the study population, the mean age was 61.4 years (SD, 4.6 years) and the mean age at menopause was 48.6 years (SD, 5.1 years). The median percent mammographic density was 9.6% (range, 0-69.2%), and the median mammographic absolute dense area was 14.8 cm^2 (range, 0-155.2 cm^2). Furthermore, mean plasma concentrations were 223.4 ng/mL (SD, 74.3 ng/mL) for IGF-I and 4,339 ng/mL (SD, 993 ng/mL) for IGFBP-3; the IGF-I/IGFBP-3 molar ratio was 0.19 (SD, 0.06). Among the women, 26% were current, 17% were past, and 57% were never hormone therapy users. Of the women, 18.4% reported a history of breast cancer in the family, whereas 9.2% reported a history of breast cancer in first-degree relatives.

Table 1 shows the crude relationship between selected variables and mean concentrations of IGF-I and IGFBP-3, or IGF-I/IGFBP-3 molar ratio, among the 977 women. The plasma concentration of IGF-I ($P_{trend} = 0.12$) and the IGF-I/IGFBP-3 molar ratio ($P_{trend} = 0.11$) decreased with increasing age, although not statistically significantly. The plasma concentration of IGF-I also decreased with increasing alcohol consumption ($P_{trend} < 0.03$). The IGF-I/IGFBP-3 ratio decreased significantly with increasing BMI ($P_{trend} < 0.002$). Plasma concentration of IGF-I and IGF-I/IGFBP-3 molar ratio showed a positive association with increasing age at menopause (both P_{trend} values <0.001). Plasma IGFBP-3 decreased ($P_{trend} < 0.002$), whereas IGF-I/IGFBP-3 ratio increased ($P_{trend} < 0.05$), with increasing frequency of smoking (Table 1). Plasma IGF-I

Table 1. Selected variables in relationship with mean plasma concentrations (SD) of IGF-I and IGFBP-3 and IGF-I/IGFBP3 molar ratio among postmenopausal Norwegian women (N = 977)

	n	IGF-I (ng/mL)	P_{trend}	n	IGFBP-3 (ng/mL)	P_{trend}	n	IGF-I/IGFBP-3 molar ratio	P_{trend}
Age (y)									
<60	403	226.7 (72.8)		403	4,340 (1,011)		403	0.20 (0.05)	
60-64	311	224.3 (81.0)		310	4,349 (996)		310	0.19 (0.06)	
65-71	263	217.1 (67.8)	0.12	262	4,324 (964)	0.86	262	0.19 (0.05)	0.11
No. children									
0	71	221.5 (74.7)		71	4,277 (870)		71	0.19 (0.06)	
1-2	389	223.7 (68.6)		388	4,372 (1,024)		388	0.19 (0.05)	
3	309	221.5 (83.0)		309	4,251 (969)		309	0.20 (0.06)	
≥4	208	226.1 (71.0)	0.73	207	4,428 (1,004)	0.57	207	0.19 (0.05)	0.99
BMI (kg/m^2), tertiles									
<25	323	216.8 (69.7)		323	4,141 (953)		323	0.20 (0.06)	
25-28	328	235.1 (82.0)		326	4,384 (996)		326	0.20 (0.06)	
≥29	326	218.1 (69.1)	0.83	326	4,489 (999)	<0.001	326	0.18 (0.05)	<0.002
Age at menopause* (y)									
<48	308	217.0 (68.0)		307	4,311 (971)		307	0.19 (0.05)	
48-50	317	214.3 (67.7)		316	4,306 (958)		316	0.19 (0.05)	
>50	327	238.5 (82.7)	<0.001	327	4,400 (1,043)	0.24	327	0.20 (0.06)	<0.001
Postmenopausal hormone therapy use									
Never	553	224.5 (69.1)		551	4,400 (958)		551	0.19 (0.05)	
Past	170	251.8 (84.5)		170	4,583 (892)		170	0.21 (0.06)	
Current	254	201.9 (71.4)	<0.003	254	4,043 (1,064)	<0.001	254	0.19 (0.06)	0.87
Smoking									
Nonsmokers	645	223.7 (74.9)		644	4,408 (988)		644	0.19 (0.06)	
Sometimes	62	223.8 (60.3)		61	4,269 (870)		61	0.20 (0.04)	
Daily smokers	270	222.3 (75.9)	0.80	270	4,188 (1,015)	<0.002	270	0.20 (0.06)	<0.05
Alcohol consumption [†]									
Teetotaler	123	236.5 (70.2)		123	4,664 (927)		123	0.19 (0.05)	
No consumption reported in the previous 12 mo	101	223.2 (78.8)		101	4,410 (1,009)		101	0.19 (0.05)	
<1.50 g/d	215	224.4 (86.1)		213	4,264 (960)		213	0.20 (0.07)	
1.50-3.79 g/d	219	220.1 (68.5)		219	4,285 (965)		219	0.19 (0.05)	
≥3.80 g/d	224	216.7 (63.3)	<0.03	224	4,233 (1,022)	<0.001	224	0.19 (0.05)	0.58

NOTE: IGFBP-3 concentration and IGF-I/IGFBP-3 molar ratio are missing for two women.
*Age at menopause is missing for 25 women.
[†] Alcohol consumption is missing for 95 women.

and IGFBP-3 concentrations were significantly lower among current users of hormone therapy compared with noncurrent users (both P values <0.001).

In multivariate analyses, both plasma IGF-I concentration (P_{trend} = 0.02) and IGF-I/IGFBP-3 ratio (P_{trend} = 0.02) were positively associated with percent mammographic density (Table 2). Women with IGF-I concentrations in the highest quartile had a higher percent mammographic density (1.5% absolute difference, 21% relative difference) compared with women with values in the lower quartiles. The corresponding differences were 1.4% absolute difference and 19% relative difference for the IGF-I/IGFBP-3 ratio. A similar association was found between IGF-I and absolute mammographic density (P_{trend} = 0.04), whereas the association with IGF-I/IGFBP-3 ratio weakened and was of borderline significance (P_{trend} = 0.06).

When we stratified the women according to current, noncurrent, and never hormone therapy use, we found that the association between IGF-I and percent or absolute mammographic density was statistically significant only among women not currently using hormone therapy (all P_{trend} values < 0.05; Table 3). The association between IGF-I/IGFBP-3 ratio and percent mammographic density was statistically significant among these women (P_{trend} = 0.03), whereas the corresponding association with absolute mammographic density was of borderline significance (P_{trend} = 0.05).

We also did analyses among current hormone therapy users stratified by the type of hormone therapy used; however, no associations between IGF-I and mammographic density were observed (data not shown).

We found similar associations between IGF-I and percent mammographic density overall and among noncurrent hor-

mone therapy users when BMI was adjusted in the multivariate model as a continuous and categorized variable (tertile or quintile) and when we excluded the 5% most extreme BMI values (2.5% highest and 2.5% lowest; data not shown). The associations with IGF-I/IGFBP-3 ratio weakened when BMI was modeled excluding the women with the most extreme BMI values (data not shown).

Discussion

This population-based cross-sectional study shows a positive association between mean plasma IGF-I concentration and percent mammographic density among women not currently taking hormone therapy after adjustment for potential confounders. This association was also present when absolute mammographic density was used as the outcome variable. For both outcome variables, the absolute difference in mammographic density between women with IGF-I concentrations in the upper and lower tertiles was small. However, the relative differences were not negligible. No associations were found between IGFBP-3 and the two measures of mammographic density.

The strengths of our study are the large sample size and the fact that it was a part of a population-based screening project with a high attendance rate (25). The reader of the mammograms was experienced and blinded to the characteristics of the women. Further, the IGF-I and IGFBP-3 analyses were done in a blinded manner at a laboratory specializing in hormone measurements. It was recently argued that the association between IGF and percent mammographic density is strongly confounded by adiposity (26) and that this is

Table 2. Adjusted mean (95% CI) percent and absolute mammographic densities by quartiles of IGF-I and IGFBP-3 concentrations and IGF-I/IGFBP-3 molar ratio among 977 postmenopausal Norwegian women

	Adjusted mean* (95% CI) percent mammographic density (%)	Adjusted mean* (95% CI) absolute mammographic density (cm²)
IGF-I (N = 977)		
Q1	7.50 (6.56-8.56)	10.71 (9.22-12.44)
Q2	8.09 (7.11-9.21)	11.58 (10.01-13.38)
Q3	8.94 (7.84-10.18)	12.80 (11.05-14.83)
Q4	9.07 (7.97-10.32)	13.08 (11.31-15.13)
P_{trend}	0.02	0.04
IGFBP-3 (n = 975)		
Q1	8.46 (7.42-9.65)	12.12 (10.45-14.06)
Q2	7.93 (6.96-9.02)	11.45 (9.89-13.25)
Q3	8.67 (7.60-9.89)	12.25 (10.56-14.21)
Q4	8.54 (7.50-9.73)	12.32 (10.64-14.26)
P_{trend}	0.69	0.73
IGF-I/IGFBP-3 molar ratio (n = 975)		
Q1	7.37 (6.47-8.41)	10.67 (9.21-12.38)
Q2	8.16 (7.17-9.29)	11.65 (10.06-13.48)
Q3	9.40 (8.25-10.72)	13.47 (11.62-15.61)
Q4	8.77 (7.72-9.96)	12.52 (10.84-14.45)
P_{trend}	0.02	0.06

NOTE: Analyses are adjusted for age at screening, number of children, age at menopause, BMI, and postmenopausal hormone therapy use (never, past, and current). IGFBP-3 concentration and IGF-I/IGFBP-3 ratio are missing for two women.
*Reported means are back-transformed from log-transformed estimated means.

only partially accounted for by adjustments for BMI, given that percent mammographic density is highly influenced by the size of the breast. Thus, another strength of our study is that we found similar associations for IGF-I and mammographic density relationship when we did the analyses using absolute mammographic density as the outcome variable.

Furthermore, the associations between IGF-I and percent mammographic density were similar when BMI was modeled as a categorized and continuous variable. We also found the expected nonlinear relationship between BMI and IGF-I previously shown in a large and several smaller studies (27-29).

One limitation is that our study was cross-sectional; therefore, we do not have information on the temporal relationship between the concentrations of IGF and mammographic density. Also, assessing mammographic density is partly based on a subjective component. However, the reader was blinded to all characteristics of the women, and we have previously shown that the reader of the mammograms had a good correlation (Pearson correlation coefficient = 0.86) for a second independent reading of percent mammographic density of 37 mammograms done for as long as 18 months after the first reading (23).

The six previous studies that had examined the relationship of IGF-I and percent mammographic density in postmenopausal women did not find any association between the two (16, 17, 19-22). These studies differed from our study as they all excluded women who were current hormone therapy users. Also, five of the six studies included <250 postmenopausal women (16, 17, 19, 21, 22) and would not have power to detect a small difference of the magnitude we found. The cross-sectional study by Diorio et al. (20) included 791 postmenopausal women who were not currently using hormone therapy from two mammography screening clinics. The study by Diorio et al. (20) is similar to our study in the following aspects: the mammographic density was assessed by a computer-assisted method (30), the mean BMI was similar, and the IGF analyses were measured by ELISA. Their study differs from ours in the aspect that almost 31% of the women in their study reported a history of breast cancer in first-degree relatives, whereas the corresponding number is 9% in our study. Otherwise, we have no explanation for why our results are discrepant from the results by Diorio et al. (20).

In our study, the IGF-I/IGFBP-3 molar ratio did not show any stronger associations with mammographic density than those associations between IGF-I and mammographic density. Thus, our findings support the recent opinion that the IGF-I/IGFBP-3 ratio is a poor surrogate for bioavailable IGF-I (5).

Table 3. Adjusted mean (95% CI) percent and absolute mammographic densities by quartiles of IGF-I and IGFBP-3 concentrations and IGF-I/IGFBP-3 molar ratio by postmenopausal hormone therapy use among 977 Norwegian women

	Adjusted mean* (95% CI) percent mammographic density (%)			Adjusted mean* (95% CI) absolute mammographic density (cm²)	
	Current hormone therapy use	Noncurrent hormone therapy use	Never hormone therapy use	Noncurrent hormone therapy use	Never hormone therapy use
IGF-I	n = 254	n = 723	n = 553	n = 723	n = 553
Q1	10.80 (8.84-13.20)	6.17 (5.24-7.27)	5.98 (5.00-7.15)	11.71 (9.13-15.01)	8.36 (6.83-10.22)
Q2	11.96 (9.34-15.31)	6.65 (5.76-7.69)	6.18 (5.23-7.30)	12.71 (9.95-16.25)	8.80 (7.29-10.63)
Q3	13.17 (10.07-17.23)	7.27 (6.31-8.38)	7.35 (6.35-8.64)	14.37 (11.32-18.25)	10.26 (8.55-12.31)
Q4	11.97 (8.91-16.08)	7.74 (6.73-8.89)	7.53 (6.34-8.95)	14.97 (11.80-18.98)	10.77 (8.87-13.09)
P_{trend}	0.38	0.03	0.03	0.03	0.04
IGFBP-3	n = 254	n = 721	n = 551	n = 721	n = 551
Q1	11.13 (9.05-13.69)	7.26 (6.20-8.51)	7.23 (6.06-8.64)	14.15 (11.07-18.09)	10.41 (8.52-12.71)
Q2	10.59 (8.38-13.37)	6.77 (5.83-7.87)	6.65 (5.59-7.91)	13.04 (10.18-16.70)	9.34 (7.68-11.36)
Q3	13.76 (10.25-18.46)	7.02 (6.10-8.06)	6.53 (5.57-7.66)	13.01 (10.21-16.58)	9.25 (7.73-11.07)
Q4	12.94 (9.88-16.94)	7.02 (6.08-8.12)	6.72 (5.62-8.02)	13.52 (10.65-17.17)	9.32 (7.63-11.39)
P_{trend}	0.23	0.88	0.55	0.75	0.46
IGF-I/IGFBP-3 molar ratio	n = 254	n = 721	n = 551	n = 721	n = 551
Q1	9.94 (7.95-12.44)	6.25 (5.36-7.28)	6.14 (5.17-7.30)	12.09 (9.45-15.47)	8.70 (7.16-10.57)
Q2	13.05 (10.05-16.94)	6.58 (5.70-7.59)	6.20 (5.25-7.33)	12.62 (9.93-16.03)	8.59 (7.13-10.36)
Q3	13.42 (10.57-17.05)	7.73 (6.67-8.96)	7.49 (6.34-8.85)	14.44 (11.31-18.44)	10.61 (8.79-12.80)
Q4	11.37 (8.78-14.72)	7.54 (6.53-8.70)	7.37 (6.17-8.80)	14.89 (11.71-18.92)	10.56 (8.65-12.90)
P_{trend}	0.34	0.03	0.06	0.05	0.07

NOTE. Analyses are adjusted for age at screening, number of children, age at menopause, and BMI. IGFBP-3 concentration and IGF-I/IGFBP-3 ratio are missing for two women.
*Reported means are back-transformed from log-transformed estimated means.

We have previously shown, in this study population, that women using a continuous estrogen plus progestin hormone therapy for ≥5 years had a 7% higher percent mammographic density compared with never hormone therapy users (31). This difference was also of a small absolute value. However, because the women in our study overall have a very low percent mammographic density, the relative difference is not negligible. The mean percent mammographic density in our study is similar to that found among non–Hispanic White postmenopausal women in the placebo group of the Women's Health Initiative study (32).

We found no association between plasma IGF-I levels and mammographic density among current hormone therapy users overall or according to type of hormone therapy used. This may be related to our finding that current users of hormone therapy had lower mean plasma IGF-I concentrations compared with noncurrent users. Previous studies have indicated that current use of p.o. hormone therapy may decrease plasma IGF-I concentration (33-37) and that this influence seem to differ according to the type of hormone therapy used (34-37). The mechanisms for the influence by hormone therapy use on plasma IGF-I levels are unclear. Another possible explanation for the lack of association between IGF-I and mammographic density among current hormone therapy users may be that the effect of IGF-I is masked by the fact that current hormone therapy users have more dense breasts (31).

In contrast to the findings in older women, IGF-I has been almost consistently shown to be associated with breast cancer risk in young women (4-10). In the recent and largest prospective study on IGF and breast cancer risk comprising 1,081 European breast cancer cases and 2,098 matched controls within the European Prospective Investigation into Cancer and Nutrition cohort, an increase in breast cancer risk was observed among women >50 years of age with increasing levels of IGF-I. This finding was attenuated to borderline significance when the analyses were restricted to women who were postmenopausal at the time of blood donation (13). In a recent meta-analysis including the studies described above, it was concluded that there was still no apparent association between IGF-I and breast cancer among postmenopausal women (5).

If, nonetheless, further prospective studies among postmenopausal women confirm that IGF-I is associated with breast cancer risk, our study indicates that mammographic density could be evaluated as an intermediate marker in studies affecting the IGF-I–breast cancer pathway.

In conclusion, we found a positive but weak association between plasma IGF-I concentration and both percent and absolute mammographic densities among postmenopausal Norwegian women. These associations were only significant among women who were currently not using hormone therapy.

Acknowledgments

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PAPER IV

Endogenous sex hormones, prolactin and mammographic density in postmenopausal Norwegian women

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The associations between endogenous sex hormone levels and breast cancer risk in postmenopausal women are well established. Mammographic density is a strong risk factor for breast cancer, and possibly an intermediate marker. However, the results from studies on the associations between endogenous sex hormones and mammographic density are conflicting. The authors examined the associations between circulating levels of sex hormones, sex hormone binding globulin (SHBG) and prolactin and mammographic densities among postmenopausal women not currently using postmenopausal hormone therapy (HT). The authors also examined if insulin-like growth factor-I (IGF-I) levels influenced the association between estrogen and mammographic density. Altogether, 722 postmenopausal participants in the Norwegian governmental mammographic screening program had endogenous hormone concentrations measured. Mammograms were classified according to percent and absolute mammographic density using a previously validated computer-assisted method. After adjustment for age, number of children, age at menopause, body mass index and HT use, both plasma concentrations of SHBG (p-trend = 0.003) and estrone (p-trend = 0.07) were positively associated with percent mammographic density. When the analyses were stratified according to median IGF-I concentration, the weak association between estrone and mammographic density was strengthened among women with IGF-I levels below median, while the association disappeared among women with over median IGF-I levels (p for interaction = 0.02). In summary, the authors found a positive association between plasma SHBG levels and mammographic densities among 722 postmenopausal Norwegian women not currently using HT. Further, the authors found a positive but weak association between plasma estrone concentration and mammographic density, which appeared to be modified by IGF-I levels.
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Several breast cancer risk factors (*e.g.*, age, age at menarche, parity, age at menopause) are believed to be related to the cumulative exposure of the breast tissue to endogenous hormonal substances.[1] Prospective studies have shown that circulating levels of sex hormones are associated with an increased risk of breast cancer in postmenopausal women not currently using postmenopausal hormone therapy (HT).[2–7] Prolactin levels have also been found to be positively associated with breast cancer risk among postmenopausal women,[8,9] while high sex hormone binding globulin (SHBG) levels have been associated with a decreased risk of breast cancer.[2,6]

Mammographic density is one of the strongest independent risk factors for breast cancer,[10–12] and possibly an intermediate marker for breast cancer.[13] Women with high mammographic density have a 4- to 6-fold increase in breast cancer risk compared with women with low mammographic density.[10–12]

The 6 studies published so far on the association between endogenous sex hormones and mammographic density among postmenopausal women have found conflicting results.[14–19]

A complex cross-talk is believed to exist between the metabolic/signaling pathways of estrogens and insulin-like growth factor (IGF) -I.[20–22] Among postmenopausal Chinese women, a study found possible synergistic effects between IGF-I and both estrogens and androgens in relation to breast cancer risk.[23] We have previously shown that IGF-I levels are positively associated with mammographic density among the postmenopausal women in this study.[24]

The objective of this cross-sectional study was to examine the associations between circulating plasma levels of sex hormones, SHBG and prolactin and mammographic densities among postmenopausal women not currently using HT. We also wanted to examine if IGF-I levels modified the association between estrogen and mammographic density, as has been suggested by laboratory studies.[20,21]

Material and methods

Study population

The Tromsø Mammography and Breast Cancer Study was conducted among postmenopausal women, aged 55–71 years, residing in the municipality of Tromsø, Norway, and attending the population-based Norwegian Breast Cancer Screening Program (NBCSP) at the University Hospital of North Norway.[25] Women were recruited in the spring of 2001 and 2002. After the women had undergone their screening mammograms, they were interviewed by a trained research nurse about reproductive and menstrual factors, previous history of cancer, smoking status and use of HT or other medications. The participants had their height measured to the nearest centimeter and weight measured to the nearest half kilogram. The women had blood samples drawn, and each was subsequently given a questionnaire to be completed at home, eliciting information on demographics, additional menstrual and reproductive factors, as well as lifestyle and dietary factors. All women signed an informed consent. The National Data Inspection Board and the Regional Committee for Medical Research Ethics approved the study. Altogether, 1,041 women were included in the study. This accounted for 70% of the women attending the NBCSP during the recruitment period.

We excluded 22 women because of previously ($n = 16$) or newly ($n = 6$) diagnosed breast cancer, and 1 woman because of ongoing chemotherapy treatment. Among the remaining 1,018 women, we were unable to retrieve the mammograms on 11 women. Thus, we obtained mammographic density readings on 1,007 women. More details are described elsewhere.[25] We further excluded 3 women because they were equivocal for menopausal status and 267 women because of current HT use ($n = 259$), or former HT use less than 3 months ($n = 8$) prior to inclusion in the study. In addition, 15 women were excluded because they had not donated blood samples, leaving 722 women for the analyses.

Mammographic classifications

The women's left craniocaudal mammogram was digitized using a Cobrascan CX-812 scanner (Radiographic Digital Imaging, Torrance, CA) at a resolution of 150 pixels per in. Percent and absolute mammographic densities were determined by an experienced reader (G.U.) using the University of Southern California Madena computer-based threshold method. This method has been described in detail and validated elsewhere.[26] Briefly, the method works as follows: The digitized mammographic image is viewed on a computer screen. A reader defines the total breast area using a special outlining tool. Next, the region of interest (ROI), excluding the pectoralis muscle, prominent veins and fibrous strands, is defined. The computer software program assigns a pixel value of 0 to the darkest (black) shade in the image and a value of 255 to the lightest (white) shade with shades of grey assigned to intermediate values. The reader then uses a tinting tool to apply a yellow tint to dense pixels with grey levels at or above some threshold X and a pixel value of ≤ 255. The reader searches for the best threshold where all pixels $\geq X$ within the ROI are considered to represent mammographic densities. The software estimates the total number of pixels, and the number of tinted pixels within the ROI. Absolute density represents the count of the tinted pixels within the ROI. Percent density, or the fraction (%) of the breast with densities, is the ratio of absolute density to the total breast area multiplied by 100. Absolute density measured in cm^2 is calculated as the number of tinted pixels within the ROI divided by the number of pixels per cm^2.

The reader of the mammograms was blinded to characteristics of the study participants.

Hormone assays

Venous samples were obtained from nonfasting participants at the day of mammographic screening. After centrifugation, plasma samples were stored at $-70°C$. All sex hormone, SHBG, prolactin and IGF-I assays were analyzed at a laboratory specialized on hormone analyses (Nutrition and Cancer group, IARC, Lyon, France), under the supervision of one of the authors (S.R.). Estradiol, estrone, androstenedione and prolactin were measured by direct double-antibody radioimmunoassays from Diagnostic Systems Laboratories (Webster, TX). Testosterone and dehydroepiandrosterone sulphate (DHEAS) were measured by direct radioimmunoassays from Immunotech (Marseille, France), and SHBG was measured by a direct "sandwich" immunoradiometric assay from Cis Bio (Gif sur Yvette, France). IGF-I was measured by enzyme-linked immunosorbent assays from Diagnostic Systems Laboratories. The IGF-I assays included an acid–ethanol precipitation to extract IGF-I from its binding proteins. Mean intrabatch and interbatch coefficients of variation were 5.4 and 15.9%, respectively, for estradiol, 5.1 and 14.7% for estrone, 4.4 and 6.8% for androstenedione, 6.0 and 7.6% for prolactin, 5.8 and 12.4% for testosterone, 6.3 and 11.4% for DHEAS, 4.2 and 11.9% for SHBG and 5.1 and 10.6% for IGF-I. The assays used for the sex hormone analyses have been validated previously.[27] Plasma concentrations of free-estradiol and free-testosterone—i.e., the fractions of hormones not linked to binding proteins in blood—were calculated from the absolute concentrations of the 2 steroids and SHBG using previously validated mass action equations.[28]

The value for estrone concentration was undetectable in 1 woman, while the values for testosterone and DHEAS were undetectable in 27 and 10 women, respectively. For analysis purposes, these women were assigned the value for the lower detection limit for the respective assays—i.e., 15 pg/mL for estrone, 0.09 ng/mL for testosterone and 10 µg/dL for DHEAS.

Statistical analyses

Mammographic densities were not normally distributed. Comparison of residual plots after square root or log transformations showed that log transformation obtained the most approximate normal distribution of mammographic densities. All hormone concentrations were also log transformed to improve the normality of the data. We used ANOVA for an unbalanced design to study the associations between plasma levels of endogenous hormones and both percent and absolute mammographic densities (Proc GLM, SAS Institute, Cary, NC). Correlations between sex hormones, SHBG, prolactin, IGF-I and body mass index (BMI) were tested using the Spearman rank correlation coefficient.

Each of the following factors was evaluated as a potential confounder of the association between the circulating hormones and mammographic densities: age at screening (continuous), age at menarche (continuous), age at menopause (continuous), number of children (continuous), age at first birth (continuous), years of education (continuous), family history of breast cancer in first degree relatives (yes, no), smoking (daily, sometimes, no), alcohol intake (grams/day), HT use (never, past use >3years ago, past use <3 years ago), plasma IGF-I concentration (continuous), assay batch (continuous) and BMI (weight in kilogram divided by height in meters squared; continuous).

We identified the aforementioned variables that were associated with levels of hormones in univariate analyses, and that also were significantly associated with mammographic density. We kept these variables in the multivariate model along with the variables that previously have been found to be associated with mammographic density in this study population.[24,25,29] This procedure left the following factors to be included in the final model: age at screening, number of children, age at menopause, BMI and HT use.

For the multivariate analyses the overall log-transformed plasma hormone levels were divided into quartile categories. Use of batch-specific cutpoints for the hormone categories did not materially alter the results. We tested for a possible effect modification by IGF-I on the association between estrogen and mammographic density by analyzing the latter associations stratified by median IGF-I levels and by adding a multiplicative interaction term to the ANOVA procedure.

The crude and adjusted mean mammographic density results were back-transformed, and are presented with 95% confidence intervals (95% CI). Reported trend test p-values correspond to analyses where the quartiles of hormone concentrations were treated as ordered variables (scored 1, 2, 3 and 4). Use of median values as category scores did not alter the p-values, and are not presented. Results were considered statistically significant if the 2-sided p-value was <0.05. We conducted all statistical analyses using SAS® 9.1 for Windows (SAS Institute).

Results

Study population characteristics

Table I shows selected characteristics, median mammographic densities and median plasma hormone concentrations among the 722 postmenopausal women in the study population. Mean age was 62 years and mean age at menopause was 48 years. Altogether, 77% of the women had never used HT, while the remaining women were former HT users with at least 3 months since last use. The median percent mammographic density was 8.0% (range: 0–61%) and the median mammographic absolute dense area was 12.2 cm^2 (range: 0–155.2 cm^2).

TABLE I – CHARACTERISTICS OF THE STUDY POPULATION (*N* = 722), GIVEN AS MEAN (±SD), FREQUENCY AND MEDIAN (RANGE) [THE TROMSØ MAMMOGRAPHY AND BREAST CANCER STUDY (2001/2002)]

	n	
Mean		
Age at screening, y	722	62.0 (4.6)
Age at menarche, y	650	13.4 (1.4)
Age at first birth[1], y	605	22.9 (3.7)
Number of children[1]	668	2.9 (1.3)
Education, y	567	9.5 (3.2)
Age at menopause, y	713	48.6 (5.1)
BMI, kg/m^2	722	27.6 (4.9)
Alcohol consumption[2], g/day	328	2.6 (3.3)
Frequency (%)		
Ever oral contraceptive use	722	49.5
Parous	722	92.5
Never postmenopausal hormone therapy use	722	77.6
Daily smokers	722	27.8
Breast cancer in 1st degree relative	722	8.0
Median		
Percent mammographic density, %	722	8.0 (0–61.0)
Absolute mammographic density, cm^2	722	12.2 (0–155.2)
Estrone, pg/mL	709	48.2 (15.0–143.6)
Estradiol, pg/mL	689	30.5 (9.7–144.7)
Free estradiol, pg/mL	687	0.7 (0.1–3.0)
Testosterone, ng/dL	703	36.1 (9.0–112.6)
Free testosterone, ng/dL	699	0.5 (0–1.9)
Androstenedione, ng/mL	714	1.0 (0.2–2.9)
DHEA sulfate, µg/dL	709	64.9 (10.0–448.2)
SHBG, nmol/L	711	51.8 (6.3–300.2)
Prolactin, ng/mL	712	9.1 (0.6–45.5)

[1]Among parous women only.–[2]Among alcohol drinkers only.

Spearman rank correlations

Table II shows the age-adjusted Spearman correlations between circulating levels of sex hormones, SHBG, prolactin, IGF-I and BMI. There were positive correlations between the circulating sex hormone levels and both estrogens and free-testosterone correlated positively with BMI. Furthermore, both free-estradiol and free-testosterone levels as well as DHEAS, were positively correlated with plasma IGF-I levels. Circulating levels of SHBG were negatively correlated with plasma levels of estrogens, free-testosterone, IGF-I as well as BMI. Further, plasma prolactin levels were negatively correlated with circulating levels of both estrogens and androgens, but correlated positively with IGF-I levels.

Endogenous hormones and mammographic density

Table III shows the associations between the sex hormones, SHBG and prolactin and the 2 measures of mammographic density. In the multivariate analyses, plasma SHBG levels were positively associated with both percent (*p*-trend 0.003) and absolute (*p*-trend 0.02) mammographic densities. Women with SHBG concentrations in the highest quartile had a statistically significantly higher percent mammographic density (2.5% absolute difference) compared with women with SHBG in the lower quartile (*p*-value = 0.007). Plasma estrone concentrations were also positively associated with both percent and absolute mammographic density, although not statistically significant (*p*-trend 0.07 and 0.12, respectively).

Analyses stratified by IGF-I levels

Table IV shows that the weak positive association found between plasma estrone concentrations and percent mammographic density overall was strengthened when analysis were restricted to women with plasma IGF-I concentrations below median, whereas the association disappeared among women with IGF-I concentrations over median (*p* for interaction = 0.02).

TABLE II – AGE ADJUSTED SPEARMAN CORRELATION COEFFICIENTS BETWEEN CIRCULATING CONCENTRATIONS OF SEX HORMONES, SHBG, PROLACTIN, IGF-I AND BMI [THE TROMSØ MAMMOGRAPHY AND BREAST CANCER STUDY (2001/2002)]

	Estradiol	Free estradiol	Testosterone	Free testosterone	Androstenedione	DHEAS	SHBG	Prolactin	IGF-I	BMI
Estrone	0.41 (*p* < 0.001)	0.42 (*p* < 0.001)	0.62 (*p* < 0.001)	0.64 (*p* < 0.001)	0.52 (*p* < 0.001)	0.58 (*p* < 0.001)	−0.23 (*p* < 0.001)	−0.11 (*p* = 0.006)	0.03 (*p* = 0.42)	0.22 (*p* < 0.001)
Estradiol	—	0.90 (*p* < 0.001)	0.37 (*p* < 0.001)	0.45 (*p* < 0.001)	0.28 (*p* < 0.001)	0.37 (*p* < 0.001)	−0.27 (*p* < 0.001)	−0.05 (*p* = 0.17)	0.04 (*p* = 0.32)	0.25 (*p* < 0.001)
Free estradiol	—	—	0.29 (*p* < 0.001)	0.60 (*p* < 0.001)	0.24 (*p* < 0.001)	0.35 (*p* < 0.001)	−0.63 (*p* < 0.001)	−0.08 (*p* = 0.04)	0.10 (*p* = 0.01)	0.43 (*p* < 0.001)
Testosterone	—	—	—	0.80 (*p* < 0.001)	0.65 (*p* < 0.001)	0.67 (*p* < 0.001)	0.01	−0.07 (*p* = 0.07)	0.05 (*p* = 0.18)	0.02 (*p* = 0.69)
Free testosterone	—	—	—	—	0.55 (*p* < 0.001)	0.62 (*p* < 0.001)	−0.54	−0.11	0.12 (*p* = 0.001)	0.33
Androstenedione	—	—	—	—	—	0.61 (*p* < 0.001)	−0.02 (*p* = 0.60)	−0.08 (*p* = 0.004)	0.05 (*p* = 0.16)	−0.02 (*p* = 0.63)
DHEAS	—	—	—	—	—	—	−0.09 (*p* = 0.02)	−0.09 (*p* = 0.04)	0.12 (*p* = 0.002)	−0.004
SHBG	—	—	—	—	—	—	—	0.09 (*p* = 0.01)	−0.15	−0.57 (*p* = 0.91)
Prolactin	—	—	—	—	—	—	—	—	0.06 (*p* < 0.001)	0.04 (*p* = 0.35)
IGF-I	—	—	—	—	—	—	—	—	—	0.007 (*p* = 0.87)

TABLE III – ADJUSTED[1] MEAN[2] (95% CI) PERCENT AND ABSOLUTE MAM-MOGRAPHIC DENSITY BY QUARTILES OF CIRCULATING SEX HORMONES, SHBG AND PROLACTIN [THE TROMSØ MAMMOGRAPHY AND BREAST CANCER STUDY (2001/2002)]

	Adjusted[2] mean[2] (95% CI)	
	Percent mammographic density (%)	Absolute mammographic density (cm[2])
Estrone	n = 700	n = 700
Q1	6.5 (5.5–7.7)	9.4 (7.8–11.8)
Q2	7.3 (6.2–8.6)	10.7 (8.9–12.9)
Q3	7.4 (6.2–8.8)	10.8 (8.9–13.1)
Q4	8.0 (6.7–9.6)	11.5 (9.5–14.0)
p-trend	0.07	0.12
Estradiol	n = 681	n = 681
Q1	7.4 (6.2–8.7)	10.7 (8.8–12.9)
Q2	6.3 (5.4–7.5)	9.2 (7.6–11.1)
Q3	8.1 (6.8–9.5)	11.6 (9.6–14.0)
Q4	7.5 (6.3–8.9)	11.3 (9.3–13.8)
p-trend	0.42	0.31
Free estradiol	n = 678	n = 678
Q1	7.8 (6.6–9.3)	11.0 (9.0–13.4)
Q2	6.4 (5.4–7.6)	9.5 (7.8–11.4)
Q3	7.8 (6.6–9.2)	11.7 (9.7–14.2)
Q4	7.0 (5.9–8.3)	10.2 (8.3–12.4)
p-trend	0.69	0.95
Testosterone	n = 695	n = 695
Q1	6.9 (5.9–8.2)	10.2 (8.5–12.3)
Q2	7.4 (6.2–8.7)	10.9 (9.0–13.2)
Q3	7.4 (6.3–8.8)	10.6 (8.7–12.8)
Q4	7.6 (6.4–9.1)	11.0 (9.1–13.4)
p-trend	0.38	0.61
Free testosterone	n = 690	n = 690
Q1	7.3 (6.1–8.6)	10.6 (8.7–12.9)
Q2	7.8 (6.6–9.2)	11.3 (9.3–13.7)
Q3	7.3 (6.1–8.6)	10.8 (8.9–13.0)
Q4	6.7 (5.7–8.0)	9.8 (8.0–11.9)
p-trend	0.41	0.46
Androstenedione	n = 705	n = 705
Q1	7.4 (6.3–8.7)	10.7 (8.9–12.9)
Q2	7.5 (6.4–8.9)	11.5 (9.5–14.0)
Q3	7.0 (6.0–8.3)	10.1 (8.4–12.2)
Q4	7.2 (6.0–8.5)	10.0 (8.2–12.2)
p-trend	0.65	0.37
DHEA sulphate	n = 700	n = 700
Q1	7.5 (6.3–8.8)	11.2 (9.3–13.5)
Q2	7.0 (5.9–8.3)	10.1 (8.4–12.2)
Q3	7.9 (6.6–9.3)	11.2 (9.3–13.6)
Q4	7.1 (6.0–8.4)	10.2 (8.5–12.4)
p-trend	0.90	0.68
SHBG	n = 702	n = 702
Q1	6.2 (5.2–7.4)	9.1 (7.5–11.1)
Q2	6.7 (5.7–7.9)	9.8 (8.1–11.8)
Q3	8.0 (6.7–9.4)	11.9 (9.8–14.4)
Q4	8.7 (7.2–10.5)	12.0 (9.8–14.8)
p-trend	0.003	0.02
Prolactin	n = 703	n = 703
Q1	6.9 (5.8–8.2)	9.7 (8.0–11.9)
Q2	7.8 (6.6–9.3)	11.2 (9.3–13.6)
Q3	7.4 (6.0–8.4)	11.2 (9.3–13.5)
Q4	7.1 (6.0–8.4)	10.4 (8.6–12.5)
p-trend	0.90	0.61

[1]Analyses are adjusted for age at screening, number of children, age at menopause, BMI and postmenopausal hormone therapy use (never, past use >3 years ago, past use <3 years ago).–[2]Reported means are back-transformed from log-transformed estimated means.

Similar associations were found when absolute mammographic density was used as the outcome variable (results not shown).

BMI modeling

Overall we found similar associations between sex-hormones, prolactin and SHBG and mammographic density when BMI was adjusted for in the multivariate model as a continuous or categorized variable (tertile or quintile), when we excluded the 5% most

TABLE IV – ADJUSTED[1] MEAN[2] (95% CI) PERCENT MAMMOGRAPHIC DEN-SITY BY QUARTILES OF CIRCULATING ESTRONE STRATIFIED BY MEDIAN PLASMA IGF-I CONCENTRATION, THE TROMSØ MAMMOGRAPHY AND BREAST CANCER STUDY (2001/2002)

	Adjusted[1] mean[2] (95% CI) percent mammographic density (%)	
	IGF-I < 229.1 ng/mL	IGF-I ≥ 229.1 ng/mL
Estrone	n = 349	n = 349
Q1	5.4 (4.3–6.9)	8.0 (6.2–10.2)
Q2	7.1 (5.6–9.0)	7.5 (6.0–9.3)
Q3	8.6 (6.7–10.9)	6.3 (4.9–8.0)
Q4	7.0 (5.4–9.0)	9.1 (7.1–11.5)
p-trend	0.02	0.61

[1]Analyses are adjusted for age at screening, number of children, age at menopause, BMI and postmenopausal hormone therapy use (never, past use >3 years ago, past use <3 years ago).–[2]Reported means are back-transformed from log-transformed estimated means.

extreme BMI values (2.5% highest and 2.5% lowest) or when we excluded the 5% highest BMI values (results not shown).

Discussion

This population-based cross-sectional study shows a positive association between plasma SHBG concentrations and percent mammographic density among women not currently using HT, after adjustment for potential confounders. Furthermore, plasma estrone concentration was positively, but weakly, associated with mammographic density. For the latter association we observed a possible effect modification by levels of IGF-I. Similar associations were present when absolute mammographic density was used as the outcome variable.

The strengths of our study are the large sample size and that it was a part of a population-based screening project with a high attendance rate.[30] The reader of the mammograms was experienced and blinded to the characteristics of the women. Furthermore, the hormone analyses were done in a blinded manner at a laboratory specialized on hormone measurements.

One limitation of our study is that we have single plasma hormone measurements. However, it has previously been indicated that 1 measurement is representative among postmenopausal women for long-term levels of estrogens and SHBG, but not so much for androgen and prolactin levels.[31] This imprecision in hormone levels would presumably result in a nondifferential misclassification, and would therefore be expected to bias the results in our study toward the null association. Another limitation is that the mean mammographic density in our study is low. However, we have previously shown a high intrarater agreement for the reader in our study (Pearson correlation coefficient = 0.86).[25] Also, in another study with a different reader, women from our study had significantly lower percent mammographic density compared with Caucasians from Hawaii and Arizona.[32]

Our findings of a positive association between SHBG levels and mammographic density is in support of most,[14,15,19] but not all,[17,18] previous studies on this association. In 2002, Boyd *et al.* reported a positive association between SHBG and prolactin and mammographic density, and an inverse association between free-estradiol and mammographic density, among 189 postmenopausal women.[14] SHBG and prolactin were also positively associated with percent mammographic density in the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial.[15,16] However, in contrast to the finding by Boyd *et al.*,[14] Greendale *et al.* found positive associations between bioavailable estradiol, as well as total estradiol and estrone and mammographic density among the 404 postmenopausal women in the PEPI trial.[15] Another American study found inverse associations between different estrogens and percent mammographic density, but only restricted to 43 overweight former HT users.[17] Two recent studies, 1 from the Nurses' Health Study and 1 from the European Prospective Investigation into

Cancer – Norfolk cohort, found no statistically significant association between endogenous sex hormones and mammographic density.[18,19] However, in the European study, a positive, but not statistically significant, association was observed between SHBG and mammographic density.[19]

The inverse association between SHBG and breast cancer risk is believed to be due to increased levels of bioavailable sex hormones.[33] However, in relation to mammographic density, it has been proposed that the positive association with SHBG may be due to a cell-membrane-associated agonistic effect of SHBG on the steroid signaling pathway in breast cells.[15] It has been suggested that the lack of an association between estrogens and mammographic density may be due to the confounding of adiposity.[19] In our study, BMI correlated positively with estrogens and negatively with SHBG and mammographic density. In a recent study, adiposity among postmenopausal women was also found to be associated with high levels of estrogens and low levels of SHBG. Furthermore, BMI was found to be a reasonable good marker of adiposity.[34] The results in our study were similar when BMI was adjusted for in the multivariate analyses as a categorized or as a continuous variable. Also, our findings were similar when absolute mammographic density was used as the outcome variable. However, it is possible that the results in our study are influenced by residual confounding by adiposity.

We find a positive, but weak, association between estrone and mammographic density, as in the PEPI trial.[15] Estrone is the most prevalent estrogen after menopause.[35] It may be that estrone, even though of less potency than estradiol, quantitatively exerts the most estrogen-related activity in regards to mammographic density among the postmenopausal women in our study. When we analyzed the association between estrone and mammographic density stratified by median IGF-I concentrations, we observed a possible effect modification by levels of IGF-I. We have previously shown a positive association between plasma IGF-I concentrations and mammographic density in this study population.[24] Laboratory studies have suggested that IGF-I can both activate and increase the number of estrogen receptors,[20,21] and that the IGF-I-ER activation may be necessary for a maximal estrogen-mediated estrogen receptors activation.[21] In a case–control study among postmenopausal Chinese women, such a synergistic effect between estrone and IGF-I was suggested in relation to breast cancer risk.[23] Thus, conversely to our findings, a plausible hypothesis exists for a possible synergism between estrone and IGF-I in relation to mammographic density. We have no explanation for the effect modification by IGF-I on the association between estrone and mammographic density found in our study, but it may be due to chance.

In conclusion, we find a positive but weak association between plasma SHBG concentrations and both measures of mammographic densities among 722 postmenopausal women not currently using HT. Further, we find a positive but weak association between plasma estrone concentration and mammographic density, possibly effect modified by levels of IGF-I.

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Appendix 1:

Request letter to participate in the TMBCS
(Original)

Forespørsel om å delta i undersøkelsen «Mammografi og brystkreft»

Institutt for Samfunnsmedisin ved Universitetet i Tromsø skal gjennomføre en undersøkelse som ser på sammenhengen mellom hormoner, livsstil, mammografimønster og brystkreft. Undersøkelsen gjøres for å få økt kunnskap om årsakene til brystkreft og mer innsikt i om det er noen kvinner som bør inviteres sjeldnere /hyppigere til mammografi-screening.

Vi vil spørre deg om å delta. Ansvarlig for undersøkelsen er lege Inger Torhild Gram, professor i forebyggende medisin. Undersøkelsen gjennomføres i samarbeid med Universitetssykehuset Nord-Norge HF og Mammografiundersøkelsen, Kreftregisteret.

Deltakelse innebærer at det blir tatt en blodprøve etter mammografering, at kroppsmål registreres og at man svarer på noen spørsmål muntlig og skriftlig. Det vil også bli innhentet opplysninger fra Tromsøundersøkelsene og fra Mammografiundersøkelsen, Kreftregisteret. Blodprøver og opplysningene vil bli lagret for mulige senere undersøkelser.

Formålet med blodprøven vil være;
* *Måle hormoner og andre stoffer i blodet som kan settes i forbindelse med mammografimønsteret (røntgenbilde av brystkjertelvevet).*
* *I framtida kunne studere de såkalte genetiske markører dvs. egenskaper i arvestoffet som kan disponere for kreft.*
* *Teste nye ideer eller hypoteser som oppstår i framtida.*

De kroppsmål som skal registreres er midje/hofte-mål, høyde og vekt. Dette er nødvendig fordi en kvinnes mammografimønster henger sammen med hennes høyde og vekt. Målingene vil bli gjort uten sko og med tøyet på. I forbindelse med blodprøve-takingen vil det bli stilt noen spørsmål om blant annet barnefødsler og bruk av hormoner og andre medisiner. Det vil også bli utdelt et skjema med spørsmål om blant annet kosthold og levesett. Du behøver ikke å svare på alle spørsmål.

Undersøkelsen er tilrådd av Regional komite for medisinsk forskningsetikk, Helseregion Nord-Norge. Alle opplysninger vil bli behandlet konfidensielt og etter de regler Datatilsynet har gitt for denne undersøkelsen.
Eventuelle framtidige undersøkelser på lagrete blodprøver og opplysninger vil bli forelagt Regional komité for medisinsk forskningsetikk og vil ikke bli gjennomført uten tilråding fra komiteen.

Det er frivillig om du vil delta i undersøkelsen. Din avgjørelse om du vil delta eller ikke, har ingen betydning for din deltagelse i mammografiscreeningen. Du kan trekke deg uten begrunnelse, og be om at opplysninger som du har gitt blir slettet, uten at dette vil få konsekvenser for deg. Undersøkelsene vi gjør er i forskningsøyemed og du vil ikke få beskjed om dine prøvesvar. Det er vårt håp at kunnskap fra denne studien skal være med å gi oss økt forståelse for hvordan brystkreft kan forebygges. Resultatene vil bli publisert i dagspressen og i internasjonale fagtidsskrifter. Du beholder en kopi av dette brevet.

Med vennlig hilsen

Inger Torhild Gram

Inger Torhild Gram, lege
Professor i Forebyggende Medisin

NAVN: . . . .
Jeg har lest informasjonen om undersøkelsen og samtykker i å delta.

Tromsø den . . . . . .   Underskrift   . . . .

Appendix 2:

Registration form (interview)
(Original)

DATO: [   ]

REGISTRERINGSSKJEMA

Fødselsår: [   ]

1.

Høyde (cm)	Vekt (kg)	Midjemål (cm) 2,5 cm over navlen	Hoftemål (cm)	Hoftemål (cm)

2. Har du født barn?   Ja ☐   Nei ☐   ANTALL ☐
(Her vil vi at du også fyller ut for dødfødte eller barn som er døde senere i livet)

3. Har menstruasjonen din stoppet i mer enn 12 mnd.?   Ja ☐   Nei ☐
   Alder da menstruasjonen opphørte?   [   ] år

4. Røyker du nå?   Ja , daglig ☐   Ja, av og til ☐   Nei ☐
   Hvis Ja,   Antall pr. dag   [   ]

5. Har du brukt noen av disse hormonpreparatene?   Ja ☐   Nei ☐
Hvis Ja (vis hormonark),

Periode	Østrogenpreparat		Styrke (mg)	Alder ved start	Brukt samme østrogenpreparat sammenhengende	
	Nr.	Navn			År	Måned
Første						
Andre						
Tredje						
Fjerde						
I dag						

Hvis Ja, når tok du sist hormontablett?..........(Dato)...............(Klokkeslett)

6. Bruker du andre medisiner daglig?   Ja ☐   Nei ☐
(Eks. kortisontabletter, medisin for lavt stoffskifte, for sukkersyke)
Hvis Ja,

Type Medisin (Navn)	Årsak

7. Har du eller har du hatt noen kreftsykdommer?
Hvis Ja, hvilken..årstall ved diagnose...................

8.

Tid for siste måltid/drikke	Tid for blodprøve

Appendix 3:

Photo-leaflet of HT regimens
(Original)

Bruk av østrogener i og etter overgangsalderen

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

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

Nr. 108. Solgt fra 1976

Dienoestrol 0,5 mg

Dienoestrol 0,01%
Nr. 109. Solgt fra 1954

Estracomb
Nr. 110. Solgt fra 1994

Cyclabil
Nr. 101. Solgt fra 1978

Nr. 111. Solgt fra 1971

Ovesterin 0,5 mg

Ovesterin 1 mg

Ovesterin 2 mg

Ovesterin 0,1 %

Nr. 102. Solgt fra 1978 Trisekvens

Trisekvens Forte Nr. 103. Solgt fra 1978

Nr. 112. Solgt fra 1989

Nr. 113. Solgt fra 1983

Nr. 114. Solgt fra 1984

Nr. 104. Solgt fra 1953

Etifollin 50 mikrog

Kliogest
Nr. 105. Solgt fra 1988

Nr. 115. Solgt fra 1995

Evorel

Nr. 117. Solgt fra 1994

Nr. 116. Solgt fra 1995

Nr. 107. Solgt fra 1967

Progynova 2 mg

Progynova 2 mg

Progynova 1 mg

Progynova 1 mg

Nr. 106. Solgt fra 1970

Estraderm
Nr. 118. Solgt fra 1989

Nr. 120. Solgt fra 1989

Nr. 119. Solgt fra 1989

Nr. 121
Solgt fra
1996.

Nr. 122
Solgt fra
1996.

Nr. 123
Solgt fra
1996.

Nr. 124
Solgt fra
1996.

MENOREST®
37,5 mikrog/24 timer, timmar, tuntia

MENOREST®
50 mikrog/24 timer, timmar, tuntia

MENOREST®
75 mikrog/24 timer, timmar, tuntia

MENOREST®
100 mikrog/24 timer, timmar, tuntia

Nr. 125
Solgt fra 1996.

Estring 2.5 mg rog/ 24 timer

Østradiol

Nr. 126
Climara 50 mg
Solgt fra
1997

Climara
50 mikrog/24 timer
Østradiolum

SCHERING

Nr. 127
Activelle
Solgt fra
1999

28 tabl.   Vnr. 54 02 03

Activelle™

Novo Nordisk
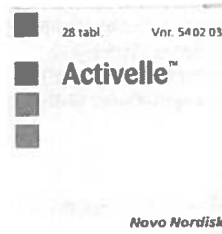
Nr. 128
Livial 2,5 mg
Solgt fra
1999

Livial®
2,5 mg tabletter

Nr. 129
Crione
Solgt fra
2000

Crinone® 8 %

TAKK FOR INNSATSEN!

Appendix 4:

Questionnaire spring 2001
(Original)

MAMMOGRAFI OG BRYSTKREFT

KONFIDENSIELT

Institutt for Samfunnsmedisin ved Universitet i Tromsø skal gjennomføre en undersøkelse som ser på sammenhengen mellom hormoner, livsstil mammografimønster og brystkreft.

Undersøkelsen gjøres for å få økt kunnskap om årsakene til brystkreft og mer innsikt i om det er noen kvinner som bør inviteres sjeldnere/hyppigere til mammografiscreeningen. Det er vårt håp at kunnskap fra denne studien skal være med å gi oss økt forståelse for hvordan brystkreft kan forebygges.

Undersøkelsen er godkjent av Datatilsynet og av Regional komite for medisinsk forskningsetikk.
Svarene brukes bare til forskning og behandles strengt fortrolig. Opplysningene kan senere bli sammenholdt med informasjon fra andre offentlige helseregistre etter de regler som Datatilsynet og Regional komite for medisinsk forskningsetikk gir.

Vi ber deg fylle ut spørreskjemaet så nøye som mulig. Det utfylte skjema sendes i vedlagte svarkonvolutt. Portoen er betalt. Sett kryss for JA i ruten nedenfor hvis du samtykker i å være med. Dersom du ikke ønsker å delta, sett kryss for NEI og returner skjemaet i vedlagt svarkonvolutt, så slipper du å bli purret på.

På forhånd takk for hjelpen!

Med vennlig hilsen
Inger Torhild Gram, lege
Professor i Forebyggende Medisin

Jeg samtykker i å delta i spørre-skjema-undersøkelsen	☐ JA
	☐ NEI

Sivil status, utdannelse, oppvekst

Er du (Sett ett kryss)
☐ gift/samboer ☐ skilt/separert ☐ enslig ☐ enke

Hvor mange års skolegang har du gjennomført?
(Ta med alle hele år du har gått på skole eller studert)
............år

Hvordan var de økonomiske forhold i oppveksten?
(Sett ett kryss)

☐ Meget gode ☐ Meget dårlige
☐ Gode ☐ Usikker
☐ Dårlige

Hvilket språk hadde dine besteforeldre?
(Sett ett eller flere kryss for hver linje)

	Norsk	Samisk	Finsk/Kvensk	Annet
Mormor				
Morfar				
Farmor				
Farfar				

Har du søsken? ☐ Ja ☐ Nei
Hvis Ja;
Hvor mange barn hadde moren din født før du ble født?
.......antall

Hvor mange jenter hadde moren din født før du ble født?
.......antall

Menstruasjonsforhold

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

Hvor mange år tok det før menstruasjonen ble regelmessig? (Sett ett kryss)

☐ Ett år eller mindre ☐ Mer enn ett år
☐ Aldri ☐ Husker ikke

Er menstruasjonen din i dag; (Sett ett kryss)
Regelmessig ....☐
Uregelmessig ....☐
Stoppet i mer enn 6 mnd. ....☐

Hvis du ikke har menstruasjon; (Sett ett kryss)
Har den stoppet av seg selv? ....☐
Operert vekk begge eggstokkene? ....☐
Operert vekk livmoren? ....☐
Annet; angi ....☐

Alder da menstruasjonen opphørte? ....år

Graviditeter, fødsler og amming

Har du født barn? ☐ Ja ☐ Nei

Hvis Ja;
Vil vi be deg om å fylle ut for hvert barn, opplysninger om fødselsår/fødselsvekt og antall måneder du ammet hvert barn (fylles også ut for dødfødte eller barn som er døde senere i livet).

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

Abort og infertilitet

Har du hatt noe svangerskap som varte mindre enn seks måneder dvs. spontanabort eller selvbestemt abort? ☐ Ja ☐ Nei

Hvis Ja;

Hvor gammel var du ved første abort? ....år

Hvor mange aborter har du hatt i alt? ....antall

Har du noen gang prøvd i mer enn 1 år å bli gravid? ☐ Ja ☐ Nei

Hvis Ja;

Hvor gammel var du? ....år

Hvor lenge prøvde du? ....år

Fikk du hormonbehandling? ☐ Ja ☐ Nei

Høyde og vekt

Det kan være vanskelig å kjenne til høyde og vekt fra oppvekst og senere i livet. Likevel ber vi deg forsøke.

Fødsel: Vekt_____gram Lengde_____cm

18 år: Vekt_____kg Høyde_____cm

Dagens: Vekt_____kg Høyde_____cm

Kroppstype i 1. klasse. (Sett ett kryss)

☐veldig tynn ☐tynn ☐normal ☐tykk ☐veldig tykk

Har du lagt på deg etter at du ble 50 år? ☐ Ja ☐ Nei

I tilfelle Ja; hvor mange kg? ....kg

Brystkreft i nærmeste familie

Hvor mange døtre har/hadde du? ....antall

Hvor mange søstre har/hadde du? ....antall

Hvor mange søstre har/hadde din mor? ....antall

Hvor mange søstre har/hadde din far? ....antall

Har noen nære slektninger hatt brystkreft?

	Ja	Nei	Vet ikke	Alder ved diagnose
datter	☐	☐	☐	
mor	☐	☐	☐	
mormor	☐	☐	☐	
farmor	☐	☐	☐	
søster	☐	☐	☐	
din mors søster	☐	☐	☐	
din fars søster	☐	☐	☐	

Prevensjonsmidler

Har du noen gang brukt p-piller, minipiller eller Levonova hormonspiral?(ikke vanlig spiral)
(Fyll ut for hver linje.)

	Alder ved start	Alder ved stopp	Antall år totalt	Aldri brukt
P-piller				
Minipiller				
Levonova				

Hormonbruk (østrogen o.l.) i overgangsalderen

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

Hvis Ja;

Begynte du å bruke disse preparatene før menstruasjonen opphørte? ☐ Ja ☐ Nei

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

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

Bruker du hormontabletter/ plaster nå? ☐ Ja ☐ Nei

Hvis Ja;
Angi navn...styrke........mg

Hvis Nei;
Hvor lenge siden er det du sluttet? ......mnd ......år

HORMONPREPARAT TIL LOKAL BRUK I SKJEDEN?

Har du noen gang brukt hormonkrem/stikkpiller? ☐ Ja ☐ Nei

Hvis Ja;

Begynte du å bruke disse preparatene før menstruasjonen opphørte? ☐ Ja ☐ Nei

Hvor lenge har du brukt hormonkrem/stikkpiller i alt? ..... år

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

Bruker du hormonkrem/ stikkpiller nå? ☐ Ja ☐ Nei

Hvis Ja;
Angi navn...styrke......mg

Hvis Nei;
Hvor lenge siden er det du sluttet? ......mnd ......år

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 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
Potet							
Gulrøtter							
Kål							
Kålrot							
Broccoli/blomkål							
Blandet salat							
Grønnsakblanding (frossen)							
Andre grønnsaker							

Hvor mange glass/kopper drikker du vanligvis av hver type?

(Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr. uke	4-6 pr. uke	1-2 pr. dag	3-4 pr. dag	5+ pr. dag
Vann						
Melk						
Appelsin juice						
Kaffe						

Kjøtt/Kjøttprodukter/Fjærkre

Hvor ofte spiser du følgende kjøtt- og fjærkreretter til middag?

(Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd	2-3 pr. mnd	1 pr. uke	2+ pr. uke
Okse, svin, får					
Kjøttdeig, pølse					
Kylling, kalkun					
Rein, elg					
Andre kjøttretter					

Fisk / fiskeprodukter

Hvor ofte pleier du å spise fisk til middag?

(Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd	2-3 pr. mnd	1 pr. uke	2 pr. uke	3+ pr. uke
Torsk, sei, hyse, lyr						
Steinbit, flyndre, uer						
Laks, ørret						
Makrell, sild						

Hvor ofte spiser 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					
Frityrfisk fiskepudding					
Plukkfisk, fiskegrateng					
Andre fiskeretter					

Hvor ofte spiser du følgende?

(Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd	2-6 pr. uke	daglig	1-3 pr. dag
Tran/kapsler/tranpiller					
Fiskekapsler					
Fisk som pålegg					

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
☐	☐	☐	☐	☐

Hvor mange ulike kosttilskudd tar du?   ....antall

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
Lettøl (glass)	☐	☐	☐	☐	☐	☐	☐
Øl (glass)	☐	☐	☐	☐	☐	☐	☐
Hvitvin (glass)	☐	☐	☐	☐	☐	☐	☐
Rødvin (glass)	☐	☐	☐	☐	☐	☐	☐
Brennevin (drinker)	☐	☐	☐	☐	☐	☐	☐

Fysisk aktivitet

Hvis du er i lønnet eller ulønnet arbeid, hvordan vil du beskrive ditt arbeid? (Ta et gjennomsnitt siste året)
(Sett ett kryss i den ruten som passer best)

☐ For det mest stillesittende arbeid?
  (f.eks. kontorarbeid)

☐ Arbeid som krever at du går/står mye?
  (Du blir ikke svett og hjertet slår ikke fortere,
  f.eks. ekspeditør, lærer, frisør)

☐ Arbeid hvor du går eller løfter mye?
  (Du svetter litt og hjertet kan slå litt fortere, f. eks. postbetjent,
  syke-, hjelpepleier.)

☐ Tungt kroppsarbeid?
  (Du svetter en del og hjertet slår raskt f.eks. tungt
  omsorgsarbeid)

Hvilken fysisk aktivitet har du i fritiden?
(Ta et gjennomsnitt siste året)
(Sett ett kryss i den ruten som passer best)

☐ Leser, ser på fjernsyn eller annen stillesittende
  beskjeftigelser?

☐ Spaserer, sykler eller beveger deg på en annen måte
  minst 2 timer i uken?
  (Her medregnes også gange eller sykling til arbeid, søndagsturer,
  m.m)

☐ Spaserer, sykler eller beveger deg påen annen måte
  minst 4 timer i uken?

☐ Trener regelmessig og flere ganger i uka?
  (Du svetter en del og hjertet slår raskt)

Hvor mange timer går du utendørs per uke?
(går til arbeid, turer i skog og mark, skiturer, løping)
(Fyll ut for hver linje)

Du blir ikke svett og hjertet slår ikke fortere   ....timer

Du svetter litt og hjertet kan slå litt fortere   ....timer

Du svetter en del og hjertet slår raskt   ....timer

Røykevaner

Har du noen gang røykt?   ☐ Ja   ☐ Nei

Hvis Ja;
Røyker du nå?
(Sett ett kryss)   ☐ Ja, daglig   ☐ Ja, av og til
                    ☐ Nei

Hvis Nei;
Hvor lenge er det siden du sluttet?   ....år

Hvor gammel var du da du begynte å røyke?   ....år

Hvor mange år har du røykt daglig i alt?   ....år

Hvis du har røykt daglig, ber vi deg om å fylle ut for hver aldersgruppe i livet hvor mange sigaretter du i gjennomsnitt røykte pr. dag i den perioden.

Alder	0-14	15-19	20-29	30-39	40-49	50-59	60+
Antall							

Røykte noen av de voksne hjemme da du vokste opp?   ☐ Ja   ☐ Nei

Har du noen gang arbeidet på røykfulle arbeidsplasser   ☐ Ja   ☐ Nei

Hvis Ja;
Hvor lenge til sammen?   ....år

Bor du sammen med noen som røyker nå?   ☐ Ja   ☐ Nei

Hvis Ja;
Hvor lenge til sammen?   ....år

Hvor lenge er du vanligvis daglig tilstede i røykfulle rom?   ....timer

Mammografiundersøkelse

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

Har du noen kommentarer til denne mammografiundersøkelsen du har vært med på?

_____

_____

_____

_____

_____

_____

_____

_____

Til slutt vil vi spørre deg om ditt samtykke til å kontakte deg på nytt pr. post. Vi vil hente adressen fra den nasjonale mammografi-screeningen.   ☐ Ja   ☐ Nei

Takk for at du ville delta i undersøkelsen!

Appendix 5:

Questionnaire spring 2002
(Original)

MAMMOGRAFI OG BRYSTKREFT

Institutt for Samfunnsmedisin ved Universitet i Tromsø skal gjennomføre en undersøkelse som ser på sammenhengen mellom hormoner, livsstil mammografimønster og brystkreft.

Undersøkelsen gjøres for å få økt kunnskap om årsakene til brystkreft og mer innsikt i om det er noen kvinner som bør inviteres sjeldnere/hyppigere til mammografiscreeningen. Det er vårt håp at kunnskap fra denne studien skal være med å gi oss økt forståelse for hvordan brystkreft kan forebygges.

Undersøkelsen er godkjent av Datatilsynet og tilrådd av Regional komite for medisinsk forskningsetikk, Helseregion Nord-Norge. Svarene brukes bare til forskning og behandles strengt fortrolig. Opplysningene kan senere bli sammenholdt med informasjon fra andre offentlige helseregistre etter de regler som Datatilsynet og Regional komite for medisinsk forskningsetikk gir.

Vi ber deg fylle ut spørreskjemaet så nøye som mulig. Dersom ingen av de oppgitte svaralternativ dekker din situasjon, sett kryss for det alternativet som ligger nærmest. Du behøver ikke å svare på alle spørsmålene. Det utfylte skjema sendes i vedlagte svarkonvolutt. Portoen er betalt. Sett kryss for JA i ruten nedenfor hvis du samtykker i å være med. Dersom du ikke ønsker å delta kan du unngå purring ved å sette kryss for NEI og returnere skjemaet i vedlagte svarkonvolutt.

På forhånd takk for hjelpen!
Med vennlig hilsen
Inger Torhild Gram, lege
Professor i Forebyggende Medisin

| **Jeg samtykker i å delta i spørre-skjema-undersøkelsen** | ☐ JA ☐ NEI |

Sivil status, utdannelse, oppvekst

Er du (Sett ett kryss)
☐ gift/samboer ☐ skilt/separert ☐ enslig ☐ enke

Hvor mange års skolegang har du gjennomført?
(Ta med alle hele år du har gått på skole eller studert)
..........år

Hvordan var de økonomiske forhold i oppveksten?
(Sett ett kryss)

☐ Meget gode ☐ Meget dårlige
☐ Gode ☐ Usikker
☐ Dårlige

Hvilket språk hadde dine besteforeldre?
(Sett ett eller flere kryss for hver linje)

	Norsk	Samisk	Finsk/Kvensk	Annet
Mormor				
Morfar				
Farmor				
Farfar				

Har du søsken? ☐ Ja ☐ Nei
Hvis Ja;
Hvor mange barn hadde moren din født før du ble født?
.......antall

Hvor mange jenter hadde moren din født før du ble født?
.......antall

Menstruasjonsforhold

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

Hvor mange år tok det før menstruasjonen ble regelmessig? (Sett ett kryss)

☐ Ett år eller mindre ☐ Mer enn ett år
☐ Aldri ☐ Husker ikke

Er menstruasjonen din i dag; (Sett ett kryss)
Regelmessig ....☐

Uregelmessig ....☐

Stoppet i mer enn 6 mnd. ....☐

Hvis du ikke har menstruasjon; (Sett ett kryss)
Har den stoppet av seg selv? ....☐

Operert vekk begge eggstokkene? ....☐

Graviditeter, fødsler og amming

Har du født barn? ☐ Ja ☐ Nei
Hvis Ja;
Vil vi be deg om å fylle ut for hvert barn, opplysninger om fødselsår/fødselsvekt og antall måneder du ammet hvert barn (fylles også ut for dødfødte eller barn som er døde senere i livet).

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

Abort og infertilitet

Har du hatt noe svangerskap som varte mindre enn seks måneder dvs. spontanabort eller selvbestemt abort? ☐ Ja ☐ Nei

Hvis Ja;

Hvor gammel var du ved første abort? ....år

Hvor mange aborter har du hatt i alt? ....antall

Har du noen gang prøvd i mer enn 1 år å bli gravid? ☐ Ja ☐ Nei

Hvis Ja;

Hvor gammel var du? ....år

Hvor lenge prøvde du? ....år

Fikk du hormonbehandling? ☐ Ja ☐ Nei

Høyde og vekt

Det kan være vanskelig å kjenne til høyde og vekt fra oppvekst og senere i livet. Likevel ber vi deg forsøke.

Fødsel:	Vekt_____gram	Lengde_____cm
18 år:	Vekt_____kg	Høyde_____cm
Dagens:	Vekt_____kg	Høyde_____cm

Kroppstype i 1. klasse. (Sett ett kryss)

☐ veldig tynn ☐ tynn ☐ normal ☐ tykk ☐ veldig tykk

Har du lagt på deg etter at du ble 50 år? ☐ Ja ☐ Nei

I tilfelle Ja; hvor mange kg? ....kg

Brystkreft i nærmeste familie

Hvor mange døtre har/hadde du? ......antall
Hvor mange søstre har/hadde du? ......antall
Hvor mange søstre har/hadde din mor? ......antall
Hvor mange søstre har/hadde din far? ......antall

Har noen nære slektninger hatt brystkreft?

	Ja	Nei	Vet ikke	Alder ved diagnose
datter	☐	☐	☐	
mor	☐	☐	☐	
mormor.....................	☐	☐	☐	
farmor......................	☐	☐	☐	
søster	☐	☐	☐	
din mors søster	☐	☐	☐	
din fars søster	☐	☐	☐	

Prevensjonsmidler

Har du noen gang brukt p-piller, minipiller eller Levonova hormonspiral?(ikke vanlig spiral)
(Fyll ut for hver linje.)

	Alder ved start	Alder ved stopp	Antall år totalt	Aldri brukt
P-piller				
Minipiller				
Levonova				

Hormonbruk (østrogen o.l.) i overgangsalderen

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

Hvis Ja;

Begynte du å bruke disse preparatene før menstruasjonen opphørte? ☐ Ja ☐ Nei

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

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

Bruker du hormontabletter/ plaster nå? ☐ Ja ☐ Nei

Hvis Ja;
Angi navn...styrke........mg

Hvis Nei;
Hvor lenge siden er det du sluttet? ......mnd ......år

HORMONPREPARAT TIL LOKAL BRUK I SKJEDEN?

Har du noen gang brukt hormonkrem/stikkpiller? ☐ Ja ☐ Nei

Hvis Ja;

Begynte du å bruke disse preparatene før menstruasjonen opphørte? ☐ Ja ☐ Nei

Hvor lenge har du brukt hormonkrem/stikkpiller i alt? ..... år

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

Bruker du hormonkrem/ stikkpiller nå? ☐ Ja ☐ Nei

Hvis Ja;
Angi navn...styrke......mg

Hvis Nei;
Hvor lenge siden er det du sluttet? ......mnd ......år

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)	☐	☐	☐	☐	☐	☐
Eksta Lett	☐	☐	☐	☐	☐	☐
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 /brus, te og vann 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	☐	☐	☐	☐	☐	☐
Ananasjuice	☐	☐	☐	☐	☐	☐
Eplejuice	☐	☐	☐	☐	☐	☐
Saft/brus med sukker	☐	☐	☐	☐	☐	☐
Saft/brus sukkerfri	☐	☐	☐	☐	☐	☐
Te	☐	☐	☐	☐	☐	☐
Vann	☐	☐	☐	☐	☐	☐

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

Er du vegetarianer?   ☐ Ja   ☐ Nei

Annet _____

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	ikke 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.) □ 1 □ 2 □ 3 □ 4+
(2 fiskeboller=1 fiskekake)
- 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						

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

Hvor ofte spiser du matvarer laget av soya?

	aldri/ sjelden	1-3 pr. mnd	1 pr. uke	2-3 pr. uke	4-6 pr. uke	7+ pr. uke
Soyabønner						
Burger/pølser						
Tofu						
Soyamelk						
Soyasaus						
Soyaprep. tilskudd						

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/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	☐	....

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 ....

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
Lettøl (glass)	☐	☐	☐	☐	☐	☐	☐
Øl ($^1/_2$ L)	☐	☐	☐	☐	☐	☐	☐
Vin (glass)	☐	☐	☐	☐	☐	☐	☐
Brennevin (drinker)	☐	☐	☐	☐	☐	☐	☐

Har du noen gang røykt? ☐ Ja ☐ Nei

Hvis Ja;
Røyker du nå?
(Sett ett kryss) ☐ Ja, daglig ☐ Ja, av og til
☐ Nei

Hvis Nei;
Hvor lenge er det siden du sluttet? ....år

Hvor gammel var du da du begynte å røyke? ....år

Hvor mange år har du røykt daglig i alt? ....år

Hvis du har røykt daglig, ber vi deg om å fylle ut for hver aldersgruppe i livet hvor mange sigaretter du i gjennomsnitt røykte pr. dag i den perioden.

Alder	0-14	15-19	20-29	30-39	40-49	50-59	60+
Antall							

Hvor lenge er du vanligvis daglig tilstede i røykfulle rom?

På arbeidet ....timer
Hjemme ....timer

Røykte noen av de voksne hjemme da du vokste opp? ☐ Ja ☐ Nei

Har du noen gang arbeidet på røykfulle arbeidsplasser ☐ Ja ☐ Nei

Hvis Ja;
Hvor lenge til sammen? ....år

Bor du sammen med noen som røyker nå? ☐ Ja ☐ Nei

Hvis Ja;
Hvor lenge til sammen? ....år

Har du noen spesielle helsemessige forhold som har påvirket ditt normale aktivitetsnivå det siste året? ☐ Ja ☐ Nei

Hvis Ja;

årsak ....

Hvor lenge til sammen? ....mnd.

Hvis du er i lønnet eller ulønnet arbeid, hvordan vil du beskrive ditt arbeid? (Ta et gjennomsnitt siste året)
(Sett ett kryss i den ruten som passer best)

☐ For det mest stillesittende arbeid?
(f.eks. kontorarbeid)

☐ Arbeid som krever at du går/står mye?
(Du blir ikke svett og hjertet slår ikke fortere, f.eks. ekspeditør, lærer, frisør)

☐ Arbeid hvor du går eller løfter mye?
(Du svetter litt og hjertet kan slå litt fortere, f. eks. postbetjent, syke-, hjelpepleier.)

☐ Tungt kroppsarbeid?
(Du svetter en del og hjertet slår raskt f.eks. tungt omsorgsarbeid)

Hvilken fysisk aktivitet har du i fritiden?
(Ta et gjennomsnitt siste året)
(Sett ett kryss i den ruten som passer best)

☐ Leser, ser på fjernsyn eller annen stillesittende beskjeftigelser?

☐ Spaserer, sykler eller beveger deg på en annen måte minst 2 timer i uken?
(Her medregnes også gange eller sykling til arbeid, søndagsturer, m.m)

☐ Spaserer, sykler eller beveger deg påen annen måte minst 4 timer i uken?

☐ Trener regelmessig og flere ganger i uka?
(Du svetter en del og hjertet slår raskt)

Hvor mange timer går du utendørs per uke?
(går til arbeid, turer i skog og mark, skiturer, løping)
(Fyll ut for hver linje)

Du blir ikke svett og hjertet slår ikke fortere ....timer
Du svetter litt og hjertet kan slå litt fortere ....timer
Du svetter en del og hjertet slår raskt ....timer

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

Oppgi hvor mange hele timer du bruker i gjennomsnitt pr. uke.

Matlagning ....timer
Rengjøring ....timer
Klesvask ....timer
Innkjøp ....timer

Brystkreft

Er du engstelig for å ha brystkreft? ☐ Ja ☐ Nei

Var du engstelig for å ha brystkreft
for ett år siden? ☐ Ja ☐ Nei

Selvundersøkelse av brystene

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

☐ Aldri
☐ 2-3 ganger pr. år
☐ 1 gang pr. måned
☐ 1 gang pr. uke
☐ Hver dag

Mammografiundersøkelse

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

Hvis Ja;
Hvor gammel var du første gangen? ....år

Hvor mange ganger har du tidligere vært undersøkt

- Etter invitasjon fra Mammografiprogrammet ....antall

- Etter henvisning fra lege ....antall

- Uten henvisning fra lege ....antall

- Etter invitasjon fra
Tromsøundersøkelsen 1986/87 ....antall

Kommentarer

Har du noen kommentarer til denne mammografi-
undersøkelsen du har vært med på?

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Til slutt vil vi spørre deg om ditt samtykke til at vi
kan sende deg en ny forespørsel om å delta i en
eventuell utvidelse av forskningsprosjektet med inn-
henting av flere opplysninger om kostholdet og/eller
nye prøver

Vi vil hente adressen fra det nasjonale Mammografi-
programmet

☐ Ja ☐ Nei

Takk for at du ville delta i undersøkelsen!

Husk å postlegge spørreskjemaet i den vedlagte svarkonvolutten

Appendix 6:

<u>English translations of:</u>

-Request letter to participate in the TMBCS

-Registration form (interview)

-Questionnaire spring 2001

(Questionnaire spring 2002 has not been translated, as the questions used were similar to those in the spring 2001 Questionnaire)

FACULTY OF MEDICINE
INSTITUTE OF COMMUNITY MEDICINE
University of Tromsø, 9037 Tromsø
Phone 77 64 48 16

INVITATION TO PARTICIPATE IN THE "MAMMOGRAPHY AND BREAST CANCER" STUDY

The Institute of Community Medicine, University of Tromsø, is performing a study looking at the relationship between hormones, lifestyle, mammographic pattern and breast cancer. The purpose of the study is to gain more knowledge about what causes breast cancer and further insight into whether some women should be invited to have their mammograms taken more or less frequently.

We hereby invite you to participate in the study. Inger Torhild Gram, M.D., Ph.D., Professor in Preventive Medicine, is the responsible project investigator. The study is performed in collaboration with the University Hospital North Norway, the Norwegian Breast Cancer Screening Program, and the Norwegian Cancer Registry.

Participation will involve the following procedures: Donation of a blood sample after the mammograms have been taken, the recording of body measurements, and answering questions both orally and in writing. Data will also be collected from the Tromsø Studies, the Norwegian Breast Cancer Screening Program and the Norwegian Cancer Registry. The blood samples and information will be stored for possible future studies.

The purpose of the blood sample is:
- Measurement of hormones and other components in the blood that can be related to the mammographic pattern (x-ray of the parenchymal tissue in the breast)
- Future studies of genetic markers, i.e. factors in the DNA that can predispose for cancers
- Test novel ideas and hypothesis that arise in the future

The body measurements to be recorded are waist and hip measurements, height and weight. This is necessary as a woman's mammographic pattern is related to her height and weight. The measurements will be taken clothed and without shoes. In relation to the drawing of blood, some questions among other things concerning childbirth, hormone use and concomitant medications. A questionnaire will be handed out, regarding among other things dietary habits and lifestyle factors. You do not have to answer all the questions.

The study is approved by the Regional Committee for Medical Research Ethics, North Norway.
The collected information will be handled confidentially and according to the rules given by The Norwegian Data Inspectorate regarding this study.

Possible future studies involving the use of stored blood samples and information from participants will be presented to the Regional Committee for Medical Research Ethics, North Norway, and not performed without their approval.

It is voluntary to participate in the study. Your decision to participate or not will not influence your participation in the Norwegian Breast Cancer Screening Program. You can withdraw without any explanation, and request that the information you have provided is deleted, with no consequences for yourself. These examinations are done for research purposes only and you will not be notified about individual results. It is our hope that at the knowledge gained from this study will increase our understanding about how to prevent breast cancer. The results will be published in the daily press and in international scientific journals.
You will have a copy of this letter.

With regards,

Inger Torhild Gram M. D.
Professor in Preventive Medicine

Name:
I have read the information about the study and consent to participate:

Tromsø   Date ....(signature)

DATE:

REGISTRATION FORM

Year of birth:

1.

Height (cm)	Weight (kg)	Waist (cm) 2,5 cm over navel	Hip (cm)	Hip(cm)

2. **Have you given birth?** ☐Yes ☐No **How many times** ☐
(We also want you to fill in for children that were stillborn or died later)

3. **Have your periods stopped for more than 12 months?** Yes☐ No☐
Age when periods stopped? ☐ **Years**

4. **Are you currently smoking?** ☐Yes, daily ☐Yes, occasionally ☐No
If yes, Number of cigarettes per day ☐

5. **Have you ever used any of these hormone therapies?** Yes☐ No☐
If yes (show leaflet),

Line	Type of estrogen preparation		Strength (mg)	Age at start	Used same estrogen preparation over time	
	Nr.	Name			Years	Months
First						
Second						
Third						
Fourth						
Today						

If yes, when did you last take a hormone tablet?..........(Date)...............(Time)

6. **Do you use other medications daily?** Yes☐ No ☐
(E.g. cortisone-tablets, medication for hypothyroidism, for diabetes)
If yes,

Type of medication (Name)	Reason

7. **Have you previously been diagnosed with cancer?**
If yes, when.......................…..........What type.........................…...........

8.

Time for last meal/drink	Time for blood sample

MAMMOGRAPHY AND BREAST CANCER

The Institute of Community Medicine, University of Tromsø, will conduct a survey on the associations between hormones, lifestyle, mammographic patterns, and breast cancer.

The survey is conducted to gain more insight into the etiology of breast cancer, into whether some women ought to be invited more seldom/more often to the mammography screening. Our hope is that knowledge from this survey will contribute with increased understanding on how to prevent breast cancer.

The survey has been approved by the National Data Inspectorate and the Regional Committee on Research Ethics.

The answers you give will only be used for research, and will be treated in strict confidentiality. The information may later be compared with information from other public health registers in accordance with the rules laid down by the National Data Inspectorate and the Regional Committee on Research Ethics.

We ask you to fill in the questionnaire as correct as possible. The filled in questionnaire is to be returned in the enclosed envelope. Postage has been prepaid. Tick YES in the box beneath if you consent to participate. If you do not wish to participate, tick NO and return the questionnaire in the enclosed envelope, and you will not be mailed a reminder.

Thank you in advance for helping us!!
Best regards
Inger Torhild Gram, M.D.
Professor of Preventive Medicine

I agree to take part in the questionnaire survey	YES ☐ NO ☐

Civil status / education/ upbringing

Are you *(Tick only one alternative)*
married/partners ☐ divorced/separated ☐ single ☐ widowed ☐

How many years of formal schooling do you have? *(Register all whole years of school/studies)*
...........Years

How would you describe your family's financial situation in your childhood/youth? *(Tick only one)*
Very good ☐ Good ☐ Poor ☐ Very poor ☐ Not sure ☐

What language did your grandparents speak?
(Tick one or more for each line)

	Norwegian	Sami	Finnish/ Kvensk	Other
Mothers mother				
Mothers father				
Fathers mother				
Fathers father				

Do you have any siblings?   Yes ☐   No ☐
If yes,
How many children did you mother have before you? ....children
How many girls did you mother have before you? ....girls

Menstruation

How old were you when you had your first period? ....years
How many years did it take before your periods became regular?
One year or less ☐   More than one year ☐
Never ☐   Don't remember ☐

Are your periods now;
Regular .... ☐
Irregular... ☐
Stopped for more than 6 months........... ☐

If you do not have periods;
Have they stopped by themselves?......... ☐
Have had both ovaries removed?.............. ☐
Have had the uterus removed?.................. ☐
Other; specify...................................... ☐

Age when periods stopped? ....years

Pregnancies, births, and breast-feeding

Have you given birth?   Yes ☐   No ☐
If yes,
We ask you to fill in information for each child's birth year, birth weight and months of breast-feeding *(fill also in for stillborns and for children who have died after birth)*.

Child	Birth year	Birth weight	Months of breast feeding
1			
2			
3			
4			
5			
6			

Abortions and infertility

Have you ever had pregnancies lasting less than six months, i.e. miscarriages or abortions?
Yes ☐   No ☐

If yes,
How old were you at the first time …….…….years
How many have you had in total? ………number

Have you ever spent more than one year trying to get pregnant?   Yes☐   No ☐
 If yes,
How old were you?   ……….…years
How long did you try?   …..…….years

Did you receive hormonal treatment?   Yes☐   No ☐

Height and weight

You might not know your height and weight from childhood onwards. We would nevertheless like you to try to answer.
Birth: Weight____grams Height____cm
At age 18:Weight____kg   Height____cm
Today: Weight____kg   Height____cm

Body type on starting school *(Tick only one)*
Very thin☐   Thin ☐ Normal☐ Fat ☐   Very fat ☐

Have you gained weight after the age of 50 years old?   Yes ☐ No☐
If yes, how many kg?   ………………………. kg

Breast cancer in the near family

How many daughters do you have? …. daughters
How many sisters do you have?   ………..sisters
How many sisters does your mother have? sisters
How many sisters does your father have?   sisters

Have any of your close relatives had breast cancer?

	Yes	No	Don't know	Age at diagnosis
Mother				
Daughter				
Sister				
Mothers mother				
Fathers mother				
Mothers sister				
Fathers sister				

Contraceptives

Have you ever been on the pill, mini-pill or Levonova IUD?*(not the regular IUD. Tick for each line.)*

	Age at start	Age at stop	Total years of use	Never used
Pill				
Mini-pill				
Levonova				

Postmenopausal hormone therapy

Have you ever used hormone pills/plasters?
Yes ☐ No ☐

If yes,
Did you initiate use before your periods had stopped?   Yes ☐   No ☐

How long have you used hormone pill/plasters in all?   ……… years
How old were you when you first used hormone pills/plasters?   ………years

Are you currently using hormone pills/plasters?
Yes ☐ No☐
If yes,
Brand……………………………………..
Strength…………mg

If no, how long has it been since you quit?
…..……..months …………years
Hormones for local vaginal application?
Have you ever used hormone creams/suppositories?   Yes ☐ No ☐
If yes,
Did you initiate use before your periods had stopped?   Yes ☐ No ☐

How long have you used hormone creams/suppositories in all?   ……...Years

How old were you when you first used hormone creams/suppositories?   ……Years

Are you currently using hormone creams/suppositories?   Yes☐   No☐
If yes,
Brand…………………………………..
Strength………mg
If no, how long since you quit?
…..……..months …………years

Diet

We would like to know your <u>usual</u> diet. For each question, tick the average number of times you have consumed each item in the last year, and how much you usually eat/drink each time.

How often do you eat fruit? *(One tick per line)*

	Never/ seldom	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2+ per day
Apples/ pears							
Oranges /citrus							
Bananas							
Other fruit							

How often do you eat vegetables? *(One tick per line)*

	Never/ seldom	1-3 per month	1 per week	2 per week	3 pr week	4-5 per week	6-7 per week
Potatoes							
Carrots							
Cabbage							
Turnip							
Broccoli/ cauliflower							
Mixed salad							
Mixed vegetables (frozen)							
Other vegetables							

How many glass/cups of the following do you usually drink? *(One tick per line)*

	Never/ seldom	1-3 per week	4-6 per week	1-2 per day	3-4 per day	5 + per day
Water						
Milk						
Orange juice						
Coffee						

Meat / meat products /poultry

How often do you eat meat for dinner?
(One tick per line)

	Never/ seldom	1 per month	2-3 per month	1 per week	2 per week	3+ per week
Beef/lamb/pork						
Minced meat /sausage						
Chicken/ Turkey						
Reindeer/ Moose						
Other meat						

Fish / Fish products

How often do you eat fish for dinner?
(One tick per line)

	Never/ seldom	1 per month	2-3 per month	1 per week	2 per week	3+ pr. week
Cod, saithe, halibut, pollack						
Wolfish, flounder, redfish						
Salmon, trout						
Mackerel, herring						

How often do you eat the following kinds of fish dish? *(One tick per line)*

	Never/ seldom	1 per month	2-3 per month	1 per week	2+ per week
Fishcakes/pudding/ balls					
Fried fish, Fish fingers					
Fish stew, Fish-pie					
Other fish dishes					

Do you eat; *(One tick per line)*

	Never/ seldom	1 per week	2-6 per week	daily	1-3 per day
Cod liver oil /pills					
Fish oil pills					
Fish as spread					

Dietary supplements

Do you use other dietary supplements?
(e.g. vitamins / minerals)   Yes☐   No☐
If yes, how often?

Never/seldom	1-3 per month	1 per week	2-6 per week	daily

How many different dietary supplements do you use ?   ......different

Alcohol

Are you a teetotaler?   Yes ☐   No ☐
If no, how often and how much did you drink on average in the last year? *(One tick per line)*

	Never/ seldom	1 per month	2-3 per month	1 per week	2-4 per week	5-6 per week	1+ per day
Light beer (glass)							
Beer (glass)							
White wine (glass)							
Red wine							

(glass)							
Liquor (drinks)							

Physical activity

If you are in paid or unpaid employment, how would you describe your work? (On average last year) *(Tick the box most suitable)*
Mostly sedentary work?................. ☐
(e.g. office work)

Work that requires a lot of
walking/standing?................. ☐
(You do not perspire, and your heart does not beat faster, e.g. shop assistant, teacher, hairdresser)

Work that requires a lot of walking and
lifting?............... ☐
(You perspire a little and your heart might beat faster, e.g. nurse / nurse assistance, postal worker.)

Heavy manual labor?............................... ☐
(You perspire quite a bit and your heart beats faster, e.g. heavy duty care)

What kind of physical activity do you have in your leisure time?
(On average last year) (Tick the box most suitable)
Reading books, watching television or other
sedentary activity? .... ☐

Walking, biking or other forms of activity for at least 2 hours a week?
(Also included walking/biking to/from work, Sunday-trips etc)
.... ☐

Walking, biking or other forms of activity for at
least 4 hours a week? ☐

Exercise regularly several times a week?...... ☐
(You sweat quite a bit and the heart beats faster)

How many hours do you walk outdoors per week? (Walk to work, in the outdoors, ski-trips, running)
(Fill in for each line)

You do not perspire, and your heart does not beat
faster ....hours
You perspire a little and your heart might beat
faster ....hours
You perspire quite a bit and your heart beats faster
..........hours

Smoking habits

Have you ever smoked?   Yes ☐   No ☐

If yes,
Do you currently smoke? (Tick only one box)
Yes, daily..................... ☐
Yes, occasionally............. ☐
No.............................. ☐

If no,
How long ago did you quit?   ......years

How old were you when you started to smoke?
......years
How many years in total have you smoked daily?
....years
If you have smoked daily, we ask you to fill out how many cigarettes you on average smoked daily for each age-period

Age (years)	0-14	15-19	20-29	30-39	40-49	50-59	60-69
Number							

Did any of the adults at home smoke during your upbringing?   Yes ☐   No ☐
If yes, for how many years?   ....years

Have you ever worked at smoke filled workplaces?   Yes ☐   No ☐
If yes, for how long?   ....years

Do you currently live with someone who smokes?
Yes ☐   No ☐
If yes, for how long?   ......years

How long are you daily in smoke filled environments?   ....hours

Mammography screening

Have you previously been to mammography examination? *(Tick only one)*
Yes ☐   No ☐

Do you have any comments on the mammography examination you currently have attended?
....

Finally, we ask if you consent to us contacting you again per mail. We will collect your address from the mammography-screening
Yes ☐   No ☐

Thank you for taking part in the survey!!

ISM SKRIFTSERIE - FØR UTGITT:

1. Bidrag til belysning av medisinske og sosiale forhold i
   Finnmark fylke, med særlig vekt på forholdene blant
   finskættede i Sør-Varanger kommune.
   Av Anders Forsdahl, 1976. (nytt opplag 1990)

2. Sunnhetstilstanden, hygieniske og sosiale forhold i Sør-
   Varanger kommune 1869-1975 belyst ved medisinal-
   beretningene.
   Av Anders Forsdahl, 1977.

3. Hjerte-karundersøkelsen i Finnmark - et eksempel på en
   populasjonsundersøkelse rettet mot cardiovasculære
   sykdommer. Beskrivelse og analyse av
   etterundersøkelsesgruppen.
   Av Jan-Ivar Kvamme og Trond Haider, 1979.

4. D. The Tromsø Heart Study: Population studies of coronary
   risk factors with special emphasis on high density
   lipoprotein and the family occurrence of myocardial
   infarction.
   Av Olav Helge Førde og Dag Steinar Thelle, 1979.

5. D. Reformer i distriktshelsetjenesten III: Hypertensjon i
   distriktshelsetjenesten.
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7.* Blodtrykksovervåkning og blodtrykksmåling.
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8.* Merkesteiner i norsk medisin reist av allmennpraktikere -
   og enkelte utdrag av medisinalberetninger av
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   Av Anders Forsdahl, 1984.

9. "Balsfjordsystemet." EDB-basert journal, arkiv og
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   Av Toralf Hasvold, 1984.

10. D. Tvunget psykisk helsevern i Norge. Rettsikkerheten ved
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    Av Georg Høyer, 1986.

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