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Estradiol and Its Metabolites and Their Association With Knee Osteoarthritis

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Objective. **To determine if levels of endogenous estrogen or estrogen metabolites are associated with an increased risk of developing knee osteoarthritis (OA) in women.**

Methods. **Serum estradiol (E_2) and 2 urinary estrogen metabolites (2-hydroxyestrone and 16α-hydroxyestrone) with radiographically defined prevalent and incident knee OA in 842 white and African American women from the Southeast Michigan Arthritis Cohort.**

Results. **The mean age and body mass index (BMI) of women in the cohort were 42.3 years and 28.5 kg/m^2, respectively. Women who developed radiographically defined knee OA had significantly greater odds of having baseline endogenous early follicular phase estradiol concentrations in the lowest tertile (<47 pg/ml; odds ratio [OR] 1.88, 95% confidence interval [95% CI] 1.07–3.51) compared with those with estradiol concentrations in the middle tertile [47–77 pg/ml]), after adjustment for age, BMI, and other covariates. Women who developed knee OA also had greater odds of having baseline urinary concentrations of 2-hydroxyestrone in the lowest tertile (OR 2.9, 95% CI 1.49–5.68) compared with women with 2-hydroxyestrone concentrations in the middle tertile), after adjustment for covariates. Women who developed knee OA were more likely to have a ratio of 16α-hydroxyestrone to 2-hydroxyestrone in the highest tertile (>0.86; OR 1.86, 95% CI 1.01–3.44 compared with women with ratios in the 0.54–0.86 range), after adjustment for other covariates.**

Conclusion. **There were significant associations of lower baseline serum estradiol and urinary 2-hydroxyestrone with developing knee OA in middle-aged women.**

Osteoarthritis (OA) of the knee is characterized by progressive degeneration of articular cartilage along with structural changes in the underlying bone, including development of marginal growths, osteophytes, and increased thickness of the bony envelope (1–3). Sex differences in OA prevalence suggest that sex hormones or alterations in reproductive hormone concentrations that occur with menopause contribute to arthritis pathology (4–6). Because OA is more prevalent in women, emerges at ages 40–50 years, and has pathobiologic underpinnings related to synthesis, inflammation, and repair, it has been suggested that reproductive hormones have a role in the natural history of developing OA. Sex hormones can impact tissues associated with knee OA through direct cellular and molecular action on the synoviocytes, chondrocytes, and bone cells, as reflected in processes such as collagen synthesis, maintenance of bone stiffness, or modulation of excess expression of cytokines (4,5).

Circulating estradiol (E_2), the primary estrogen in premenopausal and early perimenopausal women, declines with menopause. Further, products of estradiol catabolism, including 2-hydroxyestrone and 16α-hydroxyestrone, may also have important roles with respect to arthritis (7). Estradiol is preferentially converted to estrone by the 17-hydroxysteroid dehydrogenase enzyme, after which there is subsequent metabolism by the cytochrome P450 enzymes (8) (Figure 1). The 16α-hydroxyestrone acts as an estrogen agonist in some model systems, while 2-hydroxyestrone may have

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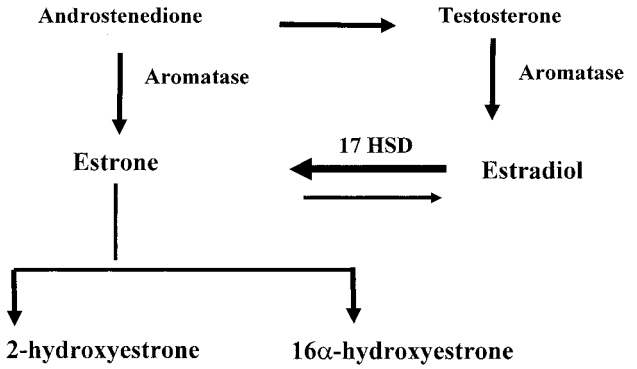

Figure 1. Pathway of estrogen metabolism including estradiol, estrone, and estrogen metabolites. 17 HSD = 17-hydroxysteroid dehydrogenase.

weak estrogenic activity (9–11). The 16α-hydroxyestrone can bind covalently and noncovalently to the estrogen receptor (ER), although with less affinity than estradiol (12). However, since 16α-hydroxyestrone has a low affinity for serum sex hormone binding globulin, it is more available for estrogen-sensitive target tissues (12). Although 2-hydroxyestrogens have reduced binding affinity for the ER (13,14), they appear to have additional important properties, including participation in oxidation/reduction actions (15) and modulating the physiologic effects of arachidonic acid metabolism and prostaglandins (16). Higher ratios of 16α-hydroxyestrone to 2-hydroxyestrone have been associated with several diseases, including reproductive cancers, systemic lupus erythematosus (SLE), liver cirrhosis, and osteoporosis (12,17–20).

The goal of this study was to evaluate the associations of serum estradiol, urinary 2-hydroxyestrone, and urinary 16α-hydroxyestrone as risk factors for knee OA in women, while controlling for effects of other known risk factors for OA.

PATIENTS AND METHODS

Sampling and study population. Premenopausal and perimenopausal women from 2 longitudinal studies were evaluated using a protocol common to both studies (21). This study includes data on 458 women from the Michigan Bone Health Study (MBHS) and 384 women from the Study of Women's Health Across the Nation (SWAN), who had radiographs of both knees as well as urine and serum specimens available for analyses of hormones. Collectively, these study groups are known as the Southeast Michigan Arthritis Cohort, and the cohort has been described previously (21).

SWAN is a population-based longitudinal study of the menopausal transition. In 1996, 543 eligible women (325 African American and 218 white) who met the age (42–52 years), menstrual status (menstrual bleeding within the previous 3 months and had not received hormone replacement therapy [HRT]), and ethnicity criteria specified by the study protocol were enrolled. In 1996–1997, a site-specific study of physical functioning, including radiographic measures of OA, was implemented in the SWAN population. Cumulatively, loss to followup of living enrollees has been $<20\%$.

MBHS, begun in 1992, is a population-based longitudinal study of musculoskeletal disease development in premenopausal and perimenopausal white women. The 664-woman sample was identified from the family records of participants in the historic Tecumseh Community Health Study (TCHS) and a 1992 Tecumseh, Michigan, community census. From the TCHS, 80% of the female offspring who were 24–44 years old in 1992 were recruited. Additionally, $>90\%$ of the eligible women from the community census who were not part of the TCHS were recruited. Loss to followup was $<15\%$ over a 10-year time period. Measures of physical functioning, including radiographically defined knee and hand OA, were obtained in the same time frame as in the Michigan SWAN population (5,21).

Approval for this study was obtained from the University of Michigan Institutional Review Board.

Scoring system. Interviews addressing pain, health status (including medications), and lifestyle, as well as phlebotomy were completed annually. Radiographs of the knee were obtained at the time of annual assessment, but 3 years apart, and were used to define 2 outcomes, the prevalence and the incidence of radiographically defined knee OA.

Anteroposterior radiographs of both knees were obtained, using General Electric (Milwaukee, WI) radiographic equipment and Kodak film (Eastman Kodak, Rochester, NY). Standard radiographic techniques were used. Radiographs of weight-bearing knee joints were evaluated by 2 readers (MH, DAJ) independently, using the Kellgren/Lawrence (K/L) scoring system (22). This scoring system is highly sensitive to the presence of osteophytes and is considered appropriate to identify the initial stages of OA, which commonly develops during middle age. Using this system, a score of 0–4 was assigned to the joints based on the degree of osteophyte formation, joint space narrowing, sclerosis, and joint deformity. According to the K/L scale, 0 = normal, 1 = doubtful OA, 2 = minimal OA, 3 = moderate OA, and 4 = severe OA. Consistency of grading between evaluators was determined and joint grading was standardized. If the 2 readers did not agree on a grade, a consensus approach including a third reader (MRS) was used. A set of "drift" films was also included to help ensure that there had been no change over time in assignment of a given K/L score to a given set of findings. Women were classified as having prevalent knee OA if they had a radiograph with a K/L score ≥ 2; women were classified as having incident knee OA if they were free of disease at baseline, but knee OA was identified in subsequent radiographs. Of the 3,768 knee radiographs evaluated according to this protocol, the readers concurred on the K/L score in 3,399 (90%); 369 radiographs had to be reread by consensus.

Exposure measures. Blood was drawn during the annual study visits, 2–7 days following menses (early follicular phase of the menstrual cycle), and after a 12-hour fast. The blood was refrigerated for up to an hour and then centrifuged to provide serum that was then frozen at -80°C until assay.

For those women with no menstrual bleeding or who were receiving HRT, blood was drawn on the anniversary of the baseline examination.

Serum estradiol concentrations were measured using a modified, offline automated chemiluminescence system E_2-6 immunoassay (ACS:180; Bayer Diagnostics, Tarrytown, NY). Inter- and intraassay coefficients of variation (CVs) were 10.6% and 6.4%, respectively, over the assay range. The estrogen metabolites 2-hydroxyestrone and 16α-hydroxyestrone were measured from urine that had been obtained in the year prior to radiographic diagnosis of knee OA. The estrogen metabolites were measured by enzyme-linked immunosorbent assay (Estramet; Immuna Care, Blue Bell, PA) from samples collected prior to radiographic assessment and were run in triplicate (23). Interassay CVs were 13.4% (3.8 ng/ml) and 11.3% (6.3 ng/ml). The intraassay CV was 5.5% over the working assay range (1.1–12.1 ng/ml).

Body mass index (BMI) was calculated as kg/m^2, with weight measured using a balance beam scale and height measured using a stadiometer. Participants were classified as current smokers, those who had ever smoked, or those who had never smoked. A history of knee injury was determined using an arthritis questionnaire to identify pain and clinical characteristics. It was administered at the same time at which radiographs were obtained. Women were classified as premenopausal, receiving HRT (including oral contraceptives), menopausal (naturally, surgically, or chemotherapy-induced), or having undergone a hysterectomy, with ovaries intact and no hormone use. Of the 107 women who had ever taken reproductive hormones, 65 were premenopausal, with the following hormone use patterns: 52 women (80%) took oral contraceptives, 7 women (11%) took a combination of conjugated estrogen and progesterone, 3 women (5%) took progesterone only, and 3 women (5%) took conjugated estrogens only. Of the 42 naturally or surgically postmenopausal women who took reproductive hormones, 37 women (88%) took conjugated estrogens, 3 women (7%) took a combination of conjugated estrogen and progesterone, and 2 women (5%) took oral contraceptives with estrogen.

Statistical analysis. All univariate, bivariate, and multivariate analyses were performed using SAS software, version 8.0 (SAS Institute, Cary, NC). The mean, median, standard deviation, and minimum and maximum values were calculated for all continuous variables, including the 2-hydroxyestrone and 16α-hydroxyestrone concentrations (and their ratio), estradiol, BMI, and baseline age. All continuous variables were evaluated for consistency with a normal distribution and were log-transformed, as appropriate, to satisfy the normality assumption in parametric testing. Frequencies and percentages were calculated for all categorical variables, including prevalence and incidence of knee OA, smoking, reproductive status, and history of knee injury.

Multivariate logistic regression models were used to examine the associations between prevalence and incidence of knee OA and estrogen metabolites and estradiol, adjusting for BMI, age, smoking history, history of knee injury, day of blood draw, and menstrual status. BMI and age were held at their baseline levels for all logistic models, and were assessed for interaction with estradiol or the metabolites with respect to prevalent or incident knee OA. No significant interactions were found, and the final logistic models included no interaction terms. Day of blood draw was initially included in each logistic model, but was excluded from final models because it was not significant, and the odds of prevalent or incident knee OA according to estradiol or estrogen metabolite concentrations were unaffected by its inclusion. Final models also did not include menstrual status, because at the followup assessment, only 7 of 58 naturally or surgically postmenopausal women (12%) presented with prevalent knee OA. Among premenopausal and early perimenopausal women, however, the odds of prevalent and incident knee OA according to estradiol and estrogen metabolite concentrations were consistent in magnitude and significance with those of the larger study population.

C statistics were used to characterize the goodness-of-fit of models. Type I error was assessed based on 2-sided tests. *P* values less than 0.05 were considered significant.

RESULTS

The baseline prevalence of knee OA in this sample was 11%, while the annual incidence was 3.2%. The mean ± SD age at baseline in the study sample was 42 ± 0.2 years (range 26–54 years). The mean ± SD BMI was 27.9 ± 0.1 kg/m^2. More than three-fourths of the women were premenopausal at the baseline examination.

The mean ± SD adjusted serum E_2 concentration was 62 ± 43 pg/ml, as shown in Table 1. While the mean serum E_2 value was ~15% lower in women with incident knee OA, this difference was not statistically significant. The mean ± SD 2-hydroxyestrone and 16α-hydroxyestrone concentrations were 11.2 ± 8.6 ng/ml and 7.8 ± 5.8 ng/ml, respectively. As shown in Table 1, the 2-hydroxyestrone and 16α-hydroxyestrone values were ~15% lower in those women with incident knee OA; these differences were statistically significant. Women with prevalent or incident knee OA also had significantly larger ratios of 16α-hydroxyestrone to 2-hydroxyestrone (mean ± SD 0.76 ± 0.47 for prevalent knee OA and 0.83 ± 0.51 for incident knee OA), compared with women without knee OA (0.68 ± 0.51).

Associations with incident knee OA. As shown in Table 2, women who developed knee OA over the 3-year period had greater odds of having baseline endogenous estradiol concentrations in the lowest tertile (<47 pg/ml; OR 1.88, 95% CI 1.07–3.51 compared with estradiol concentrations in the middle tertile [47–77 pg/ml]), after adjustment for covariates including age and reproductive status. Women who developed knee OA also had greater odds of having baseline urinary 2-hydroxyestrone concentrations in the lowest tertile (OR 2.91, 95% CI 1.49–5.68) compared with women

Table 1. Hormone concentrations classified according to knee OA, smoking, and reproductive status*

	Estradiol, mean ± SD pg/ml	2-hydroxyestrone, mean ± SD ng/ml	16α-hydroxyestrone, mean ± SD ng/ml	Ratio of 16α-hydroxyestrone: 2-hydroxyestrone, mean ± SD
Overall	62 ± 43	11.2 ± 8.6	7.8 ± 5.8	0.70 ± 0.29
Knee OA				
Prevalence (baseline)				
No knee OA (n = 745 [89])	62 ± 44	11.3 ± 8.2	7.8 ± 5.5	0.69 ± 0.27
Knee OA (n = 89 [11])	69 ± 48	10.2 ± 8.5	7.7 ± 4.7	0.76 ± 0.47†
Incidence (3-year)				
No knee OA (n = 656 [90])	63 ± 43	11.8 ± 10.2	8.0 ± 5.1	0.68 ± 0.51
Knee OA (n = 71 [10])	54 ± 38	8.3 ± 6.7†	6.8 ± 4.2†	0.83 ± 0.51†
Smoking at baseline				
Current (n = 170 [20])	61 ± 42	14.9 ± 13‡	9.3 ± 6.5‡	0.62 ± 0.39‡
Past/never (n = 661 [80])	63 ± 44	10.4 ± 7.7	7.5 ± 5.1	0.72 ± 0.51
Baseline reproductive status				
Premenopausal (n = 695 [83.5])	63 ± 42§	11.0 ± 10.5	7.6 ± 5.3	0.69 ± 0.53
Hormone therapy use (n = 107 [13])	54 ± 38§	11.9 ± 10.3	8.7 ± 6.2	0.73 ± 0.41
Postmenopausal (n = 4 [0.5])	28 ± 19§	8.3 ± 7.4	6.3 ± 4.2	0.84 ± 0.52
Hysterectomy (n = 24 [3])	103 ± 71§	16.5 ± 13.4	10.9 ± 6.9¶	0.67 ± 0.39

* A total of 842 patients were studied; however, for each category shown, data were not available on all patients (n values are the number for whom data were available; values in brackets are percents). Age- and body mass index–adjusted pairwise comparisons are based on differences between group least squares means ± SEM. OA = osteoarthritis.
† $P < 0.05$ versus no OA.
‡ $P < 0.05$ versus past/never smokers.
§ Value for each status significantly different ($P < 0.05$) from all others.
¶ $P < 0.05$ versus premenopause and postmenopause.

with 2-hydroxyestrone concentrations in the middle tertile. As a result, women who developed knee OA were also more likely to have a 16α-hydroxyestrone to 2-hydroxyestrone ratio in the highest tertile (>0.86; OR 1.86, 95% CI 1.01–3.44 compared with women with ratios in the 0.54–0.86 range), even after adjustment for

Table 2. Odds ratios and 95% confidence intervals for having levels of estradiol and the estrogen metabolites 2-hydroxyestrone and 16α-hydroxyestrone in the lowest or highest tertile, in women with prevalent or 3-year incident knee OA*

Variable	OR (95% CI)	
	Prevalent knee OA (n = 842)	Incident knee OA (n = 736)
$_{log}$estradiol (pg/ml)		
≤33rd percentile (<47 pg/ml)	1.20 (0.75–1.91)	1.88 (1.07–3.51)†
33rd–66th percentile (47–77 pg/ml)	Referent	Referent
≥66th percentile (>78 pg/ml)	1.06 (0.66–1.71)	1.04 (0.52–2.09)
c statistic	0.74	0.74
$_{log}$2-hydroxyestrone		
≤33rd percentile (<7.5 ng/ml)	1.62 (1.03–2.55)	2.91 (1.49–5.68)
33rd–66th percentile (7.6–15.3 ng/ml)	Referent	Referent
≥66th percentile (>15.4 ng/ml)	0.62 (0.37–1.03)	1.06 (0.49–2.29)
c statistic	0.76	0.75
$_{log}$16α-hydroxyestrone		
≤33rd percentile (<6.1 ng/ml)	1.37 (0.86–2.18)	1.36 (0.74–2.51)
33rd–66th percentile (6.2–9.5 ng/ml)	Referent	Referent
≥66th percentile (>9.6 ng/ml)	0.85 (0.52–1.41)	0.87 (0.44–1.71)
c statistic	0.75	0.72
$_{log}$16α-hydroxyestrone: 2-hydroxyestrone ratio		
≤33rd percentile (<0.53)	0.73 (0.45–1.21)	0.87 (0.44–1.73)
33rd–66th percentile (0.54–0.86)	Referent	Referent
≥66th percentile (>0.87)	1.65 (1.05–2.61)	1.86 (1.01–3.44)
c statistic	0.75	0.73

* All models were adjusted for age, body mass index, reproductive status, race/ethnicity, and history of knee injury. OA = osteoarthritis; OR = odds ratio; 95% CI = 95% confidence interval.
† $P < 0.05$.

Table 3. Spearman's correlations relating baseline estradiol and estrogen metabolite levels with each other and with covariates*

	Estradiol	2-hydroxyestrone	16α-hydroxyestrone	16α-hydroxyestrone: 2-hydroxyestrone ratio
Estradiol		0.27 (<0.0001)	0.16 (<0.0001)	−0.18 (<0.0001)
2-hydroxyestrone			0.67 (<0.0001)	−0.65 (<0.0001)
16α-hydroxyestrone				0.06 (NS)
Body mass index	−0.13 (0.0002)	−0.17 (<0.0001)	−0.05 (NS)	0.19 (<0.0001)
Age	−0.04 (NS)	−0.02 (NS)	−0.15 (<0.0001)	−0.11 (0.001)

* *P* values are shown in parentheses. NS = not significant.

other covariates. Similar patterns were seen with prevalent knee OA and are also shown in Table 2.

Correlations of hormone levels with BMI. Estradiol concentrations were not highly correlated with concentrations of the estrogen metabolites, although levels of the metabolites were highly and positively correlated with each other (r = 0.67) (Table 3). In this sample, BMI was negatively correlated with 2-hydroxyestrone, i.e., as BMI increased, there was less 2-hydroxyestrone. Similarly, as age increased, there was less 16α-hydroxyestrone.

DISCUSSION

There has been an ongoing debate as to whether hormone levels contribute to the pathogenesis of OA and/or serve as a biomarker of risk for the development of OA. In humans, this debate has been largely focused on HRT, under the assumption that HRT was correcting an estradiol deficiency resulting from natural or surgical menopause. It is now increasingly recognized that using HRT as a model for the impact of circulating endogenous estradiol levels in abnormal conditions is flawed because its users constitute a select and healthier subgroup of the population of women. There have been surprisingly few studies that have actually examined hormone concentrations, particularly in relation to newly developing OA among women in their 50s and 60s. There are no studies of other estrogens that have biologic activities, including the 2-hydroxyestrone and 16α-hydroxyestrone metabolites. We found lower serum estradiol concentrations as well as lower amounts of 2-hydroxyestrone in women who subsequently developed knee OA. This, in turn, led to a higher ratio of 2-hydroxyestrone to 16α-hydroxyestrone associated with developing incident knee OA, even after adjusting for age, injury, and BMI, characteristics which have been previously identified in many studies as risk factors (21).

Selected work with animal models provides support for the increased odds of developing OA with lower estradiol concentrations. It has long been reported that, in tissue culture systems, estrogen stimulated collagen synthesis in rat uteri (24). In bovine aortic smooth muscle cells, estrogen was associated with a shift in procollagen subfractions from type I to type III (25). Nasatzky and colleagues reported that in chondrocytes (and depending upon the 17β-estradiol concentration as well as the animal model used), 17β-estradiol inhibited cell proliferation, stimulated RNA synthesis, and stimulated $^{35}SO_4$ incorporation as well as collagen production (26,27). Work in baboons (28) and other animal models (29–31) suggests the presence of estrogen receptors in the chondroblasts and chondrocytes of laryngeal elastic and hyaline cartilage and in the articular cartilage of the mandibular condyle. Likewise, Tsai and Liu (29) have reported the presence of estrogen receptors in the cartilage of rabbit femoral condyles.

Our work demonstrates that lower levels of estrone metabolites, especially 2-hydroxyestrone, are also related to the development of OA. CP450 enzymes generate multiple hydroxylated products, via estrome, in estradiol catabolism. These products are hormonally inactive or less active water-soluble products, which are, in turn, excreted in the urine or feces. The lower levels of 2-hydroxyestrone may be related to lower levels of estradiol; however, the correlation between estradiol concentrations and 2-hydroxyestrone was modest (r = 0.27).

The action of higher 2-hydroxyestrone concentrations in delaying the development of knee OA potentially may be through the arachidonic acid pathway, including the inhibition of leukotriene synthesis and modulation of lipoxygenases. Rosenkrans and colleagues (32) proposed that the effects of estrogens on prostaglandins are specifically mediated through the catechol metabolites (2- and 4-hydroxyestrogens) rather than estradiol (33). This was confirmed by Alanko et al (34), who reported that while estradiol had no effect on arachidonic acid metabolism, the catechol estrogens

were much more potent inhibitors of leukotriene synthesis than thromboxane and prostaglandin E_2 synthesis. Seeger et al (35) showed that estrogen metabolites increased prostacyclin synthesis in endothelial tissues at near-physiologic concentrations. Catechol estrogens appear to modulate lipoxygenases in vivo. To inhibit leukotriene synthesis, catechol estrogens may inhibit 5-lipoxygenase by reducing the catalytically active ferric enzyme to the catalytically inactive ferrous forms (36). The inhibition of cyclooxygenase may be due to the capability of catechol estrogen to remove the intermediate radical that is essential to the cyclooxygenase mechanism (37).

There are now a limited number of reports of altered patterns of estrogen metabolism in both SLE and rheumatoid arthritis (RA) (18,19), suggesting that these alterations may be associated with symptoms (pain and inflammation) rather than disease (structural) onset and progression, as reflected in the catabolism of articular cartilage and endochondral ossification. Weidler et al (18) reported that 2-hydroxyestrone concentrations were 10 times lower in a mixed clinical sample of male and female patients with RA and SLE as compared with healthy controls. In our homogeneous population composed primarily of women in their 40s and 50s, the 2-hydroxyestrone concentrations in those with knee OA were significantly less than those in women without knee OA. However, more detailed investigation is required to establish if primary pathways are associated with symptoms, duration of symptoms, symptom severity, or with response in cartilage and bone. When we accepted every woman who reported pain without specification of the underlying pathology, a characteristic that was reported by more than one-quarter of enrollees, there was no association with pain. Greater understanding of these metabolic activities has implications for the selection of bone resorption and pain medication used in OA, in addition to the development of OA (20).

High BMI is a well-documented risk factor for the development of knee OA in both men and women, and the assumption has been largely that this risk is generated by load bearing on the knee, ankle, and foot joints (38). However, greater amounts of adipose tissue, seen among women with higher BMI, may also contribute to the estrogenic environment and influence chondrocytes or bone cells. It is now well established that androstenedione is a major precursor of estrone and that the conversion from this androgen to estrogen is undertaken by aromatase in adipose tissue (39). In menopause, estrone is produced by the peripheral aromatization of the adrenal androstenedione in the adipose tissue. In this sample, BMI was negatively correlated with 2-hydroxyestrone, i.e., as BMI increased, there was less 2-hydroxyestrone. This suggests that adipose tissue might influence an important hormonal element in the development of knee OA as well as contribute to the more traditional loading mechanism.

We noted higher estradiol levels among women who had undergone a hysterectomy (Table 1). It is thought that these levels were related to our inability to draw blood in the 2–7-day window of the early follicular phase of the menstrual cycle.

This study has strengths and limitations. The study examined hormone concentrations in women who were approaching or in the menopausal transition in relation to the development of knee OA. The hormones were related to incident knee OA but not to prevalent knee OA. We point out that this measurement of hormones temporally preceded the identification of knee OA. Potentially, concurrent measurement of hormone levels in women with prevalent knee OA would include measurements in women with knee OA that developed as a result of trauma or other causes to a point that it is recognizable on radiograph. The study time frame may not fully capture the arthritis development processes. The study also does not identify which tissues (for example, synoviocytes, chondrocytes, and bone cells) might be directly impacted by the lower levels of estradiol or 2-hydroxyestrone. The study does not identify whether the observed associations are the response to a receptor-mediated process (as in the case of lower estradiol concentrations), to a cytokine-based response (as in the case of 2-hydroxyestrone), or, potentially, to both mechanisms.

In summary, this report describes the strong associations of lower circulating estradiol and 2-hydroxyestrone levels with more knee OA (both prevalence and incidence). If findings are confirmed, then this helps motivate new areas of investigation for intervention. If the mechanistic explanation for 2-hydroxyestrone levels lies, at least in part, in arachidonic acid metabolism associated with pain and inflammation rather than receptor binding, then consideration of alternative lifestyle and therapeutic pathways to influence these metabolites becomes increasingly viable.

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