Transdermal 17-β-Estradiol and Risk of Developing Type 2 Diabetes in a Population of Healthy, Nonobese Postmenopausal Women

Rosario Rossi, md[1]
Giorgia Origliani, phd[2]
Maria G. Modena, md[1]

OBJECTIVE — Various observational and randomized studies have demonstrated a reduction in the incidence of type 2 diabetes in postmenopausal women who received estrogen orally. No studies have been performed on the incidence of type 2 diabetes in postmenopausal women treated with transdermal 17-β-estradiol. The purpose of our study was to assess the influence of transdermal 17-β-estradiol on the incidence of type 2 diabetes in a population of healthy, nonobese postmenopausal women.

RESEARCH DESIGN AND METHODS — Between January 1998 and December 2002, 673 healthy, nonobese postmenopausal women (mean age 54 ± 5 years) were enrolled: 144 (21.4%) of these took transdermal 17-β-estradiol and 529 (78.6%) had never taken hormones during their postmenopausal period. Final elaboration of the data took place in July 2003, with a mean follow-up of 3.7 ± 0.7 years (ranging from 0.5 to 5 years).

RESULTS — Type 2 diabetes developed in 60 patients during the follow-up period, which is the equivalent of 22 cases per 1,000 women-years. In the "hormones nonusers" group, diabetes developed in 10% (54 of 529 women; equivalent of 26.5 cases/1,000 women-years), whereas in the "hormones users" group, diabetes developed in 4.16% (6 of 144 women; equivalent of 12.1 cases/1,000 women-years). Transdermal 17-β-estradiol emerged as a treatment that significantly reduced the risk of developing diabetes (RR 2.19, 95% CI 1.79–3.56; $P = 0.006$).

CONCLUSIONS — Our results suggest a significant reduction in the incidence of type 2 diabetes in our population of nonobese, healthy postmenopausal women who used transdermal 17-β-estradiol. This could suggest that, in some women, the estrogen deficiency that occurs after menopause could represent a fundamental step in the process of diabetogenesis.

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Many studies have shown that postmenopausal hormone therapy is able to significantly influence glucose homeostasis (1–5), but few studies have clearly demonstrated a significant reduction in the incidence of diabetes among postmenopausal women using estrogens (6,7). All of the mentioned studies were performed using orally administered hormones. Apart from these, there is a further series of reports on the effect of transdermal 17-β-estradiol on the metabolism of glucose (8–13). Most of the latter studies (8,9,11,13) were devised to assess whether the different routes of administering estrogen had an influence on the metabolism of glucose; none of these studies have provided information on the incidence of diabetes.

Our study considered a population of healthy, middle-aged, nonobese postmenopausal women, some of whom were treated with transdermal 17-β-estradiol. The purpose of our study was to evaluate the influence of transdermal 17-β-estradiol on the incidence rate of diabetes in the same population.

RESEARCH DESIGN AND METHODS — The patients enlisted in our study were selected from women who had been referred to the "Bene Essere Donna" center, an institution dedicated to the study, prevention, and treatment of menopause-related disorders. This center is open to all women who are in the postmenopausal period (postmenopausal status defined as the absence of menstruation for ≥6 months and/or blood follicle-stimulating hormone level >40 IU/l and 17-β-estradiol levels <120 pmol/l) and are ≤60 years of age. These women, who are initially drawn to the center through local media advertising, have free access and can make queries or obtain advice about particular symptoms they are having by simply fixing an appointment beforehand. Between 1 January 1998 and 31 December 2002, we assessed 980 women.

At baseline, each participant underwent fasting blood testing for levels of total cholesterol and triglycerides as well as a 75-g oral glucose tolerance test (OGTT). Participants were eligible for inclusion in this study if they had normal result of OGTT (defined as fasting plasma glucose <110 mg/dl and 2-h plasma glucose <140 mg/dl) (14). The number of healthy postmenopausal women with normal glucose tolerance who satisfied the above-mentioned criteria was 673; mean age was 54 ± 5 years. A total of 144 (21.4%) women used transdermal 17-β-estradiol, whereas the remaining 529

(78.6%) women took no hormones. Women with hypertension, hyperlipidemia, smoking habits, and obesity were excluded from the study; women treated with oral estrogen were also excluded.

Physical examination variables measured at baseline included body weight, height, waist circumference, and systolic and diastolic blood pressure. Patient history, 12-lead electrocardiography, and echocardiography were used to exclude past or present heart disease. Participants provided questionnaire data concerning lifestyle practices and potential risk factors for cardiovascular disease.

Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or use of antihypertensive medications; hyperlipidemia was defined as total cholesterol plasma level ≥200 mg/dl and/or triglyceride level ≥170 mg/dl; and obesity as BMI ≥30 kg/m^2.

The results were finally elaborated at the end of July 2003, when the last women to enroll had been followed for at least 0.5 year; the mean follow-up was 3.7 ± 0.7 years (range 0.5–5).

Definition and ascertainment of cases

All women were seen in our outpatient clinic at regular intervals (every 6 months). At baseline and every 6-month follow-up visit, women underwent an interview, examination, and blood collection. Serum glucose measurements were performed at each visit. New cases of diabetes were identified in accordance with the criteria used in the Atherosclerosis Risk in Communities (ARIC) Study (15,16): *1*) self-reported use of hypoglycemic medications; *2*) fasting (>8 h) serum glucose level >126 mg/dl; *3*) nonfasting serum glucose level >200 mg/dl; or *4*) self-reported physician diagnosis of diabetes. For individuals classified by physician diagnosis or medication use, date of diabetes onset was considered the midpoint between the last visit when the woman was not diabetic and the first visit when the woman was diabetic. For those diagnosed by fasting or nonfasting glucose level, date of diabetes onset was the estimated date at which blood glucose level crossed the above-mentioned threshold, assuming a linear increase in glucose level between visits.

Hormone treatment

All patients who underwent hormone therapy were treated with a daily dose of 50 μg transdermal 17-β-estradiol. To limit endometrial proliferation, all women were contemporarily treated with a progestogen, supplied along with estradiol. We defined "nonusers" as patients who had never used hormones in the postmenopausal period and "users" as those who had used 17-β-estradiol for more than 3 months and who had continued therapy until the end of follow-up or until the onset of diabetes. The time period corresponding to consecutive prescriptions was used to define the duration of use of hormone therapy.

Statistical analysis

Continuous variables are presented as mean values, and categorical variables are presented as percentages. Differences in baseline characteristics between the groups were examined by ANOVA and χ^2 test, when appropriate.

Because the 673 postmenopausal women had different lengths of follow-up, person-years of exposure were calculated for each participant. For individuals in whom diabetes developed, exposure was defined as time between enrollment and diagnosis. For those who did not have diabetes, exposure was calculated as the time between enrollment and July 2003. Incidence rates of diabetes were computed as the number of cases of diabetes noticed during follow-up divided by the total exposure person-time. The relative risk of developing diabetes was computed as the ratio of the incidence rate among estrogen nonusers divided by the incidence rate among those on hormone therapy. Adjusted estimates of risk were calculated by use of the Poisson regression model, which controlled for a range of potential confounders selected a priori, including age, family history of diabetes, BMI, waist circumference, duration of postmenopausal period, years of education, alcohol consumption, and physical activity. $P < 0.05$ was considered significant.

RESULTS — Patient characteristics at baseline are shown in Table 1. The two study groups were well balanced; there were no differences between groups regarding baseline parameters.

Development of type 2 diabetes

A total of 673 patients were followed for 3.7 ± 0.7 years. None of the participants died or were lost during follow-up; none of the treated women stopped to take hormones, and none of the untreated women began taking estrogen during observation.

Diabetes developed in 60 patients during follow-up (equivalent of 22.0 cases/1,000 women-years). A total of 15 of 60 of diabetes cases (25%) were diagnosed by the physicians; the remaining 35 cases (75%) were diagnosed using measurement of serum glucose levels.

In the "hormone therapy nonuser" group, diabetes developed in 10.00% (54 of 529 women; the equivalent of 26.5 cases/1,000 women-years), whereas in the "hormone therapy users" group, diabetes developed in 4.16% (6 of 144 women; the equivalent of 12.1 cases/1,000 women-years). The relative risk (RR) of diabetes increased significantly in the group of "hormone therapy nonusers" compared with the referent group ("hormone therapy user") (RR 2.19; 95% CI 1.79–3.56; $P = 0.006$). Multiple adjustments for various risk factors attenuated the RR only slightly (RR 1.97; 95% CI 1.65–2.99; $P = 0.004$). Thus, transdermal 17-β-estradiol emerged as a treatment that significantly reduced the risk of developing diabetes (Table 2).

CONCLUSIONS — The results of our prospective study suggest that in healthy, nonobese postmenopausal women, the use of transdermal 17-β-estradiol reduces the risk of developing type 2 diabetes. The current prospective data support a possible role for postmenopausal estrogen deficiency in diabetogenesis and correspond with the data of Kanaya et al. (7), who elaborated the results of the randomized, Heart and Estrogen/progestin Study (HERS), in which orally administered conjugated equine estrogen was used. This shows that the beneficial effect of the reduction of diabetes is directly influenced by estrogens and not by the route of administration. This is not really surprising when one considers those studies that set out to compare orally and transdermally administered estrogens, which showed no difference regarding the level of fasting glycemia and insulin sensitivity (8,9,11,13).

Although our data support etiological associations, at this time, explicit mecha-

Table 1—*Baseline characteristic of the study population according to use of hormones*

	Hormone therapy users	Hormone therapy nonusers	P	95% CI for the difference
n	144	529		
Age (years)	53 ± 5	55 ± 4	0.13	−4.6 to 0.6
Time to menopause (months)	30 ± 12	37 ± 14	0.08	−15.0 to 1.0
Education (years)	14 ± 3	13 ± 4	0.44	−1.5 to 3.5
BMI (kg/m^2)	24.9 ± 2.9	25.1 ± 3.0	0.87	−2.6 to 2.2
Waist circumference (cm)	87 ± 13	91 ± 13	0.19	−10.0 to 2.0
Central obesity (%)*	49.3 (71)	57.2 (303)	0.10	−0.17 to 0.01
Family history of diabetes (%)	34.0 (49)	33.4 (177)	0.90	−0.07 to 0.09
Exercise frequency (times/week)				
Never/rarely (%)	42.3 (61)	37.0 (196)	0.31	−0.03 to 0.13
1–2 (%)	38.1 (55)	41.7 (221)	0.44	−0.13 to 0.05
≥3 (%)	19.6 (28)	21.3 (112)	0.88	−0.08 to 0.06
Alcohol consumption (g/week)	58 ± 12	57 ± 11	0.34	−1.07 to 3.07
Nonalcohol drinkers (%)	34.7 (50)	38.7 (205)	0.43	−0.12 to 0.04
Mean blood pressure (mmHg)	88 ± 7	88 ± 9	1.00	−1.59 to 1.59
Fasting total cholesterol (mg/dl)	186 ± 17	181 ± 15	0.24	−3.39 to 13.40
Fasting triglyceride (mg/dl)	144 ± 17	155 ± 15	0.07	−23.0 to 1.0
Plasma glucose (mg/dl)				
Basal	85 ± 7	86 ± 8	0.55	−4.28 to 2.38
2-h	121 ± 10	119 ± 11	0.30	−1.84 to 5.64

Data are means ± SD or % (n). *Central obesity was considered when waist circumference was >88 cm.

nisms remain speculative and require further study. Some hypotheses for our results warrant further discussion. First, it is possible that the estrogens determine a lower incidence of diabetes via the improvement of the endothelial function. Altered endothelial function lowers the permeability, and diminished peripheral blood flow may limit insulin delivery and promote insulin resistance (17,18). In fact, the interstitial concentration of insulin represents the factor limiting the effectiveness of insulin itself. In this regard, postmenopause, as a consequence of deficiency of estrogens, is associated with endothelial dysfunction (19), and by restoring the levels of estrogens, the endothelial function improves (20). It must

also be added, from this point of view, that some studies have pointed out that transdermal 17-β-estradiol seems to possess an insulin-like action at the level of the endothelial cells. After 2 h of induction of hyperglycemia, the plasma concentrations of soluble adhesion molecules are elevated both in patients with type 2 diabetes and also in healthy individuals (21). Insulin, on the contrary, decreased levels of intercellular adhesion molecule-1 in endothelial cells (22). Clinically, insulin treatment is accompanied by a reduced level of circulating soluble adhesion molecules (23). Seljeflot et al. (24) found substantial reductions in the soluble intercellular adhesion molecule, E-selectine, and vascular cell adhesion

molecule-1 after treatment with transdermal estradiol.

Another potential mechanism that may explain our results regards the effects of estrogen on glucose and insulin metabolism. We found contrasting results in the literature concerning this. In fact, not all works indicate estrogen incisive action on glucose metabolism (10,12,25,26), but some evidence demonstrates how estrogen therapy is able to improve insulin sensitivity (8,27) and to exert a beneficial effect on hepatic gluconeogenesis, reducing the hepatic production of glucose (8,28).

The reduction in the risk of developing diabetes represents an end point of utmost importance because diabetes is a

Table 2—*Incidence rates of diabetes among the study population*

	Cases of diabetes: n/N (%)	Incidence rate*	Incidence rate ratio† (crude)	P	Incidence rate ratio† (adjusted‡)	P
Total cohort	60/673 (8.91)	22.0	—	—	—	—
Hormone therapy nonusers	54/529 (10.20)	26.5	2.19§	0.006	1.97‖	0.004
Hormone therapy users	6/144 (4.16)	12.1	Referent	Referent	Referent	Referent

*Incidence rate calculated per 1,000 person-years of exposure; †incidence rate ratios derived from the comparison of the incidence rate among postmenopausal women who never use hormones and women treated with hormone therapy. Hormone therapy user group represents the referent group; ‡multiple Poisson regression model adjusted for age (continuous), family history of diabetes (yes/no), BMI (continuous), waist circumference (continuous), duration of postmenopausal period (continuous), years of education (continuous), alcohol consumption (continuous), and physical activity (never/rarely, one to two times per week, three or more times per week); §95% CI 1.79–3.56; ‖95% CI 1.65–2.99.

real public health problem in industrialized countries, mainly the U.S. (29,30), with conspicuous cardiovascular consequences and high cost in terms of mortality, morbidity, and financial resources (31). For all of these reasons, the detection of a therapy able to significantly reduce the incidence of diabetes could easily induce enthusiasm. Our findings were obtained in a nonrandomized study and, therefore, should be viewed with caution, because each treatment should be evaluated in relation to the risk/benefit ratio. In this regard, we point out that the recent randomized study, termed the Women's Health Initiative (32), a large-scale placebo-controlled trial on hormone therapy and primary prevention, has reported increased risk of coronary heart disease among healthy women assigned to estrogen plus progestin. In addition, the same treatment increased the risk of several adverse outcomes, including stroke, pulmonary embolism, and breast cancer. Unfortunately, the mentioned study, which has considered healthy women (like the present study), does not provide data about the incidence of diabetes in the studied women. Moreover, in the HERS study, an improvement in glucose tolerance and a lower incidence of diabetes noticed during follow-up (7) were not sufficient to induce a reduction of cardiovascular events after a 4-year follow-up period (33).

The points of value of our work are, in our opinion, the prospective design of the study, the large sample of women, and the ability to exactly determine the time of hormone therapy initiation. This allowed us to define current exposure and to treat it as a time-dependent covariate.

A limiting factor of our study was the fact that the postmenopausal women enrolled were relatively young and free from cardiovascular risk factors and any cardiac pathologies. The results we obtained are, therefore, only applicable to a limited number of postmenopausal women and not to all women; for that matter, the results are not applicable to men.

In conclusion, the results of this prospective study demonstrate that hormone therapy plays a role in reducing the risk of type 2 diabetes in postmenopausal women. This implies that estrogenic deficiency represents a possible step in diabetogenesis in women. The results of our study were not conclusive but are suggestive of the fact that estrogen may be con-

sidered as a diabetes prevention agent. Nevertheless, the confirmation of significant effects must be validated in formalized clinical trial with predetermined hyperglycemic end points.

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