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## Linking Physical Activity to Breast Cancer via Sex Steroid Hormones, Part 2: The Effect of Sex Steroid Hormones on Breast Cancer Risk

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## Abstract

We undertook a systematic review and appraised the evidence for an effect of circulating sex steroid hormones and sex hormone-binding globulin (SHBG) on breast cancer risk in pre- and postmenopausal women. Systematic searches identified prospective studies relevant to this review. Meta-analyses estimated breast cancer risk for women with the highest compared with the lowest level of sex hormones, and the DRMETA Stata package was used to graphically represent the shape of these associations. The ROBINS-E tool assessed risk of bias, and the GRADE system appraised the strength of evidence. In premenopausal women, there was little evidence that estrogens, progesterone, or SHBG were associated with breast cancer risk, whereas androgens showed a positive association. In postmenopausal women, higher estrogens and androgens were associated with an increase in breast cancer risk, whereas higher SHBG was inversely associated with risk. The strength of the evidence quality ranged from low to high for each hormone. Dose-response relationships between sex steroid hormone concentrations and breast cancer risk were most notable for post-menopausal women. These data support the plausibility of a role for sex steroid hormones in mediating the causal relationship between physical activity and the risk of breast cancer.

*See related reviews by Lynch et al., p. 11 and Swain et al., p. 16*

## Introduction

Physical activity may reduce breast cancer risk via its effect on sex steroid hormones (1–3). Elevated levels of sex steroid hormones have been shown to be associated with increased risk of breast cancer in observational studies (1, 4). In addition, estrogens exert mitogenic and mutagenic effects in in vitro and in vivo studies (4) and androgens can stimulate the growth of breast cancers, either by a direct action or following aromatization to estrogen. The bioavailability of these hormones is regulated by sex hormone-binding globulin (SHBG), a glycoprotein, produced primarily by the liver. SHBG binds to estrogens and androgens rendering them inactive (5). It is therefore conceivable that SHBG has the capacity to influence breast cancer risk, albeit indirectly (6).

Progestogens play an essential role in breast development and mammary gland function. Progesterone may mediate breast cancer risk indirectly, by mitigating the actions of estrogens, or by stimulating proliferation of breast tissue (7). Progesterone levels are highest during the luteal phase of the menstrual cycle (8). During the reproductive, premenopausal years, levels of estrogens and progestogens, produced by the ovaries, change according to the stage of the menstrual cycle. With the functional decline of the ovaries at menopause, production of these hormones slows and ultimately ceases (9). In contrast, androgens are produced both by the ovary and the adrenal gland; production by the latter does not occur cyclically and is unaffected by the menopause (10), so levels of androgens are more stable than levels of estrogens and progestogens.

Steroids are synthesised de novo from cholesterol (Fig. 1). Progestogens give rise to androgens and glucocorticoids, with estrogens arising from the aromatization of testosterone (11). All but estrogens act as a substrate for another class of steroid hormone. A series of

metabolites within each class of hormone are produced during the conversion process (12). The identification of glucocorticoid receptors in breast tissue has led to speculation that glucocorticoids may have a role to play in breast cancer development (13).

We have reported the results of our systematic review assessing the effect of physical activity on steroid hormone levels (14). Here, we synthesize and appraise the evidence to determine whether circulating levels of sex steroid hormones or SHBG influence breast cancer risk, in pre- or postmenopausal women.

## Materials and Methods

The methods for this review have been reported previously (3) and have been registered on PROSPERO (CRD42020146736). In brief, systematic searches of Medline (Ovid) and Embase were performed up to August 2019. Search terminology is provided in Supplementary Table ST1. Prospective studies that examined the association between five endogenous sex steroid hormones and breast cancer incidence were eligible for inclusion. Sex steroid hormones included estrogens and estrogen metabolites, progestogens, and androgens. Sex hormone-binding globulin and glucocorticoids were also included. Following duplicate removal, two reviewers screened all titles and abstracts and then full texts with consensus being the basis for inclusion. Data were extracted and entered into pre-piloted tables. Risk of bias was assessed using the Risk of Bias in Nonrandomized Studies-of Exposures (ROBINS-E) tool (15). To rate the overall quality of the evidence for each sex hormone–breast cancer pathway, the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system was used (16). Random effects meta-analyses were used to estimate breast cancer risk for women with the highest compared with the lowest reported categories of sex hormone levels. Subgroup analyses were performed to examine whether effect estimates differed between pre- and postmenopausal women and, where possible, for menstrual cycle stage and breast cancer subtype, for all sex hormone–breast cancer pathways. The DRMETA Stata package was used to perform a one-stage random-effects dose–response metaanalysis of summarised data using restricted cubic splines, to graphically represent the shape of associations for each sex hormone–breast cancer pathway, provided there were sufficient studies ( $n > 3$ ) that presented consistently defined and discernible hormone values for each category of breast cancer risk (17–19). Sensitivity analyses excluded studies with serious overall risk of bias or moderate risk of bias for exposure classification (i.e, hormone measurement). All effect estimates generated by the meta-analyses are presented as relative risk and 95% confidence intervals (RR, 95% CI), although these have been derived from studies that present a mix of RR, odds ratios (OR), and hazard ratios (HR). Where there were multiple publications based on a single cohort that examined sex steroid hormones and breast cancer, we extracted data from the most recent publication. All statistical analyses were performed using Stata version 16 (Stata Corporation).

## Results

### Search results

A PRISMA chart (Fig. 2) provides details of the screening process for articles and the number excluded (and reasons for exclusion) at each stage, for each steroid hormone and SHBG. Of 14,659 results returned across the searches for all five sex steroid hormones, there were 64 papers arising from 32 cohorts and one Mendelian randomization study that assessed the effect of steroid hormones or SHBG on breast cancer risk.

### Study characteristics

Study characteristics are provided in Supplementary Methods and Material (Supplementary Table S2A and S2B). Eight studies (21 publications) included premenopausal women with sample sizes from 66 to 1,933 (20–40). Twenty-four studies (49 publications), included postmenopausal women with sample sizes from 87 to 1,375 (21–24, 28, 29, 40–82). Exposures examined included measurement of endogenous estradiol ( $n = 27$ ), free estradiol ( $n = 10$ ), bioavailable estradiol ( $n = 3$ ), urinary estradiol ( $n = 4$ ), estrone ( $n = 15$ ), urinary estrone ( $n = 4$ ), estrone sulfate ( $n = 4$ ), 2-hydroxyestrone (2-OH-E1;  $n = 11$ ), 16 $\alpha$ -hydroxyestrone (16 $\alpha$ -OH-E1;  $n = 11$ ), additional 2- and 16-pathway metabolites ( $n = 6$ ), progesterone ( $n = 4$ ), SHBG ( $n = 24$ ), testosterone ( $n = 22$ ), free testosterone ( $n = 15$ ), androstenedione ( $n = 12$ ), dehydroepiandrosterone (DHEA;  $n = 3$ ), and dehydroepiandrosterone sulphate (DHEAS;  $n = 13$ ). The Mendelian randomization study included 122,977 cases and 105,974 controls. It examined the effect of genetically determined SHBG concentrations on breast cancer risk (83).

### Risk of bias

Assessments of risk of bias assessments are presented in Supplementary Methods and Material (Supplementary Table S3). All cohort studies were judged to have at least moderate levels of bias owing to potential confounding of the effect of sex steroid hormones on breast cancer risk. Seven studies were judged to have serious bias due to confounding as they did not adjust for measures of body composition and lifestyle factors such as alcohol intake (21, 46, 56, 70, 73, 74, 78). Additional sources of identified bias studies included poor sensitivity, inter- or intra-assay variation of the assays used to measure hormones (20, 27, 48, 53, 55), and missing data for more than 10% of participants (21, 25, 31). All of the studies used valid and reliable means to ascertain breast cancer incidence, except for two, where no information on this was provided (64, 76). Overall ROB was assessed as moderate in 25 cohort studies and serious in 5 studies. The Mendelian randomization study was judged to have low risk of bias overall. However, we note that ROBINS-E has not been designed to assess risk of bias in Mendelian randomization studies; we are unaware of a suitable tool to appraise bias in these studies. Removing studies with an overall serious risk of bias or a moderate risk of bias for hormone measurement from the meta-analyses did not change our findings.

## Effects of steroid hormones and SHBG on breast cancer risk

Forest plots, comparing breast cancer risk for women with the highest quantile compared with the lowest level of sex hormones, are presented in Supplementary Figures SF1–SF4. Dose–response curves are presented in Fig. 3 (premenopausal estradiol, testosterone, free testosterone, DHEAS, and SHBG) and Fig. 4 (postmenopausal estradiol, free estradiol, estrone, androstenedione, testosterone, free testosterone, DHEAS, and SHBG).

### SHBG

There was little evidence that SHBG levels were related to breast cancer risk in premenopausal women when comparing risk in those with the highest levels of SHBG with the lowest (RR = 0.96; 95% CI = 0.78–1.14;  $I^2 = 0\%$ ) or when examining dose–response curves (Fig. 3E). In contrast, there was evidence that postmenopausal women with the highest levels of SHBG had reduced risk of breast cancer compared with women with the lowest levels (RR = 0.54; 95% CI = 0.45–0.64;  $I^2 = 29\%$ ; Supplementary Methods and Materials; Fig. 1A). This association was dose-dependent (Figs. 4H). Meta-analyses found no differences between higher SHBG levels and the development of either estrogen receptor–positive or negative breast cancer. A Mendelian randomization study found that genetically predicted SHBG decreased the odds of developing breast cancer overall (OR = 0.94; 95% CI = 0.90–0.98) and ER<sup>+</sup> breast cancer (OR = 0.92; 95% CI = 0.87–0.97), but increased the risk of ER<sup>–</sup> breast cancer (OR = 1.09; 95% CI = 1.00–1.18 per 25 nmol/L higher SHBG concentrations; ref. 83).

### Estrogens

There was little evidence that estrogens had an effect on breast cancer risk in premenopausal women (Supplementary Materials SF2). In contrast, postmenopausal women with the highest levels of the major estrogens had increased risk of breast cancer risk compared with those with the lowest risk. This was most evident for estradiol (RR = 1.88; 95% CI = 1.63–2.14;  $I^2 = 0\%$ ), estrone (RR = 1.74; 95% CI = 1.37–2.11;  $I^2 = 7.5\%$ ), free estradiol (RR = 1.86; 95% CI = 1.53–2.18;  $I^2 = 0\%$ ), urinary estradiol (RR = 1.59, 95% CI = 1.19–1.98;  $I^2 = 0\%$ ), and urinary estrone (RR = 1.50; 95% CI = 1.09–1.91;  $I^2 = 8\%$ ). For estradiol and free estradiol, increased breast cancer risk was more evident for ER<sup>+</sup> (estradiol RR = 1.75; 95% CI, 1.21–1.78;  $I^2 = 29\%$ ; free estradiol RR = 1.92; 95% CI = 1.42–2.44;  $I^2 = 0\%$ ) than ER<sup>–</sup> (estradiol RR = 1.37; 95% CI, 0.96–1.98;  $I^2 = 0\%$ ; free estradiol RR = 1.50; 95% CI, 0.87–2.12;  $I^2 = 0\%$ ) breast cancer. Associations between postmenopausal levels of estradiol, free estradiol, and estrone with overall breast cancer risk appeared to be dose-dependent (Fig. 4A–C). There was also a suggestion of increased breast cancer risk for postmenopausal women with higher levels of bioavailable estradiol (RR = 2.19; 95% CI = 0.96–3.41;  $I^2 = 0\%$ ) and estrone sulfate (RR = 1.90; 95% CI = 0.53–3.28;  $I^2 = 57\%$ ).

Eleven studies (nine in postmenopausal women) examined the relationship between circulating or urinary estrogen metabolites and breast cancer risk. For the most common metabolites examined, breast cancer risk increased with increasing concentrations of 2-hydroxyestrone (circulating: RR = 1.51; 95% CI = 1.07–1.96;  $I^2 = 0\%$ , urinary: RR = 1.24; 95% CI = 0.99–1.48;  $I^2 = 0\%$ ), but not for 16 $\alpha$ -hydroxyestrone (circulating: RR = 1.01; 95% CI = 0.81–1.21;  $I^2 = 4\%$ , urinary: RR = 1.38; 95% CI = 0.81–1.95;  $I^2 =$

24%) or the ratio of 2-hydroxyestrone: 16 $\alpha$ -hydroxyestrone (RR = 0.99; 95% CI = 0.76–1.21) in postmenopausal women. There were a limited number of studies that examined additional metabolites from the 2-, 4-, and 16-pathways. Of these, higher breast cancer risk was seen with higher levels of estriol, 16-ketoestradiol, and 16-epiestriol. However, high heterogeneity was noted for most metabolites. Meta-analysis was not possible for premenopausal women. Findings from one study suggest that most midluteal urinary estrogen metabolite concentrations are not positively associated with breast cancer risk (33). Because of the small number of studies, the measurement of hormones in different biological fluids (urine and blood) and issues with unit conversion, we were unable to complete dose–response curves for estrogen metabolites.

### Progestogens

Meta-analysis did not identify an association between levels of progesterone and breast cancer risk (RR = 0.98; 95% CI, 0.74–1.23;  $I^2$  = 0%). The absence of heterogeneity from the data, meant that data from premenopausal women, including samples taken from both the follicular and luteal phases, and postmenopausal women could be meta-analyzed together. When meta-analysis was restricted to luteal phase progesterone (RR = 1.09; 95% CI = 0.90–1.29;  $I^2$  = 0%).

### Androgens

Women with the highest androgen levels had increased risk of breast cancer compared with women with low androgen levels, regardless of menopause status. In premenopausal women, women with the highest levels of testosterone (RR = 1.44; 95% CI = 1.11–1.77;  $I^2$  = 14%) and free testosterone had increased breast cancer risk (RR = 1.25; 95% CI = 1.00–1.50;  $I^2$  = 0%). In contrast, higher levels of DHEAS were not associated with breast cancer risk in premenopausal women (RR = 1.07; 95% CI = 0.82–1.33;  $I^2$  = 0%). These findings were also evident in the dose–response curves (Fig. 3B–D). In postmenopausal women, women with higher levels of androstenedione (RR = 1.43; 95% CI = 1.09–1.77;  $I^2$  = 0%), testosterone (RR = 1.45; 95% CI = 1.20–1.70;  $I^2$  = 21%), free testosterone (RR = 1.99; 95% CI = 1.65–2.32;  $I^2$  = 0%) and DHEAS (RR = 1.64; 95% CI = 1.35–1.93;  $I^2$  = 0%) had increased breast cancer risk compared with women with lower levels of these hormones. These associations appeared to be dose dependent (Fig. 4D–G). Effect estimates for postmenopausal testosterone and free testosterone were stronger for ER<sup>+</sup> (testosterone RR = 1.35; 95% CI = 1.04–1.65;  $I^2$  = 16%; free testosterone RR = 1.50; 95% CI = 1.40–2.44;  $I^2$  = 0%) than ER<sup>–</sup> breast cancer (testosterone RR = 1.08; 95% CI = 0.73–1.4;  $I^2$  = 0%; free testosterone RR = 1.50; 95% CI = 0.87–2.12;  $I^2$  = 0%). DHEA was only measured in a small number of studies ( $n$  = 3, 2 of premenopausal and 1 postmenopausal women), but there was little evidence of an effect on breast cancer risk seen (RR = 1.08; 95% CI = 0.77–1.40; heterogeneity  $I^2$  = 0.00%).

### Glucocorticoids

Cortisol was the endogenous glucocorticoid investigated in the studies identified. None of these studies met the inclusion criteria for the current analysis (Fig. 2).

## Grade

Results of the GRADE appraisal are presented in Table 1. As evidence for postmenopausal SHBG came from both observational and Mendelian randomization studies, and as a dose–response relationship was identified for postmenopausal women, the quality of evidence for a postmenopausal SHBG–breast cancer association was graded as high. For estrogens, progesterone, and androgens, the evidence comes exclusively from observational studies. As such, evidence for these associations was initially graded as low quality according to the GRADE criteria. Where a dose–response effect was reported, the quality of evidence was upgraded to moderate. Furthermore, the evidence for a postmenopausal estradiol–breast cancer association was graded up to high owing to the large effect estimates generated in the meta-analysis. No sex hormone–breast cancer association met the criteria to be downgraded.

## Discussion

Our analysis of the existing literature indicates that, estrogens, progesterone and SHBG do not appear to affect a woman’s breast cancer risk during the reproductive years. This changes, however, once a woman enters menopause. Postmenopausal women with higher levels of estrogens were found to have an increased risk of breast cancer, whereas higher levels of SHBG were associated with a reduced breast cancer risk. These appeared to be dose-dependent associations. In contrast, both pre- and postmenopausal women with higher levels of androgens had an increased risk of breast cancer. We were unable to determine whether glucocorticoids affect breast cancer risk due to a paucity of literature on the topic and the failure of existing studies to meet inclusion criteria. The quality of evidence (based on GRADE) for estrogens is low in premenopausal but high for postmenopausal women, whereas the evidence for SHBG and testosterone evidence is moderate to high for both groups.

A strength of this review is the use of the WCRF International/ University of Bristol framework (84), which incorporates a risk of bias assessment and a quality assessment, to synthesise the evidence for the sex steroid hormone pathway and breast cancer risk. Other strengths include examination of estrogen metabolites and analysis of dose–response relationships. Only prospective observational studies and a single Mendelian randomization study met the criteria for inclusion. Cross-sectional and case–control studies were excluded. We used the text-mining program TeMMPo (84) to prioritize potential mediators for these systematic reviews. TeMMPo ranked potential mediators based on the quantity of evidence available for exposure–mediator and mediator–outcome relationships. While this ensured that mediators with the most published evidence were reviewed, consideration of only the top 20 mediators might mean that some novel or less extensively studied mediators were overlooked. We restricted study populations to apparently “healthy” women, excluding those with preexisting menstrual or metabolic disorders. The type and quality of assays used for hormone measurements have evolved over time and where low levels of sensitivity and large inter- and intra-assay variabilities were reported, risk estimates were affected. Some studies reported either excluding breast cancer cases diagnosed within the first year or two of sex steroid hormone assessment, or conducting sensitivity analyses that did this. However, some studies did not consider this potential source of reverse causation. We were also unable to

account for duration of past exogenous hormone use, although we did exclude studies where participants were currently using oral contraceptives or hormone replacement therapy.

Higher levels of estrogens were associated with increased risk of breast cancer in post- but not premenopausal women. This highlights the complexity of sex steroid hormones on breast cancer risk, linking it to a woman's reproductive stage of life. While ovarian estrogen biosynthesis ceases during menopause (9), estrogen produced by adipose tissue, including that found in the breast, will likely contribute to systemic levels (85). This suggests that estrogens produced by the ovaries may not be the key drivers of breast cancer risk. It is important to note, however, that it is not possible to assess cumulative exposure to estrogen pre-menopause and that some studies fail to adjust for menstrual cycle stage when reporting hormone levels. Unlike estrogens, androgens do not decline at menopause (10). This was reflected in the association we reported between androgen levels and breast cancer risk in both pre- and postmenopausal women. Androgens can act either directly, to stimulate the development of breast cancer or they may act as a substrate for aromatase facilitating the conversion of androgens to estrogens (Fig. 1).

The findings of this systematic review are largely consistent with prior reviews and key studies of the effect of sex steroid hormones on breast cancer risk. An analysis of pooled data from nine prospective studies reported that postmenopausal women with higher levels of estrogens and androgens, or lower levels of SHBG, had an increased risk of breast cancer (86). Furthermore, our finding that endogenous estrogens were associated with increased breast cancer risk for post-menopausal women is consistent with a recent examination of the type and timing of menopausal hormone therapy on breast cancer risk (87). This study demonstrated that breast cancer risk in postmenopausal women increased with exogenous estrogen therapy, with or without a progestogen supplement. In addition, our finding that higher levels of circulating SHBG were associated with lower risk of breast cancer is supported by a prior systematic review (6). However, unlike findings from our review, a pooled analysis of data from seven prospective studies found that estrogen levels were positively associated with premenopausal breast cancer risk (88). As we have noted above, assessing lifetime (total) premenopausal estrogen exposure is challenging and disparity between studies in the timing of blood collection for hormone measurements may help to explain the discrepancy in findings.

We reviewed studies that assessed circulating levels of sex steroid hormones, not the site of hormone production. The ovary is not the only site of androgen production in women; the adrenal glands account for approximately 50% of testosterone production and also produce DHEA (10). While testosterone and androstenedione fluctuate in premenopausal women, with higher levels midmenstrual cycle and in the luteal phase, DHEAS levels remain stable throughout the menstrual cycle, declining only with age (89). DHEA, DHEAS, and androstenedione are not directly androgenic; they must be converted to testosterone. Androgen levels remain relatively stable during the transition to menopause (10). With a stable supply of androgens, extragonadal sites such as breast adipose tissue (85) can aromatize androgens to estrogens, and it is this locally produced estrogen, which likely contributes to breast cancer development in postmenopausal women.



These results, considered alongside our concurrent review of physical activity on sex steroid hormones (14), support the biological plausibility of the physical activity–sex steroid hormone–breast cancer pathway. Our meta-analyses of randomized controlled trials investigating the effects of physical activity on sex steroid hormone production identified small decreases in estrogens, progesterone and androgens, as well as an increase in SHBG following exercise. These reductions appeared to be evident in both pre- and postmenopausal women. Considered collectively, the findings from our two reviews (14) provide robust evidence supporting the hypothesis that physical activity’s effect on breast cancer risk is mediated, at least in part, by the sex steroid hormone pathway

There remain some gaps in our understanding of this mechanistic pathway. In order to obtain more accurate effect estimates for the sex steroid hormone–breast cancer pathway, future research will need to take a more considered approach to the selection of confounders and ensure that sensitive and reproducible assays are used (90, 91). As Mendelian randomization studies examining the effect of estrogens, progestogens, and androgens on breast cancer risk become more commonplace causal inference will improve (92). For the overall physical activity–sex steroid hormone–breast cancer pathway, causal mediation techniques can be utilized to quantify precisely how much of the effect of physical activity on breast cancer risk is explained by sex hormones (93). However, sex steroid hormones represent only one pathway by which physical activity can affect breast cancer risk. Additional reviews are underway to examine the biological plausibility of inflammation and insulin signalling as mediators in the physical activity–breast cancer pathway (3).

The evidence suggests that there is no association between circulating estrogens, progestogens, and breast cancer risk in premenopausal women. In contrast, circulating estrogens, likely produced by the adipose tissue, and androgens, produced by the adrenal gland, either directly or as substrate for aromatization to estrogen, may be responsible for driving an increase in the risk of breast cancer in postmenopausal women. Higher levels of SHBG are associated with reduced risk of breast cancer after menopause, and may affect breast cancer risk by fine-tuning exposure to androgens and estrogens. It is biologically plausible, and supported by our reviews on these topics, that physical activity reduces breast cancer risk via sex steroid hormone pathways.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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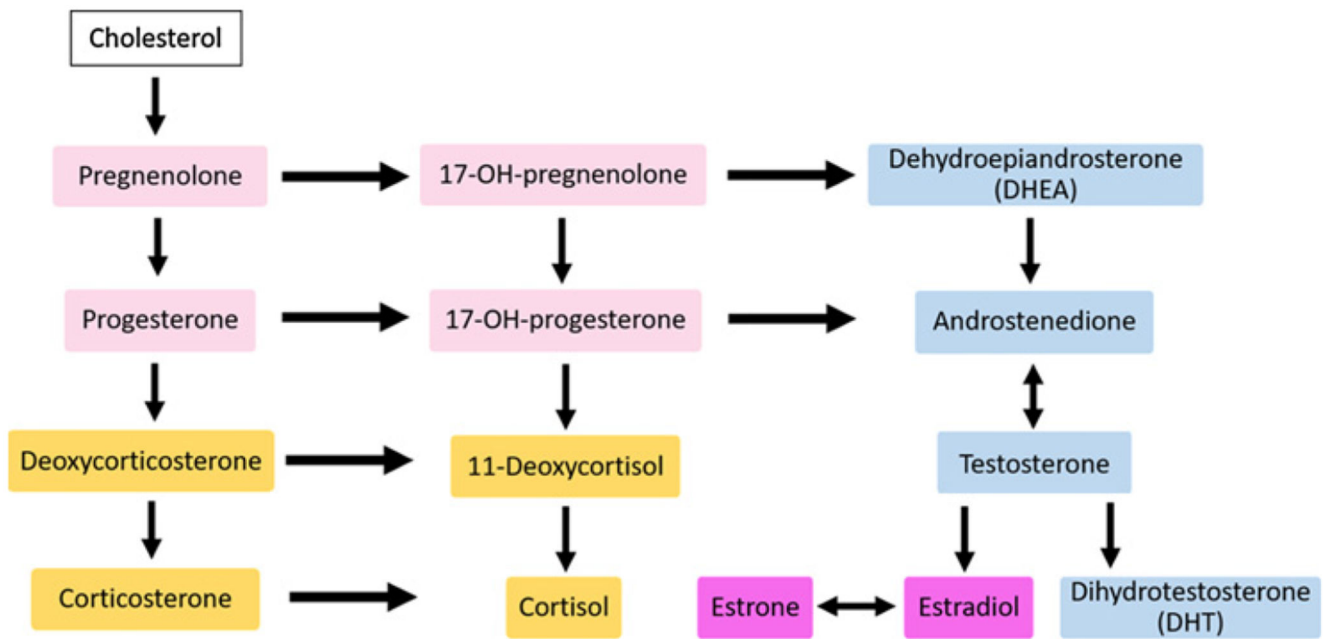
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**Figure 1.** Schematic diagram of the steroidogenic pathway. The relationship between different classes of steroid hormones. The adrenal glands produce glucocorticoids and androgens; the premenopausal ovaries produce progesterone, androgens, and estrogens. Dual arrows indicate that hormonal conversion occurs in both directions.



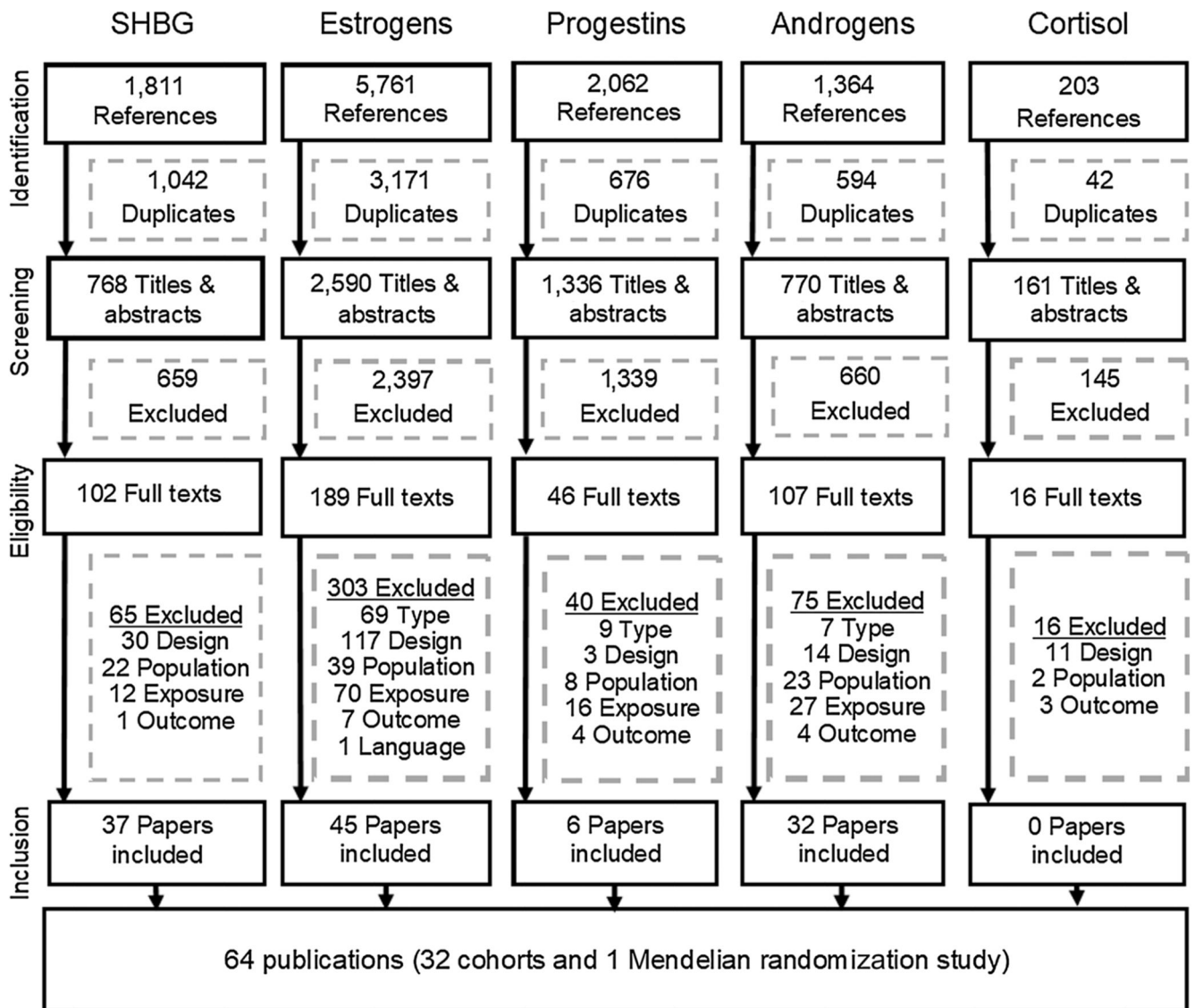
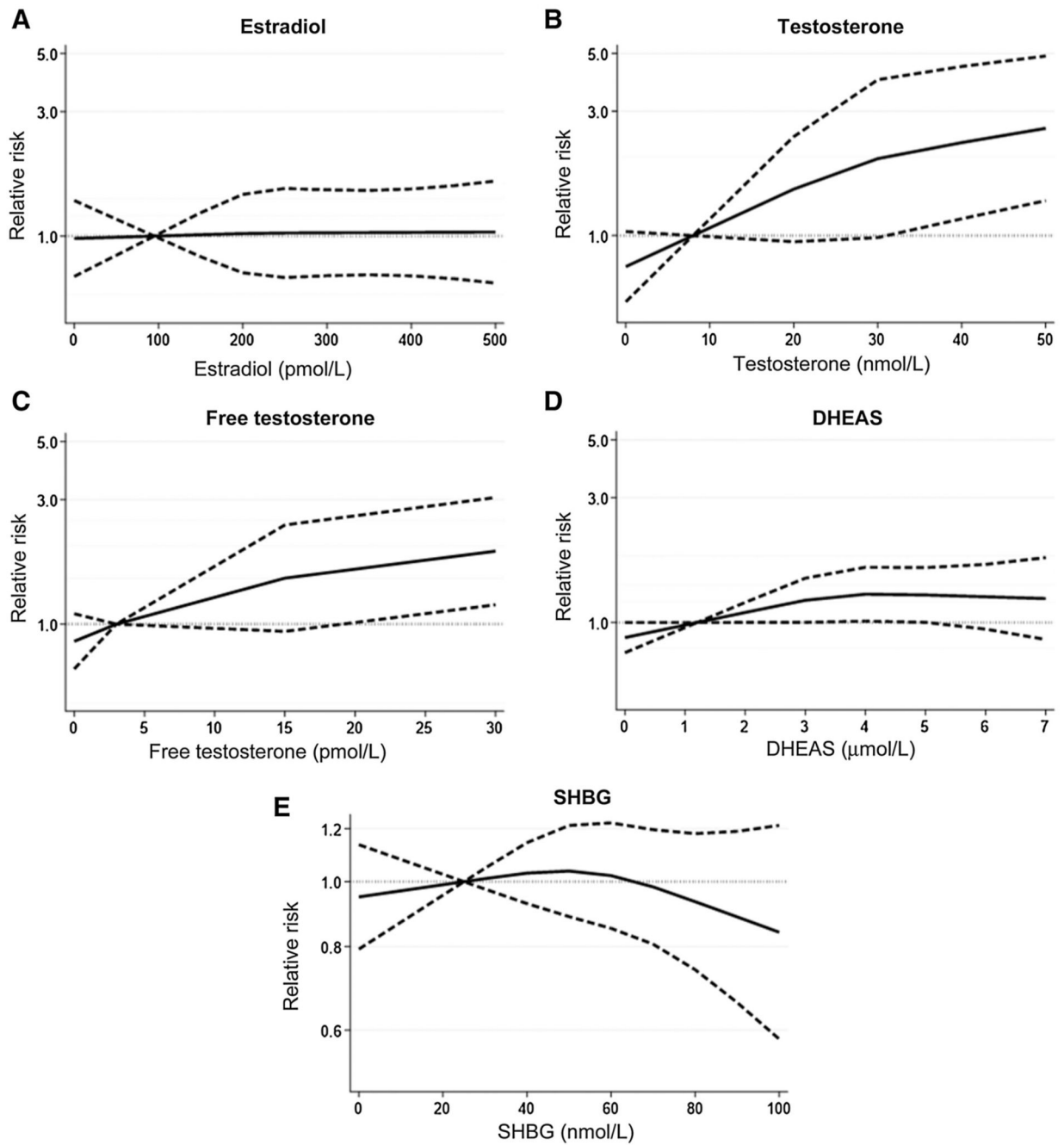
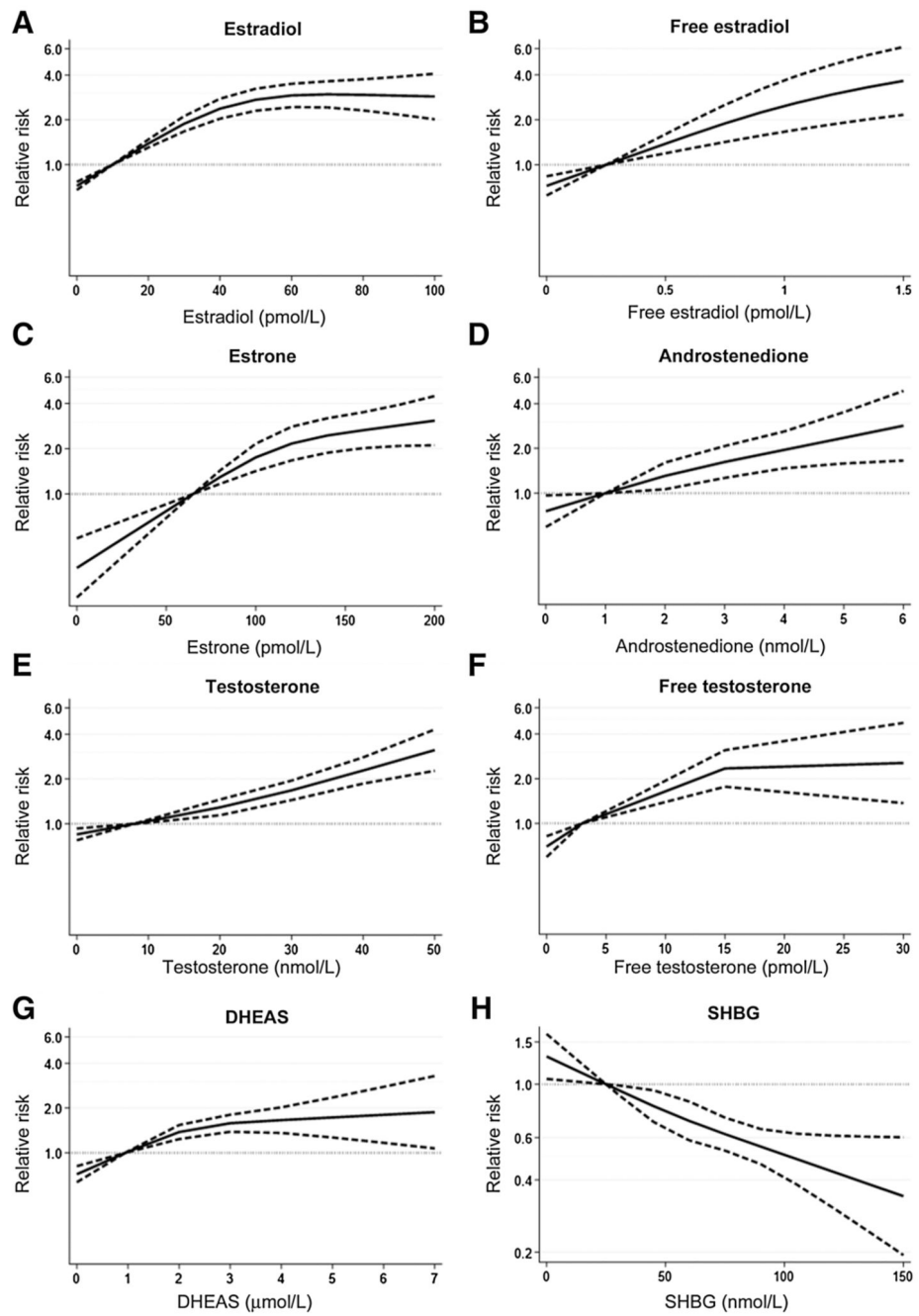


Figure 2. PRISMA flow diagram of literature search, screening, and study selection.



**Figure 3.**

A–E, Dose–response curves for effects of sex steroid hormones on breast cancer risk in premenopausal women.



**Figure 4.** A–H, Dose–response curves for effects of sex steroid hormones on breast cancer risk in postmenopausal women.

**Table 1**  
**GRADE evidence.**

Hormone, menopausal status	Study type, number, participant number	Effect estimates (RR, 95% CI)	Quality of evidence
<b>SHBG</b>			
Pre	Observational, 6 (5,769)	0.96 (0.78–1.14)	Moderate
Post	Observational, 16 (11,211)	0.54 (0.45–0.64)	High (based on both study types)
	Mendelian randomization, 1 (228,951)	0.98 (0.97–0.99)	
<b>Estradiol</b>			
Pre	Observational, 6 (4,730)	0.96 (0.75–1.19)	Low
Post	Observational, 19 (11,814)	1.88 (1.63–2.14)	High
<b>Urinary estradiol</b>			
Post	Observational, 4 (2,373)	1.59 (1.19–1.98)	Low
<b>Estrone</b>			
Pre	Observational, 2 (1,964)	1.00 (0.66–1.34)	Low
Post	Observational, 12 (6,462)	1.74 (1.37–2.11)	Moderate
<b>Free estradiol</b>			
Pre	Observational, 2 (3,831)	0.95 (0.66–1.23)	Low
Post	Observational, 6 (5,228)	1.86 (1.53–2.18)	Moderate
<b>Bioavailable estradiol</b>			
Post	Observational, 3 (1,493)	2.19 (0.96–3.41)	Low
<b>Urinary estrone</b>			
Post	Observational, 4 (2,373)	1.50 (1.09–1.91)	Low
<b>Estrone sulphate</b>			
Post	Observational, 4 (1,910)	1.90 (0.53–3.28)	Low
<b>2-Hydroxyestrone</b>			
Post	Observational, 7 (5,288)	1.24 (0.90–1.29)	Low
<b>16<math>\alpha</math>-Hydroxyestrone</b>			
Post	Observational, 7 (5,288)	1.01 (0.81–1.21)	Low
<b>2-Hydroxyestrone: 16<math>\alpha</math>-hydroxyestrone</b>			
Post	Observational, 4 (3,487)	1.07 (0.83, 1.30)	Low
<b>Progesterone</b>			
Pre and post	Observational, 6 (4,307)	0.98 (0.74–1.23)	Moderate
<b>Testosterone</b>			
Pre	Observational, 5 (5,459)	1.44 (1.11–1.77)	Moderate
Post	Observational, 14 (9,816)	1.45 (1.20–1.70)	Moderate
<b>Free testosterone</b>			
Pre	Observational, 5 (5,193)	1.25 (1.00–1.50)	Moderate
Post	Observational, 7 (5,230)	1.99 (1.65–2.32)	Moderate
<b>Androstenedione</b>			
Pre	Observational, 2 (1,105)	1.90 (1.05–2.75)	Moderate
Post	Observational, 8 (4,210)	1.43 (1.09–1.77)	Moderate
DHEA	Observational, 3 (4,409)	1.08 (0.77–1.40)	Low

Hormone, menopausal status	Study type, number, participant number	Effect estimates (RR, 95% CI)	Quality of evidence
DHEAS			
Pre	Observational, 4 (4,166)	1.07 (0.82–1.33)	Low
Post	Observational, 8 (5,770)	1.64 (1.35–1.93)	Moderate