Fatty Liver Disease and Changes in Dense Breasts in Pre- and Postmenopausal Women

Fatty Liver Disease and Changes in Dense Breasts in Pre- and Postmenopausal Women: The Kangbuk Samsung Health Study

Running title: Fatty liver and breast density

Yoosun Cho, MD, PhD;[1] Yoosoo Chang, MD, PhD;[2,3,4*] Hyun-Suk Jung;[1] Chan-won Kim; [1] Hyungseok Oh;[1] Eun Young Kim, MD;[1] Hocheol Shin, MD, PhD;[2,5] Sarah H. Wild[6]; Christopher D Byrne [7,8]; and Seungho Ryu, MD, PhD[2,3,4*]

[1]Total Healthcare Center, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

[2]Center for Cohort Studies, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

[3]Department of Occupational and Environmental Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

[4]Department of Clinical Research Design & Evaluation, Samsung Advanced Institute for Health Sciences & Technology, Sungkyunkwan University, Seoul, Republic of Korea

[5]Department of Family Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

[6]Usher Institute, University of Edinburgh, Edinburgh, U.K.

[7]Nutrition and Metabolism, Faculty of Medicine, University of Southampton, Southampton, U.K.

[8]National Institute for Health Research Southampton Biomedical Research Centre, University Hospital Southampton, Southampton, U.K.

[*] **Address correspondence to**

Seungho Ryu, MD, PhD, Department of Occupational and Environmental Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Samsung Main Building B2, 250, Taepyung-ro 2ga, Jung-gu, Seoul 04514, Republic of Korea

Tel: +82-2-2001-5137; fax: +82-2-757-0436; e-mail: sh703.yoo@gmail.com

Yoosoo Chang, MD, PhD, Department of Occupational and Environmental Medicine, Kangbuk Samsung

Hospital, Sungkyunkwan University School of Medicine, Samsung Main Building B2, 250, Taepyung-ro 2ga, Jung-gu, Seoul 04514, Republic of Korea

Tel: +82-2-2001-5139; fax: +82-2-757-0436; e-mail: yoosoo.chang@gmail.com

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Abstract

Purpose: While increased breast density is a risk factor for breast cancer, the effect of fatty liver disease on breast density is unknown. We investigated whether fatty liver is a risk factor for changes in breast density over ~4 years of follow-up in pre- and postmenopausal women.

Methods: This study included 74,781 middle-aged Korean women with mammographically determined dense breasts at baseline. Changes in dense breasts were identified by more screening mammograms during follow-up. Hepatic steatosis (HS) was measured using ultrasonography. Flexible parametric proportional hazards models were used to determine the adjusted hazard ratios (aHRs) and 95% confidence intervals (CIs), and a Weibull accelerated failure time model (AFT) was used to determine the time ratios (TRs) and 95% CIs.

Results: During a median follow-up of 4.1 years, 4,022 women experienced resolution of the dense breasts. The association between HS and dense breast resolution differed by the menopause status (*P* for interaction<0.001). After adjusting for body mass index and other covariates, the aHRs (95% CI) for dense breast resolution comparing HS to non-HS were 0.81 (0.70–0.93) in postmenopausal women, while the association was converse in premenopausal women with the corresponding HRs of 1.30 (1.18–1.43). As an alternative approach, the multivariable-adjusted TR (95% CI) for dense breast survival comparing HS to non-HS were 0.81 (0.75-0.87) and 1.19 (1.06-1.33) in premenopausal and postmenopausal women, respectively.

Conclusion: The association between HS and changes in dense breasts differed with the menopause status. HS increased persistent dense breast survival in postmenopausal women but decreased it in premenopausal women. menopause status.

Keywords: dense breast, mammogram, mammographic breast density, resolution of dense breast, persistent dense breast, cohort study

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3) Figures: 1

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD), the most common chronic liver disease, is a multisystem disease, associated with increased risks of cardiometabolic morbidity and mortality from all-cause, cardiovascular disease, and extrahepatic cancers in addition to liver-related morbidity and mortality [1, 2]. Moreover, NAFLD may be an additional risk factor beyond obesity for extrahepatic cancers including breast cancer [3-6], which is the most common female cancer worldwide [7, 8]. According to a recent meta-analysis, NAFLD was associated with a nearly 40% higher risk of breast cancer [9].

The results of a large-scale cohort study of Korean women suggested that a high fatty liver index (FLI), a proxy marker of NAFLD, was associated with increased breast cancer risk in postmenopausal but not premenopausal women [10]. Another cohort study demonstrated a positive association between NAFLD and breast cancer risk [3]; however, the role of menopause status in the association was not investigated. A recent meta-analysis found that body mass index (BMI), a measure of general adiposity, was differently associated with breast cancer risk before and after menopause [11, 12]. However, studies on the differential effect of fatty liver on breast cancer risk by menopause status are very limited.

Breast density is a strong risk factor associated with a 4–5-fold increased risk of breast cancer in both pre- and postmenopausal women [13-15]. However, breast density is not a static condition and the extent of breast density decreases with age by an average of 1 cm^2/year, with a sharp decline over menopause; however, about 30% of postmenopausal women have high breast density [16, 17]. Additionally, longitudinal changes in mammographic density may be more helpful than a single measure to identify risk for breast cancer [18]. Several cohort studies found that both dense breasts at baseline and their persistence over time may be independent risk factors for breast cancer [18-20]. Therefore, it is crucial to determine the risk factors of persistently increased breast density to understand its underlying mechanism.

We hypothesized that fatty liver, as either an ectopic fat marker or hepatic manifestation of metabolic syndrome, affects changes in the breast density, resolution, or persistence of dense breasts; the latter is a strong risk factor for breast cancer; and this association would differ by menopause status. Therefore, we investigated the association between hepatic steatosis (HS) and changes in dense breasts among pre- and postmenopausal women with dense breasts at baseline who participated in a regular health screening program, including repeated mammograms, while accounting for the interaction of this association with the menopause status.

METHODS

Study population

This cohort study included Korean adults who participated in a health examination annually or biennially at Kangbuk Samsung Hospital Total Healthcare Centers in Seoul and Suwon, South Korea. We initially restricted the study population to 93,727 women, aged ≥35 years with mammographic dense breasts at baseline who underwent a health check-program including mammograms between 2011 and 2017 and underwent at least one follow-up mammogram through December 31, 2019. The prevalence of dense breasts among premenopausal women was >93%, which was in agreement with previous reports [21, 22]; thus, the study population was restricted to women who had dense breasts at baseline and were followed up for the resolution or persistence of dense breast. After excluding participants who met the exclusion criteria (Figure 1), 74,781 participants were included in the analysis. This study was approved by the Institutional Review Board of Kangbuk Samsung Hospital (IRB No. KBSMC 2021-01-038), which waived the required for informed consent due to the use of anonymized retrospective data routinely collected during the health screening process.

Data collection

Baseline demographic characteristics, lifestyle behaviors, diet, reproductive factors, medical condition, and medication use were assessed via a standardized, structured, self-administered questionnaire. Parity was defined as the number of previous pregnancies, including live births and stillbirths. Menopause was defined as amenorrhea lasting for ≥12 months; additionally, women aged ≥55 years without data for menopause were considered to be postmenopausal [23]. Education levels were categorized as less than college graduate or college graduate or more. Smoking (never, former, and current smoker) and alcohol consumption (<10 g/day, 10–<20 g/day, or ≥20 g/day)[24] were also assessed. Physical activity level was collected using the validated Korean version of the International Physical Activity Questionnaire short form [25] and the participants were categorized into one of three categories: inactive, minimally active, or health-enhancing physical activity (HEPA) meeting either of the two criteria: (i) vigorous-intensity activity on ≥3 days per week totaling ≥1,500 MET min/week, or (ii) 7 days with any combination of walking, moderate-intensity, or vigorous-intensity activities achieving at least 3,000 MET min/week [25]. Sleep quality score (≥6 for ‘poor’ and <6 for ‘good’) was assessed using Pittsburgh Sleep Quality Index (PSQI), a validated, self-administered questionnaire used to evaluate sleep quality during the past month [26].

Blood pressure (BP) and anthropometric parameters were measured by a trained nurse. Obesity was defined as BMI ≥25 kg/m^2 according to the Asian-specific criteria. Hypertension was defined as a systolic BP ≥140 mmHg, diastolic BP ≥90 mmHg, or current use of BP-lowering medication. Fasting serum lipid profiles, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), albumin, platelet, insulin, and high-sensitivity C-reactive protein (hsCRP) levels were also assessed. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as fasting blood insulin (mU/mL) × fasting blood glucose (mmol/L)/22.5. Diabetes was defined as fasting blood glucose ≥126 mg/dL or current use of insulin or anti-diabetic drugs.

Mammographic measures and definitions of breast density

Standard four-view mammograms (bilateral craniocaudal and mediolateral oblique views) for both baseline and follow-up visits were conducted using a full-field digital mammography system on the same day as other health examinations (Senograph 2000D/DMR/DS, General Electric Company, Milwaukee, WI, USA or Selenia, Hologic, Marlborough, MA, USA) in Suwon and Seoul centers [19]. Experienced breast imaging radiologists reported the findings according to the American College of Radiology Breast Imaging Reporting and Data System (BI-RADS). According to the BI-RADS, breast density was categorized into four groups: 1) almost entirely fat (≤25% fibroglandular tissue), 2) scattered fibroglandular (26–50%), 3) heterogeneously dense (51–75%), or 4) extremely dense (>75%) [27]. We classified women with dense breasts as those with "heterogeneously dense" or "extremely dense" breasts [28]. Resolution of dense breasts was defined as the presence of dense breasts at baseline but not during follow-up. Otherwise, "persistently" dense breasts were defined as participants with dense breasts at baseline and throughout follow-up.

Liver ultrasound measures and definition of fatty liver

Abdominal ultrasounds were performed by experienced radiologists who were unaware of the study aims. HS was diagnosed based on the following standard criteria: a diffuse increase in fine echoes in the liver parenchyma compared to the kidney or spleen parenchyma, deep beam attenuation, and bright vessel walls [29]. The inter-observer and intra-observer reliability values of the diagnosis of HS were substantial (kappa statistic of 0.74) and excellent (kappa statistic of 0.94), respectively [30]. We used Fibrosis-4 (FIB-4), a non-invasive liver fibrosis score, to evaluate HS severity [31]. The FIB-4 index was calculated as follows: FIB-4 = (age

[years]×AST [U/L]) / (platelet count ($\times 10^9$/L)×ALT (U/L)$^{1/2}$) [32]. The FIB-4 cutoff points were ≥2.67 for a high probability of advanced liver fibrosis, <1.3 for a low probability of advanced fibrosis, and 1.30–2.66 for an intermediate probability of advanced fibrosis [31, 32].

Statistical analysis

The primary endpoint was the resolution of dense breasts during follow-up (no resolution indicated the persistence of dense breasts). Since the association between HS and changes in breast density differed significantly by menopause status, all analyses were presented separately in pre- and postmenopausal women. For analysis of the association between HS and incident resolution of dense breasts, if the resolution occurred during follow-up, subsequent observations were not incorporated in further analysis. Each participant was followed from the baseline exam until either resolution or the last health exam before December 31, 2019. Incident resolution rate was calculated as the number of incident resolution cases divided by the follow-up duration in person-years. Since the onset of dense breast resolution occurred at an unknown time point between the visit when the resolution of dense breasts was identified and the previous visit, we used a flexible parametric proportional hazards model with natural cubic splines of log time with 3 internal knots at the 25th, 50th, and 75th percentiles which accounts for interval-censored events [33]. The adjusted hazard ratio (aHR) with a 95% confidence interval (CI) for the incident resolution of dense breast was estimated.

We performed a sensitivity analysis to estimate the relative mean survival of dense breast by hepatic steatosis according to menopause status using a Weibull accelerated failure time (AFT) model [34]. Along with a Cox proportional hazards (PH) model, an AFT is an alternative method of survival analysis describing the estimated delay until an event occurs with exposure relative to the reference group [35]. We calculated time ratios (TRs) of persistent dense breast survival with HS by menopause status. For example, a time ratio of 2 would mean that the time until an event occurs is twice as long in the exposure group (HS group) relative to the control group (non-HS group).

Model 1 was adjusted for age, health screening examination center (Seoul or Suwon), year of screening exam, alcohol consumption, smoking, physical activity, total energy intake, education level, history of hypertension, history of diabetes, sleep duration, sleep quality, age at menarche, female hormone medication, and parity. Model 2 was adjusted for all covariates in model 1 plus BMI as BMI is a strong risk factor for NAFLD regardless of menopause, but may differently affect breast cancer risk according to menopause status [36].

Additionally, we performed an analysis on longitudinal associations between HS and subsequent changes in breast density over time according to the menopause status using a linear mixed model with random intercept and random slopes while treating BI-RADS scores 1–4 as continuous outcomes. Predefined subgroup analyses were also performed in non-obese women with BMI (<25 kg/m^2) and women without significant alcohol intake defined as ≥20 g/day. Likelihood ratio tests were used to test interactions by menopause (premenopausal vs. postmenopausal women), comparing models with and without multiplicative interaction terms. All analyses were conducted using STATA version 16.0 (StataCorp LP, College Station, TX, USA). We defined the P-value for statistical significance as a two-sides P<0.05.

RESULTS

At baseline, the mean ages (standard deviation) of the 68,130 premenopausal women and 6,501 postmenopausal women with dense breasts at baseline were 39.9 (4.2) and 54.1 (7.5) years, respectively (Table 1). Both pre- and postmenopausal women with HS tended to be older and less educated, have a lower alcohol intake, higher total daily calorie intake, higher prevalence of obesity, hypertension, and diabetes, higher BMI, BP, glucose, total cholesterol, AST, GGT, and hsCRP levels, and HOMA-IR compared to those without HS. Postmenopausal women with HS tended to be less physically active and were less likely to receive menopausal hormone treatment than those without HS.

During a median follow-up of 4.1 years (interquartile range: 2.2-6.5 years), 4,022 women experienced resolution of dense breast (resolution rate, 10.0 per 1000 person-years for premenopausal women; 39.8 per 1000 person-years for postmenopausal women) (Table 2). The association between HS and dense breast resolution differed by menopause status (*P* for interaction<0.001). After adjusting for BMI and other confounders (model 2), the aHRs (95% CI) for dense breast resolution comparing HS to non-HS were 0.81 (0.70-0.93) in postmenopausal women. This association was the opposite in premenopausal women. Specifically, the aHRs for dense breast resolution comparing HS and to non-HS were 1.30 (1.18–1.43).When the change in HS status and other covariates during follow-up were treated as time-varying covariates, the associations of HS severity and dense breast resolution between simple HS, referred to HS with a low probability of advanced hepatic steatosis defined as an FIB-4 index of <1.3, and dense breast resolution were similarly observed. Furthermore, in an alternative approach based on AFT to estimate time ratios of dense breast survival with HS by menopause status

instead of hazard ratios, HS was associated with persistent dense breast survival in the postmenopausal women, whereas an inverse association was observed in premenopausal women (Table 3). After adjusting for BMI and other covariates, the TRs (95% CI) for dense breast survival comparing HS to non-HS were 0.81 (0.75-0.87) in premenopausal women but 1.19 (1.06-1.33) in postmenopausal women (*P* for interaction<0.001).

Even in women without significant alcohol consumption (defined as ≥20 g/day), the association between NAFLD and dense breast resolution was also observed and was also modified by menopause (*P* for interaction <0.001). Specifically, aHRs (95% CI) for dense breast resolution comparing NAFLD to no NAFLD was 1.28 (1.15–1.42) for premenopausal women and 0.83 (0.70–0.99) for postmenopausal women. Additionally, among non-obese women (BMI <25 kg/m^2), the association between HS and dense breast regression was also observed, with aHRs (95% CI) for dense breast regression comparing HS to non-HS of 1.37 (1.20–1.57) for premenopausal women and 0.81 (0.66–0.98) for postmenopausal women.

In an alternative approach estimating subsequent changes in breast density as a continuous variable, the average breast density decreased over 5 years in pre- and postmenopausal women with/without HS (Supplementary Table 1). Compared to women without HS, those with HS showed a significantly greater decrease in breast density category over 5 years among both pre- and postmenopausal women. However, this difference was stronger in premenopausal women than in postmenopausal women with a corresponding mean 5-year BI-RADS score (95% CI) of -0.086 (-0.094~-0.077) in premenopausal women and -0.028 (-0.053~-0.003) in postmenopausal women.

When the association between HS severity and changes in the dense breast were further analyzed (Supplementary Table 2), HS with intermediate/high FIB-4 was not significantly associated with changes in dense breasts in pre- or postmenopausal women. In premenopausal women, the aHRs for dense breast resolution comparing HS plus a low FIB-4 score and HS plus an intermediate/high FIB-4 score to non-HS were 1.29 (1.17–1.42) and 1.52 (1.02–2.27), respectively, but this association was attenuated and no longer significant in the time-dependent model. In postmenopausal women, the corresponding aHRs for dense breast resolution were 0.75 (0.64–0.89) and 0.96 (0.77–1.20), respectively.

Other explanatory factors which significantly predict resolution of breast density were age, menopause, alcohol

intake of ≥20 g/day, current smoking, physical activity (specifically HEPA, health-enhancing physical activity), higher total daily energy intake, parity, and BMI (Supplementary Table 3). However, even after adjustment for these variables, the association between HS and resolution of breast density remained significant.

DISCUSSION

In this study, we demonstrated that HS was associated with the resolution of dense breast in premenopausal women but with persistence of dense breasts in postmenopausal women. When changes in the HS status and other covariates during follow-up were treated as time-varying covariates, similar associations were observed. In an alternative approach based on time ratios, HS was also positively associated with persistent dense breast survival in postmenopausal women but inversely associated with it in premenopausal women. However, the association among postmenopausal women was inconsistent across analyses using dense breast as a binary endpoint and that using the BI-RADS category as a continuous variable. Our findings indicate that the association between fatty liver disease and breast density differs according to the menopause status.

However, the involvement of fatty liver in resolution of dense breasts and variation of the association according to the menopause status is still unclear. No previous studies have addressed the association between fatty liver disease and breast density. However, some studies have suggested an association between fatty liver disease and breast cancer. In the Asian population, a large population-based study involving a 7-year follow-up demonstrated that a high fatty liver index, an index of NAFLD, was associated with an increased incidence of breast cancer in postmenopausal women, whereas no such association was found in premenopausal women [10]. According to a cohort study in Korea, almost two times higher risk of breast cancer was observed in individuals with ultrasound-diagnosed NAFLD than in those without, independent of age, smoking status, serum lipid profiles, and other confounders [3].. However, the impact of menopause status on this association has not been investigated [3]. A case-control study in Korea demonstrated that NAFLD was significantly associated with breast cancer, independent of laboratory and reproductive factors, and although obesity and NAFLD are correlated in patients with breast cancer, the association between NAFLD and breast cancer was observed only in women without obesity [37]. Evidence on the association between NAFLD and breast cancer risk has been emerging. However, whether or not the risk of breast cancer due to NAFLD could differ according to the menopause status or metabolic factors, such as obesity is still unclear.

Although our study did not explore the association between NAFLD and breast cancer incidence, it is the first study to demonstrate a relationship between fatty liver and mammographically established dense breasts, a potential surrogate marker of breast cancer. Our findings suggest that changes in dense breast tissue caused by fatty liver disease are independent of BMI and differ according to the menopause status. Metabolic syndrome and insulin resistance, key factors of NAFLD, were associated with mammographic density [38]. Studies suggested that breast density can mediate some associations between established breast cancer risk factors and breast cancer, although the extent can vary with risk factors and the menopause status [39, 40]. Currently, it is unclear how fatty liver disease is involved in the resolution of dense breasts and why this association varies with the menopause status.

Considering that fatty liver disease is closely associated with obesity, it might be inversely associated with mammographic density in premenopausal women. Several studies involving pre- and postmenopausal women have shown an inverse association between BMI and the breast density volume percentage but positive correlation with the non-dense volume [36, 41, 42]. Other cross-sectional studies have shown inverse associations between waist circumference, abdominal obesity, and mammographic density in premenopausal women [38, 43]. Therefore, individuals with fatty liver, an ectopic fat marker, may have high levels of fat tissue and low mammographic percent density, regardless of the amount of dense breast tissue. In our study, the increased resolution of mammographic density in premenopausal women with HS remained significant after adjusting for BMI, indicating an independent association between NAFLD and mammographic density consistent with those of other adiposity indices with mammographic density.

The reasons for this association between NAFLD and the resolution of dense breasts in premenopausal women are not fully understood, although a relationship between NAFLD, female hormones, and breast density has been proposed. Estradiol, the most predominant form of circulating estrogen, is mainly synthesized and secreted by the ovaries in premenopausal women [44]. Anovulation is more likely in overweight or obese women than in non-obese women [45], possibly inducing abnormal patterns of female hormones. Obese premenopausal women have lower estrogen levels, which is due to the high uptake of estradiol into adipose tissue and high liver clearance of estrogen [46]. Similarly, premenopausal women with fatty liver disease had lower circulating estradiol levels compared to premenopausal women without fatty liver disease [47], partly explaining the

observed association between HS and the resolution of dense breasts in premenopausal women in our study.

In postmenopausal women, HS was associated with the persistence of mammographic density. In postmenopausal women, instead of estrogen production, androgen production in the ovary becomes predominant [48]. Adipose tissue might also synthesize androgens through increased expression of 17β-hydroxysteroid dehydrogenase type 5, a catalytic enzyme that plays a role in the conversion of androstenedione to testosterone [49]. A longitudinal study demonstrated that the levels of androgens, testosterone, and their derivatives were inversely associated with an annual decrease in mammographic density over time in women [50]. Therefore, elevated androgen levels in postmenopausal women with fatty liver disease might explain the association between NAFLD and persistence of dense breast. However, further studies are required to elucidate the mechanism underlying the association between NAFLD and changes in breast density according to the menopause status.

The "resolution" of a dense breast is not a sudden, permanent change but is more likely to be a gradual change such that a quantitative measure of breast density as a continuous variable may be more relevant to examine changes in breast density over time. In a linear mixed model assessing changes in dense breasts as continuous measures, the decrease in breast density over 5 years in the HS group was greater than that in the non-HS group in both pre- and postmenopausal women. The decrease in breast density in postmenopausal women was contradictory to the findings of the main analysis using resolution of dense breasts as a binary outcome, which showed that HS increased the risk of persistent dense breasts in postmenopausal women. Possible reasons for this discrepancy are as follows. First, most women still had dense breasts (BI-RADS score 3 or 4) over 5 years, and a slight decrease in density resulting in change of category of dense breast would differ from a decrease that results in categorization as non-dense breasts. Second, a discrepancy between BI-RADS breast density categorization and binary categorization ("dense" vs. "non-dense") might occur. For example, the extent of decrease in BI-RADS scores from 3 to 2 and 4 to 3 is one grade change, but the clinical significance is different; the former results in classification as a "non-dense" breast, while the latter as a "dense" breast, requiring consideration of supplementary tests to find any lesions masked by dense breast tissues. Moreover, considering the characteristics of the BI-RADS classification, even if the overall assessment of breast density results in a breast being classified as "non-dense," a radiologist can assign it as a dense breast if there is any focally dense

area [51]. Therefore, a change in the breast density according to BI-RADS category may not always correspond to an overall change in the quantitative measurement of breast density. BI-RADS density category, a standard for reporting breast density in clinical practice, is a qualitative measure of mammographic density. BI-RADS breast density is estimated visually and may reflect the quantitative value of density as well as other features including the distribution and parenchymal pattern, whereas automated quantitative measures algorithmically assess absolute dense areas or volume [52]. It is unclear which aspects can explain the discrepancy between findings based on the binary endpoint of dense breast and those based on BI-RADS categories as continuous variables. Unfortunately, information on automated quantitative measures was unavailable because our study was based on de-identified, retrospective cohort data of individuals who participated in a routine health screening, and in this, only the BI-RADS category of breast density assessed by radiologists was available. Future studies using quantitative measures of mammographic density combined with BI-RADS breast density measures would help better understand the association between HS and subsequent changes in both qualitative and quantitative breast density measures and their significance in the breast cancer risk stratified by the menopause status.

Additionally, changes in dense breasts were not significantly influenced by HS severity, showing no clear dose-response between them. Sample sizes of women with NAFLD and those with a high FIB-4 score and the number of incident cases in each group were small. HS severity was evaluated by the FIB-4 index rather than histopathology. Therefore, further studies with larger sample sizes and histological confirmation of HS severity are required to elucidate the dose-response relationship between HS severity and changes in dense breasts.

Previous studies have suggested that factors such as aging, elevated BMI, high physical activity [17], high parity, postmenopausal status [53], and current smoking status (due to anti-estrogenic effect) [54] were associated with low mammographic density. In our study, factors including aging, menopause, current smoking, physical activity, parity, and BMI were significantly associated with the resolution of breast density, in line with the previous findings. However, the association between HS and breast density resolution remained significant after adjusting for confounders including BMI, indicating an independent association between NAFLD and changes in breast density.

Our study has some limitations. First, histological information on liver steatosis and fibrosis from liver biopsy

was unavailable. The non-invasive diagnosis of fatty liver disease based on ultrasonography findings has been validated, has shown acceptable accuracy, and is widely used in population-based studies [55]. Second, breast density was determined based on BI-RADS scores, a qualitative measure, but no quantitative measure was utilized. However, qualitative density assessments by radiologists have a reasonable and moderate agreement with automated software measurements and are widely used in clinical settings and large-scale population studies [18, 56]. Additionally, we did not perform intra- and inter-observer reliability tests among the radiologists. Different radiologists were involved over time; however, they were unaware of the study's aims. Third, as the menopause status was collected from a self-reported questionnaire, misclassification was possible. Finally, as our data included healthy and middle-aged, well-educated Korean women with easy access to healthcare resources, the generalizability of our findings may be limited. However, to the bests of our knowledge, this is the first study to investigate the association between fatty liver disease and resolved or persistent dense breasts according to the menopause status. Our findings suggest a potential explanation for the different associations between fatty liver disease and the breast cancer risk reported in previous studies by suggesting that the effect depends on the menopause status.

CONCLUSIONS

This cohort study demonstrated that HS was associated with the resolution of dense breasts in premenopausal women and with persistence of dense breasts in postmenopausal women. In an alternative approach based on AFT to estimate time ratios of dense breast survival with HS, HS increased persistent dense breast survival in postmenopausal women but decreased it in premenopausal women. However, associations among postmenopausal women were inconsistent across analyses using dense breast as a binary endpoint and that using BI-RADS category as a continuous variable. Further investigations using both qualitative and quantitative breast density measures are required to establish how associations between fatty liver disease and changes in breast density by menopause status may differently affect the breast cancer risk in pre- and postmenopausal women.

DECLARATIONS

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Conflicts of interest/Competing interests

All authors declare that they have no conflicts of interest

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Code availability

STATA Code is available upon request.

Author's contributions

YCho, YChang, and SR planned, designed and implemented the study, including quality assurance and control. SR analyzed the data and designed the study's analytic strategy. HS and SR supervised field activities. YCho, SR, YChang, CWK, HJ, EK and HO conducted the literature review and prepared the Materials and Methods and Discussion sections of the text. YCho and YChang drafted the manuscript. All authors interpreted the results and SR, SHW, and CDB contributed to critical revisions of the manuscript. All authors approved the final version of this manuscript.

Ethics approval

This study was approved by the Institutional Review Board of Kangbuk Samsung Hospital (IRB No. KBSMC 2021-01-038), which waived the required for informed consent due to the use of anonymized retrospective data routinely collected during the health screening process.

Consent to participate

Not applicable

Consent for publication

Not applicable

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Additional file 1:

Supplementary Table 1. 5-year change in BI-RADS score for women with HS and without HS according to the menopause status among women with BI-RADS score 3 or 4

Supplementary Table 2. Hazard ratios (95% CI) of dense breast resolution by HS and its severity based on the FIB-4 index

Supplementary Table 3. Hazard ratios (95% CI) of dense breast resolution with explanatory factors.

Figure 1. A flow chart of study population; A total of 74,781 patients were finally parcitipated in this study.

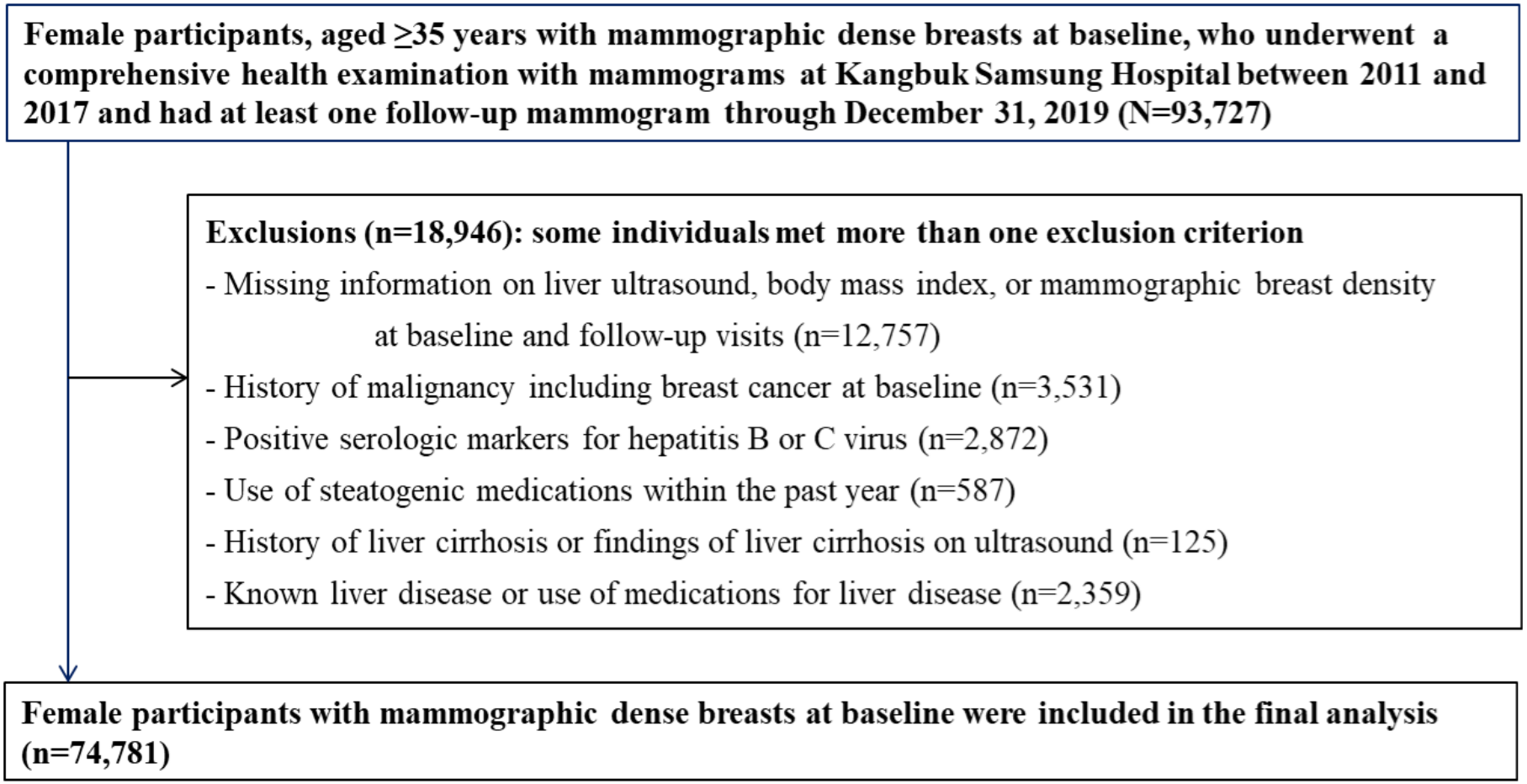
Female participants, aged ≥35 years with mammographic dense breasts at baseline, who underwent a comprehensive health examination with mammograms at Kangbuk Samsung Hospital between 2011 and 2017 and had at least one follow-up mammogram through December 31, 2019 (N=93,727)
Exclusions (n=18,946): some individuals met more than one exclusion criterion
- Missing information on liver ultrasound, body mass index, or mammographic breast density at baseline and follow-up visits (n=12,757)
- History of malignancy including breast cancer at baseline (n=3,531)
- Positive serologic markers for hepatitis B or C virus (n=2,872)
- Use of steatogenic medications within the past year (n=587)
- History of liver cirrhosis or findings of liver cirrhosis on ultrasound (n=125)
- Known liver disease or use of medications for liver disease (n=2,359)
Female participants with mammographic dense breasts at baseline were included in the final analysis (n=74,781)

Table 1. Baseline characteristics according to hepatic steatosis among pre- and postmenopausal women with dense breasts at baseline.

	Premenopausal women (n = 68,130)		Postmenopausal women (n = 6,501)	
Characteristics	No hepatic steatosis	Hepatic steatosis	No hepatic steatosis	Hepatic steatosis
Number	60,976	7,154	4,822	1,679
Age (years) [a]	39.7 (4.1)	41.6 (4.8)	53.7 (7.6)	55.3 (6.8)
Ever/current smoker (%)	10.7	11.3	8.5	7.4
Alcohol intake (%)[c]	6.0	5.4	5.0	5.3
HEPA (%)	14.9	14.4	22.7	18.1
High education level (%)[d]	81.6	72.1	48.9	40.6
Hypertension (%)	2.6	10.1	16.80	31.54
Diabetes (%)	0.6	8.0	4.4	16.4
Obesity (%)	6.7	49.6	11.0	45.4
Parity (%)[e]	10.2	13.1	22.4	24.9
Female hormone medication	1.7	1.8	8.5	5.7

Poor sleep quality (%)	20.2	21.2	22.8	24.2
Sleep duration (hours) [a]	6.8 (1.2)	6.6 (1.2)	6.5 (1.3)	6.5 (1.3)
Age at menarche (years) [a]	13.8 (1.5)	13.8 (1.5)	14.9 (1.7)	14.9 (1.7)
Body mass index (kg/m^2) [a]	21.2 (2.4)	25.3 (3.3)	22.0 (2.5)	24.9 (2.8)
Systolic BP (mmHg)[a]	101.8 (10.8)	109.4 (13.2)	108.5 (13.8)	114.8 (13.9)
Diastolic BP (mmHg)[a]	65.2 (8.2)	69.9 (9.6)	68.9 (9.2)	72.2 (9.2)
Glucose (mg/dL) [a]	91.2 (8.7)	99.4 (19.9)	94.5 (12.02)	104.1 (23.8)
Total cholesterol (mg/dL) [a]	184.9 (30.0)	200.1 (35.1)	205.6 (35.4)	212.6 (40.0)
LDL-C (mg/dL) [a]	112.6 (27.7)	131.1 (32.5)	127.8 (32.4)	139.6 (36.9)
HDL-C (mg/dL) [a]	73.1 (23.1)	64.3 (30.9)	76.3 (29.0)	66.6 (34.1)
Triglyceride (mg/dL) [b]	71 (56–93)	114 (82–159)	81 (61–109)	124 (90–171)
AST (U/L) [b]	17 (14–19)	18 (15–23)	19 (17–23)	21 (18–27)
ALT (U/L) [b]	13 (10–16)	18 (13–25)	13 (10–16)	18 (13–25)
GGT (U/L) [b]	13 (10–17)	19 (14–27)	15 (12–21)	21 (16–32)
Albumin (g/dL) [a]	4.53 (0.24)	4.53 (0.24)	4.56 (0.25)	4.59 (0.25)
Platelet ($\times 10^9$/L) [a]	258.8 (57.3)	290.7 (63.4)	245.0 (52.5)	266.4 (56.4)
hsCRP (mg/L) [b]	0.3 (0.2–0.5)	0.8 (0.4–1.5)	0.3 (0.2–0.7)	0.8 (0.4–1.4)
HOMA-IR[b]	1.03 (0.70–1.45)	1.89 (1.28–2.77)	0.93 (0.62–1.36)	1.71 (1.15–2.46)
Total calorie intake (kcal/d) [b,f]	1,352 (1004-1725)	1,427 (1065-1827)	1,349 (1010-1687)	1,428 (1104-1811)

Data are expressed as [a]mean (standard deviation), [b]median (interquartile range), or percentage.
[c] ≥20 g/day; [d]≥College graduate; [e]≥3 times; [f]Among 44,962 participants with plausible estimated energy intake levels (within three standard deviations from the log-transformed mean energy intake).
Abbreviations: ALT, alanine aminotransferase; BP, blood pressure; GGT, gamma-glutamyl transpeptidase; HDL-C, high-density lipoprotein-cholesterol; HEPA, health-enhancing physically active; hsCRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein-cholesterol

Table 2. Hazard ratios (95% CI) of dense breast resolution by hepatic steatosis (HS)

	Person-years (PY)	Resolution cases	Resolution rate (/ 10^3 PY)	Age-adjusted HR (95% CI)	Multivariable-adjusted HR (95% CI)[a]		HR (95% CI)[b] in a model using time-dependent variables
					Model 1	Model 2	
Premenopause							
No HS	278002.7	2291	8.2	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
HS	30732.7	795	25.9	2.72 (2.51-2.96)	2.62 (2.41-2.84)	1.30 (1.18-1.43)	1.33(1.21 -1.46)
Postmenopause							
No HS	17592.1	632	35.9	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
HS	5916.0	304	51.4	1.28 (1.12-1.47)	1.26 (1.09-1.44)	0.81 (0.70-0.93)	0.82 (0.71-0.94)

P<0.001 for the overall interaction between menopause status and hepatic steatosis for dense breast resolution (multivariable-adjusted model 2).

[a] Estimated from parametric proportional hazard models for dense breast resolution with hepatic steatosis. Multivariable model 1 was adjusted for age, center, year of screening exam, alcohol consumption, smoking, physical activity, total energy intake, education level, hypertension, diabetes, sleep duration, sleep quality, age at menarche, female hormone medication, and parity; model 2: model 1 plus adjustment for BMI

[b]Estimated from parametric proportional hazard models for dense breast resolution with hepatic steatosis, smoking, alcohol consumption, physical activity, BMI, total energy intake, hypertension, diabetes, sleep duration, sleep quality, female hormone medication, and parity as time-dependent categorical variables and baseline age, center, year of screening exam, education level, and age at menarche as time-fixed variables.

Abbreviations: HS, hepatic steatosis; BMI, body mass index; CI, confidence interval; HR, hazard ratio

Table 3. Relative mean survival (95%, CI) of dense breast relative to hepatic steatosis (HS) according to menopause status.

	Time ratios (95% CI) [a]	
	Model 1	Model 2
Premenopause		
No HS	1.00 (reference)	1.00 (reference)
HS	0.45 (0.42-0.49)	0.81 (0.75-0.87)
Postmenopause		
No HS	1.00 (reference)	1.00 (reference)
HS	0.83 (0.74-0.93)	1.19 (1.06-1.33)

P<0.001 for the overall interaction between menopause status and hepatic steatosis for relative mean survival of dense breast (multivariable-adjusted model 2).

[a] Relative mean survival is estimated in a Weibull accelerated failure time model so that estimates above 1 indicate persistent dense breast survival. For example, the estimate of 0.45 indicates a 55% shorter mean persistent dense breast survival associated with HS vs. No HS and the estimate of 1.19 indicates a 19% longer mean persistent dense breast survival associated with HS vs. No HS

Multivariable model 1 was adjusted for age, center, year of screening exam, alcohol consumption, smoking, physical activity, total energy intake, education level, hypertension, diabetes, sleep duration, sleep quality, age at menarche, female hormone medication, and parity; model 2: model 1 plus adjustment for BMI

Abbreviations: HS, hepatic steatosis; BMI, body mass index; CI, confidence interval

Supplementary Table 1. 5-year change in BI-RADS score for women with HS and without HS according to the menopause status among women with BI-RADS score 3 or 4

	Premenopause		Postmenopause	
	No HS	HS	No HS	HS
Mean BI-RADS score (95% CI)[a]				
Baseline	3.864 (3.822~3.907)	3.848 (3.801~3.894)	3.701 (4.572~3.830)	3.700 (3.564~3.836)
Year 5	3.842 (3.800~3.885)	3.740 (3.694~3.786)	3.557 (3.428~3.686)	3.528 (4.393~3.664)
5-year difference (year 5 – baseline)	-0.022 (-0.025~-0.019)	-0.107 (-0.116~-0.099)	-0.144 (-0.157~-0.130)	-0.172 (-0.194~-0.149)
Difference in mean BI-RADS score (95% CI)[a]				
Baseline	0 (reference)	-0.017 (-0.029~-0.004)	0 (reference)	-0.001 (-0.029~0.027)
Year 5	0 (reference)	-0.102 (-0.114~-0.091)	0 (reference)	-0.029 (-0.054~-0.003)
Difference in differences (year 5 –	0 (reference)	-0.086 (-0.094~-0.077)	0 (reference)	-0.028 (-0.053~-0.003)

baseline)

[a] Estimated from linear mixed models with random intercept and random slopes used with BI-RADS 1–4 as the continuous outcome

Multivariable model was adjusted for HS, smoking, alcohol consumption, physical activity, BMI, total energy intake, hypertension, diabetes, sleep duration, sleep quality, female hormone medication, and parity as time-dependent variables and baseline age, center, year of screening exam, education level, and age at menarche as time-fixed variables.

Abbreviations: HS, hepatic steatosis; BMI, body mass index; CI, confidence interval

Supplementary Table 2. Hazard ratios (95% CI) of dense breast resolution by HS and its severity based on the FIB-4 index

	PY	Resolved cases	Resolution rate (/10^3 PY)	Age-adjusted HR (95% CI)	Multivariable-adjusted HR (95% CI)[a]		HR (95% CI)[b] in a model using time-dependent variables
					Model 1	Model 2	
Pre-menopause							
No HS	278,002.7	2,291	8.2	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
HS	30,038.3	770	25.6	2.72 (2.51–2.96)	2.61 (2.40–2.84)	1.29 (1.17–1.42)	1.31 (1.20–1.44)
HS plus FIB-4 ≥1.3	694.5	25	36.0	2.90 (1.95–4.31)	2.76 (1.86–4.10)	1.52 (1.02–2.27)	1.37 (0.98–1.93)
Post-menopause							
No HS	17,592.1	632	35.9	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
HS	4,657.6	208	44.7	1.24 (1.06–1.45)	1.19 (1.02–1.40)	0.75 (0.64–0.89)	0.77 (0.66–0.91)
HS plus FIB-4 ≥1.3	1,258.4	96	76.3	1.40 (1.13–1.74)	1.43 (1.15–1.78)	0.96 (0.77–1.20)	0.88 (0.71–1.09)

P<0.001 for the overall interaction between the menopause status and hepatic steatosis and its severity for dense breast resolution (multivariable-adjusted model 2)

[a] Estimated from parametric proportional hazard models for dense breast resolution with hepatic steatosis and its severity. Multivariable model 1 was adjusted for age, center, year of screening exam, alcohol consumption, smoking, physical activity, total energy intake, education level, hypertension, diabetes, sleep duration, sleep quality, age at menarche, female hormone medication, and parity; model 2: model 1 plus adjustment for BMI

[b] Estimated from parametric proportional hazard models for dense breast resolution with hepatic steatosis and its severity, smoking, alcohol consumption, physical activity, BMI, total energy intake, hypertension, diabetes, sleep duration, sleep quality, female hormone medication, and parity as time-dependent categorical variables and baseline age, center, year of screening exam, education level, and age at menarche as time-fixed variables

Abbreviations: HS, hepatic steatosis; Fibrosis-4, FIB-4; BMI, body mass index; CI, confidence interval; HR, hazard ratio; PY, person-years

Supplementary Table 3. Hazard ratios (95% CI) of dense breast resolution with explanatory factors.

	Multivariate-adjusted HR (95% CI)[a]	
	Model 1	Model 2
Age per one-year increment	1.07 (1.06-1.07)	1.06 (1.05-1.07)
Hepatic steatosis	2.62 (2.41-2.84)	1.30 (1.18-1.43)
Menopause	1.59 (1.40-1.81)	1.59 (1.40-1.81)
Hepatic steatosis	0.48 (0.41-0.56)	0.62 (0.53-0.73)
Alcohol consumption		
<10 g/day	1.00 (reference)	1.00 (reference)
10-20 g/day	1.09 (0.97-1.23)	1.03 (0.92-1.17)

≥20 g/day	1.20 (1.04-1.39)	1.12 (0.97-1.30)
Smoking		
Never	1.00 (reference)	1.00 (reference)
Ever	1.09 (0.96-1.23)	1.07 (0.94-1.20)
Current	1.21 (0.98-1.50)	1.25 (1.01-1.55)
Physical activity		
Inactive	1.00 (reference)	1.00 (reference)
Minimal	0.99 (0.92-1.07)	0.99 (0.92-1.07)
HEPA	1.10 (1.01-1.20)	1.07 (0.98-1.17)
Total energy intake		
Q1	1.00 (reference)	1.00 (reference)
Q2	1.00 (0.83-1.21)	1.00 (0.83-1.21)

Q3	1.05 (0.88-1.25)	1.01 (0.85-1.20)
Q4	1.03 (0.87-1.22)	0.98 (0.83-1.16)
Q5	1.09 (0.92-1.29)	1.00 (0.85-1.19)
Education level	0.93 (0.86-1.01)	1.05 (0.97-1.14)
History of hypertension	1.25 (1.12-1.40)	1.06 (0.95-1.19)
History of diabetes	1.08 (0.89-1.31)	1.05 (0.86-1.27)
Sleep duration		
≤5 hours	1.12 (1.00-1.25)	1.02 (0.92-1.14)
6 hours	1.09 (1.00-1.18)	1.05 (0.97-1.14)
7 hours	1.00 (reference)	1.00 (reference)
8 hours	0.98 (0.89-1.09)	0.99 (0.89-1.09)
≥9 hours	0.92 (0.77-1.11)	0.95 (0.79-1.14)

Poor sleep quality	1.05 (0.96-1.14)	1.08 (0.99-1.18)
Age at menarche		
10-11 years	1.41 (1.09-1.82)	1.20 (0.93-1.55)
12-13 years	1.12 (0.92-1.37)	1.05 (0.86-1.29)
14-15 years	0.94 (0.77-1.14)	0.94 (0.78-1.15)
16-17 years	0.94 (0.76-1.15)	0.97 (0.79-1.19)
≥18	1.00 (reference)	1.00 (reference)
Female hormone medication	1.04 (0.87-1.25)	1.10 (0.92-1.32)
Parity		
0	1.00 (reference)	1.00 (reference)
1	1.28 (1.06-1.54)	1.40 (1.16-1.68)
2	1.51 (1.27-1.79)	1.63 (1.37-1.94)

≥3	1.92 (1.59-2.30)	1.99 (1.66-2.40)
BMI per one kg/m^2 increment		1.19 (1.18-1.20)

[a] Estimates from parametric proportional hazard models for dense breast resolution with hepatic steatosis. Multivariate model 1 was adjusted for age, center, year of screening examination, alcohol consumption, smoking, physical activity, total energy intake, education level, hypertension, diabetes, sleep duration, sleep quality, age at menarche, female hormone medication, and parity; model 2: model 1 along with adjustment for BMI

Abbreviations: HS, hepatic steatosis; BMI, body mass index; CI, confidence interval; HR, hazard ratio; HEPA, health-enhancing physical activity