Table S1: Comparison of baseline characteristics between subjects for whom we did and did not retrieve mammograms, by case-control status

| | Cases | | Controls | |
| --- | --- | --- | --- | --- |
| | Mammogram retrieved | | Mammogram retrieved | |
| | Yes | No | Yes | No |
| *Mean (SD)* | | | | |
| Age at entry | 58.2 (7.1) | 53.5 (10.9) | 58.4 (6.5) | 56.0 (11.4) |
| Body mass index | 26.0 (4.7) | 25.2 (4.3) | 26.0 (4.7) | 25.3 (4.3) |
| | | | | |
| *Number (%)* | | | | |
| Eligible for hormone assays | 265 (52.8) | 48 (29.1) | 343 (61.7) | 68 (48.2) |
| Provided blood sample | 492 (98.0) | 157 (95.2) | 548 (98.6) | 131 (92.9) |
| Postmenopausal at blood draw | 402 (80.1) | 77 (46.7) | 449 (80.8) | 84 (59.6) |
| Not on postmenopausal hormone therapy at blood draw | 394 (78.5) | 134 (81.2) | 487 (87.6) | 124 (87.9) |
| Family history of breast cancer | 141 (28.1) | 45 (27.3) | 106 (19.1) | 35 (24.8) |
| Cases with invasive breast cancer | 431 (85.9) | 138 (83.6) | | |
| | | | | |
| Total subjects | 502 (100) | 165 (100) | 556 (100) | 141 (100) |

Table S2a: Association between oestradiol, testosterone and SHBG levels and absolute mammographic dense area among control subjects: Breakthrough Generations Study, United Kingdom

| Characteristic | Absolute mammographic dense area, quartiles (a) | | | | % increase per quartile (b) | P-value trend (b) |
|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | | |
| No. of controls | 83 | 82 | 83 | 82 | | |
| Median absolute dense area, cm$^2$ | 5.1 | 14.9 | 28.4 | 47.6 | | |
| Mean age at mammogram, yrs | 61.6 | 61.3 | 60.5 | 60.5 | | |
| Mean BMI at cohort entry, kg/m$^2$ | 27.3 | 25.3 | 25.8 | 25.7 | | |
| *Geometric mean, adjusted for laboratory batch and age at mammogram* | | | | | | |
| Oestradiol, pmol/L | 21.7 | 19.7 | 18.6 | 18.3 | -5.5 | 0.077 |
| Free oestradiol, pmol/L | 0.53 | 0.46 | 0.44 | 0.42 | -7.4 | 0.026 |
| Testosterone, nmol/L | 0.66 | 0.58 | 0.53 | 0.58 | -4.9 | 0.153 |
| Free testosterone, nmol/L | 0.010 | 0.008 | 0.007 | 0.008 | -8.6 | 0.017 |
| SHBG, nmol/L | 37.8 | 44.0 | 43.6 | 47.8 | 7.2 | 0.001 |
| *Geometric mean, additionally adjusted for BMI* | | | | | | |
| Oestradiol, pmol/L | 19.9 | 20.8 | 19.2 | 18.6 | -2.8 | 0.307 |
| Free oestradiol, pmol/L | 0.48 | 0.49 | 0.45 | 0.42 | -4.3 | 0.130 |
| Testosterone, nmol/L | 0.65 | 0.58 | 0.55 | 0.58 | -3.6 | 0.294 |
| Free testosterone, nmol/L | 0.009 | 0.008 | 0.008 | 0.008 | -6.5 | 0.063 |
| SHBG, nmol/L | 39.7 | 42.7 | 42.9 | 47.4 | 5.6 | 0.007 |

(a) Q1: <9.31; Q2: 9.31-21.73; Q3: 21.74-37.0; Q4: >37.0 cm$^2$

(b) Percentage change in sex hormone or SHBG level per quartile increase in absolute mammographic dense area, p-value based on Wald test.

Table S2b: Association between oestradiol, testosterone and SHBG levels and absolute mammographic non-dense area among control subjects: Breakthrough Generations Study, United Kingdom

| Characteristic | Absolute mammographic non-dense area, quartiles (a) | | | | % increase per quartile (b) | P-value trend (b) |
|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | | |
| No. of controls | 83 | 82 | 82 | 83 | | |
| Median absolute non-dense area, cm$^2$ | 60.9 | 97.3 | 130.4 | 175.0 | | |
| Mean age at mammogram, yrs | 60.1 | 61.2 | 61.4 | 61.2 | | |
| Mean BMI at cohort entry, kg/m$^2$ | 23.0 | 25.0 | 26.2 | 29.9 | | |
| *Geometric mean, adjusted for laboratory batch and age at mammogram* | | | | | | |
| Oestradiol, pmol/L | 14.4 | 18.0 | 22.3 | 25.3 | 21.0 | <0.001 |
| Free oestradiol, pmol/L | 0.32 | 0.42 | 0.53 | 0.63 | 25.2 | <0.001 |
| Testosterone, nmol/L | 0.52 | 0.53 | 0.68 | 0.61 | 7.3 | 0.044 |
| Free testosterone, nmol/L | 0.007 | 0.007 | 0.010 | 0.009 | 14.7 | <0.001 |
| SHBG, nmol/L | 51.8 | 44.1 | 41.8 | 36.2 | -10.7 | <0.001 |
| *Geometric mean, additionally adjusted for BMI* | | | | | | |
| Oestradiol, pmol/L | 17.6 | 19.5 | 22.6 | 19.3 | 4.9 | 0.159 |
| Free oestradiol, pmol/L | 0.40 | 0.46 | 0.53 | 0.46 | 5.9 | 0.100 |
| Testosterone, nmol/L | 0.56 | 0.56 | 0.70 | 0.56 | 3.2 | 0.455 |
| Free testosterone, nmol/L | 0.007 | 0.008 | 0.010 | 0.008 | 5.4 | 0.233 |
| SHBG, nmol/L | 46.9 | 42.5 | 41.7 | 41.4 | -3.9 | 0.107 |

(a) Q1: <78.4; Q2: 78.4-113.5; Q3: 113.6-152.2; Q4: >152.2 cm$^2$

(b) Percentage change in sex hormone or SHBG level per quartile increase in absolute mammographic non-dense area, p-value based on Wald test.

Table S3: Odds ratios of incident breast cancer in relation to absolute mammographic dense area, Breakthrough Generations Study, United Kingdom

| | Absolute mammographic dense area (cm$^2$), quartiles (a) | | | | P trend (b) |
|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | |
| *Number (%)* | | | | | |
| Cases | 40 (15.8) | 60 (23.7) | 69 (27.3) | 84 (33.2) | |
| Controls | 83 (25.2) | 82 (24.9) | 83 (25.2) | 82 (24.9) | |
| *Odds ratio (95% CI)* | | | | | |
| Multivariate(c) | 1.00 | 1.62 (0.93-2.82) | 1.92 (1.11-3.33) | 2.13 (1.24-3.66) | 0.003 |
| *Additional adjustment for:* | | | | | |
| Oestradiol(d) | 1.00 | 1.70 (0.93-3.12) | 1.93 (1.05-3.53) | 2.20 (1.22-3.94) | 0.005 |
| Free oestradiol(d) | 1.00 | 1.72 (0.94-3.17) | 1.93 (1.05-3.54) | 2.21 (1.23-3.98) | 0.005 |
| Testosterone(d) | 1.00 | 1.73 (0.94-3.18) | 2.00 (1.09-3.68) | 2.22 (1.23-3.99) | 0.005 |
| Free testosterone(d) | 1.00 | 1.80 (0.98-3.31) | 2.12 (1.15-3.88) | 2.30 (1.28-4.14) | 0.004 |
| SHBG(d) | 1.00 | 1.80 (0.98-3.29) | 2.07 (1.13-3.79) | 2.39 (1.32-4.32) | 0.002 |

(a) Q1: <9.31; Q2: 9.31-21.73; Q3: 21.74-37.0; Q4: >37.0 cm$^2$

(b) Based on Wald-test on square-root transformed percentage density, continuous

(c) Covariates:  year of entry to the study (≤2005, 2006, 2007, ≥2008); age at entry (5-year age groups); blood in post (0-1,2, ≥3 days, not known); years breast cancer-free in study (0-6); body mass index, kg/m2 (continuous); parity (nulliparous; age at first birth<25y/1-2 children; age at first birth<25y/≥3 children; age at first birth 25-29y/1-2 children; age at first birth 25-29y/≥3 children; age at first birth ≥30y/1-4 children); family history of breast cancer (yes, no); age at menarche (≤12, 13,14,15-20, not known); age at menopause (<45, 45-49, 50-54, ≥55, not known); years of postmenopausal hormone therapy (never, <2. 2-5, 6-9, ≥10, not known); alcohol units (0, <5, 5-14, ≥15 grams per day, not known)

(d) Adjustment for (c) and quartile of respective sex hormone or SHBG level and laboratory batch

4

Table S4: Odds ratios for breast cancer risk in relation to sex hormone and SHBG level and absolute mammographic dense area, Breakthrough Generations Study, United Kingdom

| Sex hormone or SHBG level (tertiles) | Mammographic dense area, tertiles (a) | | | | | |
|---|---|---|---|---|---|---|
| | 1st | | 2nd | | 3rd | |
| | Cases/ controls | OR(b) (95% CI) | Cases/ controls | OR(b) (95% CI) | Cases/ controls | OR(b) (95% CI) |
| *Oestradiol (c)* | | | | | | |
| 1st | 18/33 | 1.00 | 22/38 | 1.19 (0.48-2.96) | 30/38 | 1.59 (0.66-3.79) |
| 2nd | 16/40 | 0.85 (0.32-2.25) | 27/30 | 2.29 (0.92-5.69) | 34/37 | 1.80 (0.75-4.32) |
| 3rd | 22/36 | 1.81 (0.68-4.81) | 39/40 | 3.03 (1.25-7.38) | 45/34 | 3.83 (1.55-9.45) |
| | | | | | | P interaction(d)= 0.769 |
| *Free oestradiol (c)* | | | | | | |
| 1st | 19/35 | 1.00 | 25/33 | 1.60 (0.66-3.90) | 34/41 | 1.75 (0.76-4.03) |
| 2nd | 14/41 | 0.69 0.26-1.86) | 30/37 | 2.13 (0.90-5.07) | 30/30 | 2.02 (0.84-4.88) |
| 3rd | 23/33 | 1.99 (0.75-5.25) | 33/38 | 2.46 (1.01-5.96) | 45/38 | 2.98 (1.24-7.17) |
| | | | | | | P interaction(d)= 0.709 |
| *Testosterone(c)* | | | | | | |
| 1st | 13/35 | 1.00 | 22/36 | 1.80 (0.68-4.73) | 35/39 | 2.11 (0.85-5.26) |
| 2nd | 23/34 | 1.70 (0.65-4.43) | 26/43 | 1.95 (0.76-5.01) | 37/33 | 3.02 (1.20-7.65) |
| 3rd | 20/41 | 1.37 (0.52-3.62) | 40/30 | 3.83 (1.52-9.64) | 37/38 | 2.89 (1.16-7.24) |
| | | | | | | P interaction(d)= 0.643 |
| *Free testosterone  (c)* | | | | | | |
| 1st | 14/36 | 1.00 | 26/37 | 2.36 (0.93-5.96) | 31/37 | 2.47 (1.00-6.12) |
| 2nd | 16/34 | 1.87 (0.69-5.04) | 19/38 | 2.15 (0.81-5.70) | 36/38 | 2.96 (1.22-7.18) |
| 3rd | 26/40 | 2.05 (0.80-5.26) | 43/34 | 3.90 (1.62-9.41) | 42/35 | 4.01 (1.63-9.86) |
| | | | | | | P interaction(d)= 0.863 |

| Sex hormone or SHBG level (tertiles) | Mammographic dense area, tertiles (a) | | | | | |
|---|---|---|---|---|---|---|
| | 1st | | 2nd | | 3rd | |
| *SHBG (c)* | | | | | | |
| 1st | 26/44 | 1.00 | 27/36 | 1.28 (0.56-2.94) | 35/28 | 2.45 (1.04-5.77) |
| 2nd | 19/30 | 0.98 (0.39-2.48) | 37/38 | 1.86 (0.79-4.34) | 40/39 | 1.50 (0.67-3.37) |
| 3rd | 11/36 | 0.44 (0.16-1.19) | 24/35 | 1.11 (0.44-2.81) | 34/43 | 1.20 (0.51-2.84) |
| | | | | | | P interaction(d)= 0.585 |

OR: Odds ratio; CI: Confidence Interval

(a) Tertiles, 1st: <12.70; 2nd: 12.70-30.27; 3rd: >30.27 cm$^2$
(b) Covariates: year of entry to the study (≤2005, 2006, 2007, ≥2008); age at entry (5-year age groups); blood in post (0-1,2, ≥3 days, not known); years breast cancer free in study (0-6); body mass index, kg/m2 (continuous); parity (nulliparous; age at first birth<25y/1-2 children; age at first birth<25y/≥3 children; age at first birth 25-29y/1-2 children; age at first birth 25-29y/≥3 children; age at first birth ≥30y/1-4 children); family history of breast cancer (yes, no); age at menarche (≤12, 13,14,15-20, not known); age at menopause ((<45, 45-49, 50-54, ≥55, not known); years of postmenopausal hormone therapy (never, <2. 2-5, 6-9, ≥10, not known); alcohol units (0, <5, 5-14, ≥15 grams per day, not known); laboratory batch.
(c) 1st, 2nd, 3rd tertile respectively, oestradiol: <14.77, 14.77-24.45, >24.45 pmol/L; free oestradiol: <0.35, 0.35-0.61, >0.61 pmol/L; testosterone: <0.49, 0.49-0.84, >0.84 nmol/L; free testosterone: <0.0064, 0.0064-0.0115, >0.0115 nmol/L; SHBG: <35.2, 35.2-52.7, >52.7 nmol/L
(d) Based on log-likelihood ratio test

Table S5: Odds ratios for breast cancer risk in relation to sex hormone and SHBG level and percentage mammographic density and absolute dense area, Breakthrough Generations Study, United Kingdom

| | Percentage mammographic density (a) | | | | Absolute mammographic dense area (a) | | | |
|---|---|---|---|---|---|---|---|---|
| | <median | | ≥median | | <median | | ≥median | |
| Sex hormone | No cases/ controls | OR (95% CI)(b) | No cases/ controls | OR (95% CI)(b) | No cases/ controls | OR (95% CI)(b) | No cases/ controls | OR (95% CI)(b) |
| Oestradiol (c) | | | | | | | | |
| <median | 44/78 | 1.00 | 71/84 | 1.44 (0.81-2.54) | 46/78 | 1.00 | 65/83 | 1.40 (0.79-2.48) |
| ≥median | 65/87 | 1.71 (0.93-3.12) | 82/81 | 2.11 (1.17-3.78) | 54/86 | 1.44 (0.78-2.70) | 88/79 | 2.41 (1.34-4.34) |
| | | | | P (interaction)=0.700 P(RERI)=0.953 | | | | P (interaction)=0.663 P(RERI)=0.358 |
| Free oestradiol (c) | | | | | | | | |
| <median | 42/77 | 1.00 | 72/88 | 1.41 (0.79-2.50) | 45/80 | 1.00 | 65/84 | 1.41 (0.79-2.52) |
| ≥median | 67/88 | 1.80 (0.97-3.32) | 81/77 | 2.30 (1.26-4.21) | 55/84 | 1.59 (0.84-2.99) | 88/78 | 2.65 (1.45-4.85) |
| | | | | P (interaction)=0.881 P(RERI)=0.881 | | | | P (interaction)=0.675 P(RERI)=0.328 |
| Testosterone (c) | | | | | | | | |
| <median | 45/87 | 1.00 | 66/81 | 1.22 (0.68-2.21) | 39/80 | 1.00 | 68/87 | 1.41 (0.78-2.54) |
| ≥median | 64/79 | 1.38 (0.77-2.47) | 87/86 | 1.95 (1.11-3.42) | 61/85 | 1.43 (0.79-2.58) | 85/77 | 2.44 (1.36-4.38) |
| | | | | P (interaction)=0.716 P(RERI)=0.510 | | | | P (interaction)=0.626 P(RERI)=0.318 |
| Free testosterone (c) | | | | | | | | |
| <median | 40/78 | 1.00 | 68/87 | 1.20 (0.66-2.18) | 36/75 | 1.00 | 67/89 | 1.44 (0.79-2.63) |
| ≥median | 69/88 | 1.40 (0.76-2.56) | 85/80 | 2.09 (1.15-3.78) | 64/90 | 1.56 (0.84-2.89) | 86/75 | 2.67 (1.46-4.90) |
| | | | | P (interaction)=0.584 P(RERI)=0.359 | | | | P (interaction)=0.667 P(RERI)=0.301 |
| SHBG (c) | | | | | | | | |
| <median | 73/95 | 1.00 | 73/70 | 1.28 (0.74-2.21) | 61/92 | 1.00 | 80/69 | 1.92 (1.11-3.34) |
| ≥median | 36/71 | 0.62 (0.33-1.16) | 80/97 | 0.95 (0.55-1.67) | 39/73 | 0.79 (0.42-1.47) | 73/95 | 1.05 (0.60-1.84) |
| | | | | P (interaction)=0.636 P(RERI)=0.871 | | | | P (interaction)=0.373 P(RERI)=0.249 |

OR: Odds ratio; CI: Confidence Interval; RERI: Relative Excess Risk due to Interaction (Rothman, 1986)

(a) For percentage density: 1st: <16.8 2nd: ≥16.8 percent, for dense area 1st <21.7, 2nd ≥21.7 cm$^2$

(b) Covariates: year of entry to the study (≤2005, 2006, 2007, ≥2008); age at entry (5-year age groups); blood in post (0-1,2, ≥3 days, not known); years breast cancer free in study (0-6); body mass index, kg/m2 (continuous); parity (nulliparous; age at first birth<25y/1-2 children; age at first birth<25y/≥3 children; age at first birth 25-29y/1-2 children; age at first birth 25-29y/≥3 children; age at first birth ≥30y/1-4 children); family history of breast cancer (yes, no); age at menarche (≤12, 13,14,15-20, not known); age at menopause ((<45, 45-49, 50-54, ≥55, not known); years of postmenopausal hormone therapy (never, <2. 2-5, 6-9, ≥10, not known); alcohol units (0, <5, 5-14, ≥15 grams per day, not known); laboratory batch.

(c) Categorised based on the median value among control subjects, oestradiol: <18.86, ≥18.86 pmol/L; free oestradiol: <0.45, ≥0.45 pmol/L; testosterone: <0.63, ≥0.63 nmol/L; free testosterone: <0.0088, ≥0.0088 nmol/L; SHBG: <43.5, ≥43.5 nmol/L

(d) Based on log-likelihood ratio test

Rothman KJ (1986) *Modern Epidemiology*. Brown and Company: Boston M.A.