# Exogenous Estrogen Exposures and Changes in Diabetic Retinopathy

## The Wisconsin Epidemiologic Study of Diabetic Retinopathy

BARBARA E.K. KLEIN, MD, MPH
RONALD KLEIN, MD, MPH
SCOT E. MOSS, MA

**OBJECTIVE** — To investigate whether the use of exogenous estrogen is associated with changes in the severity of diabetic retinopathy and the incidence of macular edema.

**RESEARCH DESIGN AND METHODS** — The study design involved observation of two well-defined cohorts of women with diabetes. One group was diagnosed with diabetes at <30 years of age and used insulin (younger-onset group), and the other group was diagnosed at ≥30 years of age with no criteria regarding therapy (older-onset group). Subjects received standard examinations, medical interviews, and retinal photography in 1980–1982. Specific questions about exogenous hormone exposure were added to the study questionnaire at the first follow-up examination 4 years after the baseline examination. Change in the severity of retinopathy 6 and 10 years after the 4-year follow-up examination were examined regarding the use of oral contraceptives at the first follow-up examination in the younger-onset group and at 6 years after the first follow-up examination regarding hormone replacement therapy in the older-onset group.

**RESULTS** — Changes in the severity of retinopathy and incidence of macular edema were unrelated to either type of estrogen exposure in univariable and multivariable analyses.

**CONCLUSIONS** — These data are compatible with the hypothesis that the medications used by our population do not affect the severity of diabetic retinopathy or macular edema.

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Hormonal events or exposures may influence the course of diabetic retinopathy in women (1,2). Pregnancy is the most notable of these exposures and is associated with an increased risk and progression of retinopathy. Data suggest that puberty may influence the development of diabetic retinopathy (2,3). Exogenous hormone exposures in women affect risk factors for vascular disease in general. In women taking oral contraceptives, serum lipids (4) and glycemia (5) may be altered, and blood pressure may be elevated (6). In women receiving hormone replacement therapy (HRT), changes in serum lipids (7), glucose (8), insulin (8), and retinal vasculature (9) have been found. In women with type 2 diabetes, decreased $HbA_1$ has been associated with taking 17 β-estradiol (10). Although these data suggest a salutary effect, in another study of women who survived one atherosclerotic cardiovascular event and who were randomly assigned to begin HRT, an increased risk of having another such event was evident within the first year of use of HRT (11). Also, data indicate that HRT has an effect on endometrial cancer (12), and other data suggest that both HRT and oral contraceptives affect the risk of breast cancer (13). Nevertheless, the use of these hormone preparations is appealing and is widespread among women with and without diabetes because of their beneficial effects, such as pregnancy control from oral contraceptives and a diminished risk of osteoporosis (14) and a possible decreased risk of primary cardiovascular events (15) from HRT.

We previously examined the association in prevalence data between the use of oral contraceptives and the severity of diabetic retinopathy in women with younger-onset diabetes who participated in the Wisconsin Epidemiologic Study of Diabetic Retinopathy. We found no relationship between exposure and retinal lesions (16). Herein we describe our experience at a follow-up 10 years later. In addition, we describe the change in severity of diabetic retinopathy in women with older-onset diabetes during a 6-year interval regarding the use of HRT.

## RESEARCH DESIGN AND METHODS

### Population

This population has been described in previous reports (17–20). Briefly, the population consisted of a sample selected from 10,135 diabetic patients who received primary care in an 11-county area in southern Wisconsin from 1979 to 1980. This sample consisted of a younger-onset group (women diagnosed at <30 years of age, all of whom took insulin, $n$ = 576) and an older-onset group (a random sample of women stratified by duration of diabetes, 439 took insulin and 540 did not, $n$ = 979).

Of the younger-onset women, 484 participated at the baseline examination (1980–1982), 441 participated 4 years later, 388 participated at the 10-year follow-up, and 322 participated at the 14-year follow-up. Comparisons of participants at

From the Department of Ophthalmology and Visual Sciences, University of Wisconsin Medical School, Madison, Wisconsin.

Address correspondence to Barbara E.K. Klein, MD, MPH, Department of Ophthalmology and Visual Sciences, University of Wisconsin-Madison, 610 N. Walnut St., 460 WARF, Madison, WI 53705-2397. E-mail: kleinb@epi.ophth.wisc.edu.

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**Abbreviations:** dBP, diastolic blood pressure; HRT, hormone replacement therapy; sBP, systolic blood pressure.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances

**Table 1—*Baseline characteristics of women participating at the baseline, 4-year, and 10-year examinations***

| | Baseline | | 4-Year | | 10-Year | |
|---|---|---|---|---|---|---|
| Cohort characteristics | *n* | Mean ± SD | *n* | Mean ± SD | *n* | Mean ± SD |
| Younger onset | | | | | | |
| Age (years) | 484 | 29.6 ±13.1 | 441 | 28.7 ±12.2 | 388 | 28.0 ± 11.8 |
| Duration (years) | 484 | 15.3 ± 10.6 | 441 | 14.7 ± 10.1 | 388 | 14.1 ± 9.8 |
| $HbA_1$ (%) | 464 | 10.9 ± 2.1 | 424 | 10.8 ± 2.1 | 371 | 10.8 ± 2.1 |
| sBP (mmHg) | 482 | 122.0 ± 20.0 | 440 | 121.0 ± 18.0 | 387 | 119.0 ± 17.0 |
| dBP (mmHg) | 480 | 78.0 ± 11.0 | 439 | 79.0 ± 11.0 | 386 | 78.0 ± 10.0 |
| Older onset | | | | | | |
| Age (years) | 734 | 67.2 ± 11.4 | 542 | 65.4 ± 11.2 | 308 | 61.6 ± 10.8 |
| Duration (years) | 734 | 12.0 ± 8.3 | 542 | 11.4 ± 8.2 | 308 | 9.8 ± 7.1 |
| $HbA_1$ (%) | 690 | 9.8 ± 2.0 | 508 | 9.7 ± 2.0 | 287 | 9.5 ± 2.0 |
| sBP (mmHg) | 733 | 149.0 ± 24.0 | 542 | 147.0 ± 24.0 | 308 | 144.0 ± 20.0 |
| dBP (mmHg) | 730 | 78.0 ± 12.0 | 540 | 79.0 ± 11.0 | 307 | 80.0 ± 11.0 |

baseline and at the 4- and 10-year follow-up examinations are shown in Table 1. Of the older-onset women, 734 participated at the baseline examination, 542 participated at the 4-year follow-up, and 308 participated at the 10-year follow-up. We did not examine the older-onset group at the 14-year follow-up. Comparisons among participants at baseline and at the 4- and 10-year follow-up examinations are shown in Table 1. Time between the baseline and 10-year follow-up examinations was 10.1 ± 0.4 years (means ± SD). We collected data concerning many of the exposures of interest to this investigation at the 4-year follow-up examination in 1984–1986. Therefore, change in retinopathy was determined with respect to measures at that examination. Data in Table 1 referring to baseline data reflect the actual baseline examination in 1980–1982 and are provided so that the reader can compare the characteristics at baseline of the subjects who participated in subsequent follow-up examinations.

## Procedures

All examinations were performed in a mobile examination van in or near the city in which the participants lived. Blood pressure was measured with a Hawksley random-zero sphygmomanometer (Hawksley & Sons, West Sussex, U.K.) by using the Hypertension Detection and Follow-Up Program protocol (21).

Other relevant parts of the examination consisted of measuring height and weight, dilating the pupils, taking stereoscopic color fundus photographs of seven standard fields (22), administering a structured interview, performing a semiquantitative determination of protein in the urine by using Labstix (Ames, Elkhart, IN), and determining $HbA_1$ with a microcolumn technique (Isolab, OH) (23). We followed the tenets of the Declaration of Helsinki. Subjects gave informed consent, and an institutional human experimentation committee approved the protocol.

## Definitions

Current age was the age at the 1984–1986 examination. Age at diagnosis of diabetes was the age when a physician first recorded the diagnosis on the participant's chart or on a hospital record. The duration of diabetes was the period between the age at diagnosis and the age at the 1984–1986 examination. The mean systolic blood pressure (sBP) was the average of the two sBP determinations, and the mean diastolic blood pressure (dBP) was the average of the two dBP determinations. In people aged ≥25 years at the 1984–1986 examination, hypertension was defined as a mean sBP of ≥160 mmHg, and/or a mean dBP of ≥95 mmHg, and/or a history of hypertension involving the use of antihypertensive medications. In people aged ≤25 years, hypertension was defined as a mean sBP of ≥140 mmHg, and/or a mean dBP of ≥90 mmHg, and/or a history of hypertension involving the use of antihypertensive medications. Incidence of hypertension was computed for people not classified as having hypertension as defined above in 1984–1986. Incidence cases involved subjects who developed hypertension at one of the follow-up examinations. Proteinuria was defined as a urine protein concentration of ≥0.30 g/l measured with a reagent strip.

At the 4-year follow-up, all subjects were asked when they began to menstruate and whether they were currently having menstrual cycles. If not, they were asked when the cycles stopped and whether this was due to surgery or natural menopause. Subjects who reported having surgery were asked whether their ovaries were removed. All subjects aged ≥14 years were asked whether they had ever taken estrogen or estrogen-progesterone combination drugs, whether they were currently taking such drugs, and, if so, for how long. The names of specific estrogen and/or estrogen-progesterone combinations were also recorded. If a subject reported an age at onset of menses and also responded that she was not currently having menstrual cycles, then she was assumed to be menopausal. These subjects were classified as receiving HRT if the name of the estrogen and/or estrogen-progesterone drugs they reported taking

**Table 2—*The 10-year progression and incidence of retinal end points and hypertension by use of oral contraceptives in younger-onset women aged ≥18 years in 1984–1986: the Wisconsin Epidemiologic Study of Diabetic Retinopathy***

| End point 10 years later | Use of oral contraceptives | *n* | % Reaching the end point | *P* value |
|---|---|---|---|---|
| Progression of retinopathy | Never | 176 | 65.5 | 0.40 |
| | Ever | 82 | 69.7 | |
| Progression to proliferative diabetic retinopathy | Never | 176 | 34.6 | 0.11 |
| | Ever | 82 | 25.1 | |
| Incidence of macular edema | Never | 168 | 16.2 | 0.64 |
| | Ever | 80 | 14.0 | |
| Incidence of hypertension | Never | 164 | 27.7 | 0.78 |
| | Ever | 80 | 25.4 | |

**Table 3—*The 6-year progression and incidence of retinal end points and hypertension by HRT in older-onset women: the Wisconsin Epidemiologic Study of Diabetic Retinopathy***

| End point 6 years later | HRT | *n* | % Reaching the end point | *P* value |
|---|---|---|---|---|
| Incidence of retinopathy | Never | 70 | 50.0 | 0.39 |
| | Ever | 14 | 64.3 | |
| Progression of retinopathy | Never | 226 | 47.8 | 0.67 |
| | Ever | 24 | 54.2 | |
| Progression to proliferative diabetic retinopathy | Never | 226 | 15.0 | 0.22 |
| | Ever | 24 | 4.2 | |
| Incidence of macular edema | Never | 172 | 19.2 | 0.58 |
| | Ever | 24 | 12.5 | |
| Incidence of hypertension | Never | 92 | 28.3 | 0.34 |
| | Ever | 12 | 41.7 | |

was classified as HRT according to *Drug Facts and Comparisons* (24).

A history of diuretic or antihypertensive medication use and the specific names of these medications were obtained from the participant. If we had any question about these medications, we verified the data via a physician's report. Cigarette smoking status was determined as follows: the subject was classified as having never smoked if she had smoked <100 cigarettes in her lifetime, the subject was classified as an ex-smoker if she smoked >100 cigarettes in her lifetime but had stopped smoking before the 1984–1986 examination, or the subject was classified as currently smoking if she had not stopped smoking. BMI was the subject's weight in kilograms divided by height in meters squared. Retinopathy status was determined by grading fundus photographs in a masked fashion according to a modification of the Airlie House classification scheme (25). For this study, the severity of retinopathy was classified into 13 levels of severity ranging from no retinopathy (level 10) to end-stage proliferative retinopathy with visual loss (level 85). The retinopathy level for a participant was derived by concatenating the levels for the two eyes, giving the eye with the higher level greater weight, and providing a 15-step scale when all levels of proliferative retinopathy were grouped as one level. Progression to proliferative retinopathy was estimated from all people who were free of this complication at the 4-year follow-up examination. For individuals with nonproliferative or no retinopathy, progression was defined as an increase in the retinopathy severity by two steps or more at either of the follow-up examinations. Macular edema was present or absent based on grading for this specific lesion.

### Statistical analyses

We included only data for women aged ≥18 years in the analyses. Some younger-onset participants who were observed at the 4- and 10-year examinations did not participate in the 14-year examination. Thus, censored observations are included. To compute the rates of the end points while using the information we had, we used the product-limit method (26). To test for trends in incidence among subgroups of the population, we used Mantel-Haenszel's nonzero correlation stratified by follow-up period. We also used these methods to calculate relative risks (27). Thus, the relative risks do not necessarily agree with the incidence produced by the product-limit method. In the older-onset group, because only two time points were available, we estimated incidence as the simple proportion of the number of new events divided by the number at risk. Multivariable analyses for predicting incidence were based on the discrete linear logistical model in the younger-onset group and the logistical model in the older-onset group (28).

**RESULTS** — In the younger-onset group, no significant associations were evident among the use of oral contraceptives at the 4-year examination and progression of retinopathy, progression to proliferative retinopathy, or incidence of macular edema 10 years later (Table 2). We found no evidence of a relationship between the use of oral contraceptives and subsequent incidence of hypertension during the same interval. After classifying the medications according to high- or low-dose estrogen and progestin, we found no effect of dosage on retinopathy and no effect of reported duration of use.

For the older-onset group, we found no association between the use of HRT (which mostly involved conjugated estro-

**Table 4—*Odds ratio for use of oral contraceptives in younger-onset women aged ≥18 years controlling for other risk factors***

| End point 10 years later | Odds ratio (95% CI) | *P* value |
|---|---|---|
| Progression of retinopathy | 1.21* (0.73–2.02) | 0.45 |
| Progression to proliferative diabetic retinopathy | 0.54† (0.27–1.08) | 0.08 |
| Incidence of macular edema | 0.99‡ (0.46–2.14) | 0.99 |
| Incidence of hypertension | 1.05§ (0.56–1.94) | 0.89 |

*Controlling for $HbA_1$ and mean diastolic blood pressure; †controlling for age, $HbA_1$, and severity of retinopathy; ‡controlling for $HbA_1$ and severity of retinopathy; §controlling for $HbA_1$ and proteinuria.

**Table 5—*Odds ratio for HRT in older-onset women controlling for other risk factors***

| End point 6 years later | Odds ratio (95% CI) | *P* value |
|---|---|---|
| Incidence of retinopathy | 1.83* (0.43–7.72) | 0.41 |
| Progression of retinopathy | 1.19† (0.43–3.24) | 0.74 |
| Progression to proliferative diabetic retinopathy | 0.28‡ (0.03–2.77) | 0.28 |
| Incidence of macular edema | 0.48§ (0.11–2.08) | 0.33 |
| Incidence of hypertension | 1.91‖ (0.50–7.26) | 0.34 |

*Controlling for age and $HbA_1$; †controlling for age, duration, and $HbA_1$; ‡controlling for $HbA_1$ and severity of retinopathy; §controlling for $HbA_1$; ‖controlling for age and $HbA_1$.

gen) and the retinal end points, nor did we find an association with the development of hypertension 6 years after the 4-year follow-up (Table 3). We found no relationship between duration of use of HRT and retinopathy.

Because other risk factors may have influenced the association between the hormone exposures and the study end points, we performed multiple logistical regression. In the younger-onset group, no evidence of a relationship between use of oral contraceptives and retinal lesions or hypertension existed (Table 4) after considering other associated characteristics. In the older-onset group, the use of HRT was not significant in explaining the development of the retinal end points or the development of hypertension (Table 5) after considering other associated characteristics.

**CONCLUSIONS** — The most important limitations of our investigation result from its intrinsic nature as an observational study. Thus, selection factors may be personal and related to medical care or advice that influence exposure to the drugs of interest, the patterns of their use, and the other risk factors for the end points of interest. An opportunity may exist in the future to evaluate the effects of HRT on retinopathy in an auxiliary study of the Women's Health Initiative (29), the Women's Health Initiative Sight Examination, which includes some women with diabetes.

The primary reason for our initial study was to determine the sequence and rates of progression of diabetic retinopathy during the years of its evolution and not the hypotheses explored herein. In addition, the number of women exposed to the drugs of interest was small. Thus, we cannot infer that our data are strong evidence of a lack of effect of the exposures of interest to diabetic retinopathy, macular edema, or hypertension, but they are compatible with no effect. These drugs are widely used and their intended effects are clearly beneficial. We have no evidence to be wary of their use in women with diabetes.

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