

Impaired Endothelial Function in Young Women with Premature Ovarian Failure: Normalization with Hormone Therapy

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Normal menopause is associated with vascular endothelial dysfunction, an early stage of atherosclerosis. The effect of premature ovarian failure (or premature menopause) on endothelial function in young women is unknown. Endothelial function was assessed in 18 women with premature ovarian failure before and after 6 months of hormone therapy and was compared with the endothelial function of 20 age- and body mass index-matched premenopausal women. Brachial artery diameter was measured both during hyperemia (an index of endothelium-dependent vasodilation) and in response to glyceryl trinitrate (an index of endothelium-independent vasodilation). Flow-mediated dilation was significantly lower in women with premature ovarian failure at baseline (increase in brachial artery diameter during hyperemia by $3.06 \pm 4.33\%$) than in control women (increase by $8.84 \pm 2.15\%$; $P < 0.0005$).

Glyceryl trinitrate-induced vasodilation did not differ between the groups. After hormone therapy for 6 months, flow-mediated dilation was improved in women with premature ovarian failure, increasing by more than 2-fold ($7.41 \pm 3.86\%$; $P < 0.005$ compared with pretreatment) and reaching normal values (P not significant compared with control women). Glyceryl trinitrate-induced vasodilation did not change after treatment in women with premature ovarian failure. Young women with premature ovarian failure have significant vascular endothelial dysfunction. Early onset of endothelial dysfunction associated with sex steroid deficiency may contribute to the increased risk of cardiovascular disease and mortality in young women with premature ovarian failure. Hormone therapy restores endothelial function within 6 months of treatment. (*J Clin Endocrinol Metab* 89: 3907–3913, 2004)

CARDIOVASCULAR DISEASE, INCLUDING coronary artery disease, stroke, and peripheral vascular disease, is the leading cause of death among women. The incidence of cardiovascular disease is very low in normal premenopausal women, but it increases sharply with age, especially after menopause (1). This disparity between premenopausal and postmenopausal women has been attributed to the cardioprotective effect of endogenous estrogen. Estrogen exerts protective effects on the cardiovascular system, including total cholesterol- and low density lipoprotein (LDL) cholesterol-lowering (2), antioxidant (3), and vasodilating (4) effects.

Premature ovarian failure (or premature menopause) is characterized by amenorrhea, infertility, sex steroid deficiency, and elevated gonadotropins in women less than 40 yr of age (5). It affects 1% of women by age 40 yr and 0.1% by age 30 yr (6). Young women with premature ovarian failure have estrogen deficiency for more years than do naturally

menopausal women, thereby resulting in a significantly higher risk for bone loss (7, 8) and cardiovascular disease (1, 9). Women with premature ovarian failure have a higher risk of premature death (10, 11), mainly due to increased cardiovascular mortality (12–14).

Normal menopause is associated with endothelial dysfunction, and there is substantial evidence that chronic estrogen treatment improves vascular endothelial function in postmenopausal women (15). Endothelial dysfunction contributes to the development of atherosclerosis, and the magnitude of this defect predicts cardiovascular events (15).

The effect of premature ovarian failure on endothelial function in young women is unknown. We undertook this study 1) to determine whether women with premature ovarian failure have endothelial dysfunction compared with age-matched premenopausal women, and 2) to investigate the effect of hormone therapy on endothelial function in these young women with ovarian failure.

Subjects and Methods

Study population

This was a cohort study evaluating endothelial function in women with premature ovarian failure before and 6 months after hormone therapy. We studied women with premature ovarian failure and age-

Abbreviations: BMI, Body mass index; FMD, flow-mediated dilation; GTN, glyceryl trinitrate; HDL, high density lipoprotein; LDL, low density lipoprotein; POF, premature ovarian failure.

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and body mass index (BMI)-matched women with normal ovarian function (control population) at the Michaelideion Cardiac Research Center, University of Ioannina (Ioannina, Greece). We measured brachial artery diameter both during hyperemia (an index of endothelium-dependent vasodilation) and in response to glyceryl trinitrate (an index of endothelium-independent vasodilation).

Women with premature ovarian failure

To be eligible for our study, women with premature ovarian failure had to fulfill the following inclusion criteria: 1) diagnosis of premature ovarian failure before the age of 40 yr; 2) at least 4 months of amenorrhea; 3) two FSH levels above 40 mIU/ml (40 IU/liter; confirmed on two separate occasions, at least 1 month apart); 4) normal 46,XX karyotype; 5) no contraindications for hormone therapy; 6) no evidence of any disease other than hypothyroidism, which is very common in this group of women (women with hypothyroidism had to be under T₄ replacement, with normal thyroid function tests during screening evaluation); 7) no prior treatment in the past 6 months known to affect vascular endothelial function [hormone therapy or other hormones (apart from T₄), vitamins, antioxidants, and cardiovascular medications]; 8) little or no alcohol use (fewer than two drinks per day); and 9) BMI (kilograms per meter squared) between 19 and 30. Smoking history was recorded, but it was not an exclusion criterion. All subjects underwent gynecological examination, including transvaginal ultrasound. On the basis of gynecological examination, the patients had to fulfill the following criteria: no suspicion of malignancy, no endometrial pathology, endometrial thickness less than 5 mm, and the presence of normal ovaries and breasts. All women included in this study had secondary ovarian failure, and the interval since the development of ovarian failure was recorded. The bone mineral density of the lumbar spine was measured at baseline, as clinically indicated, by dual energy x-ray absorptiometry. None of the women underwent exercise or dietary modifications before or during the study.

Women with normal ovarian function (controls)

Healthy age- and BMI-matched regularly menstruating women (menstrual cycles between 21 and 35 d) who did not use alcohol, were not taking medications, and were not using hormonal contraception served as the controls. None had a history of diabetes or hypertension.

Study design

Endothelial function was evaluated in all women with premature ovarian failure at baseline and 6 months after the initiation of hormone therapy. Endothelial function was also assessed in control women. Circulating lipid and hormone concentrations were measured at baseline in women with premature ovarian failure and in controls and at 6 months after initiation of hormone therapy in women with premature ovarian failure.

Study protocol 1: comparison of endothelial function in women with premature ovarian failure at baseline (before the initiation of hormone therapy) and in control women. In women with premature ovarian failure, blood samples were drawn after the physical examination, and endothelial function was assessed the next day. In control women, blood samples were drawn during the early follicular phase of their menstrual cycle (d 3–5), and endothelial function was assessed the next day.

Assessment of endothelial function began at 0830 h, with subjects fasted for at least 14 h. Current smokers refrained from smoking for at least 14 h before the study. The subjects rested supine in a quiet, air-conditioned room (constant temperature, 22–25°C) for 30 min before endothelial function was assessed as described below.

Study protocol 2: effect of hormone therapy on endothelial function in women with premature ovarian failure: comparison of endothelial function in women with premature ovarian failure following hormone therapy and control women. Women with premature ovarian failure, who participated in the study, received oral estrogen/progestogen cyclic treatment (0.625 mg conjugated equine estrogen daily plus 5 mg medroxyprogesterone acetate cyclically). Medication was taken every morning for 6 months. Adherence to medication was checked by tablet counts. A second physical examination, including gynecolog-

ical examination, was performed at the end of the 6-month period. The presence of predictable vaginal bleeding and breast tenderness in each woman was recorded at the end of the study. Measurement of endothelial function and circulating lipid and hormone concentrations were performed at the end of the 6-month period, as described above. Women with premature ovarian failure were evaluated during the estrogen-only phase of the hormone cycle (not while on progestins), so that they had comparable sex steroid levels with the normal controls.

The study was approved by the ethics committee of the University Hospital of Ioannina, and all study participants provided written informed consent.

Serum hormone and lipid measurements

For the measurement of FSH and LH, microparticle enzyme immunoassays were used (AxSYM Estradiol, AxSYM FSH, and AxSYM LH, respectively; Abbott Laboratories, Abbott Park, IL). Total T₃, free T₄, and TSH were also measured by immunoassay on the AxSYM analyzer (Abbott Laboratories). Serum total cholesterol and triglycerides were determined by enzymatic colorimetric assay (Olympus AU560; Diagnostica, Hamburg, Germany). High density lipoprotein (HDL) cholesterol was determined enzymatically in the supernatant after dextran-magnesium-induced precipitation of other lipoproteins. LDL cholesterol was calculated using the Friedewald formula (16).

Assessment of endothelial function

Endothelial function was assessed in all women by measurement of flow-mediated dilation (FMD) of the brachial artery in response to hyperemia of the hand, using, in principle, the method established by Celermajer *et al.* (17). The procedure was performed according to recently published guidelines (18). All studies were performed by the same operator, who was unaware of the gonadal hormone status of the women. Optimal imaging of the right brachial artery was obtained using an echo-Doppler ultrasound (Ultrasound HDI 5000, ATL, Bophell, WA) and a 5- to 12-MHz transducer. Images were recorded on superVHS videotape (VCR AG-MD 835; Panasonic, Osaka, Japan). Off-line analysis and measurement of end-diastolic arterial diameter using electronic calipers from the anterior to the posterior m-line was performed by another blinded operator.

Images were acquired at baseline (after 30-min supine rest), during hand hyperemia 90 sec after deflation of a wrist cuff inflated to supra-systolic pressure (to at least 50 mm Hg above systolic pressure) for 5 min for measurement of FMD, and at 4 min after 400 µg sublingual glyceryl trinitrate (GTN) for measurement of nitrate-induced, endothelium-independent vasodilation. All hemodynamic measurements were confirmed as having returned to baseline 15 min after the release of the wrist cuff before administering GTN. Brachial artery blood flow was also measured by continuous wave Doppler as the product of the mean velocity corrected for Doppler angle and the internal brachial artery diameter. FMD was calculated as the percent increase in arterial diameter during hyperemia compared with the diameter at rest, whereas the peak flow at 15 sec after cuff release was used as the measure of hyperemic flow. Heart rate and blood pressure (by brachial sphygmomanometry) were also measured during the study.

Reproducibility

In our laboratory, the intra- and interobserver variabilities for repeated measurements of brachial artery diameter are 0.10 ± 0.11 and 0.09 ± 0.17 mm, respectively. In studies performed on 2 separate days (5–7 d apart) in eight subjects, the mean difference in brachial vasodilation was 0.5 ± 2.8% for the endothelium-dependent response and 0.7 ± 1.8% for the endothelium-independent response, whereas the correlation coefficients of the two repeat measurements were 95% and 91%, respectively. The within-subject coefficients of variation of the endothelium-dependent and endothelium-independent responses were 4.9% and 3.2%, respectively.

Statistical analysis

Data are presented as the mean ± sd. An unpaired *t* test and χ^2 test were used to compare continuous and categorical variables, respec-

tively, between women with premature ovarian failure and control women. A paired *t* test and χ^2 test were used to compare continuous and categorical variables, respectively, in women with premature ovarian failure before and after hormone therapy. *P* < 0.05 was considered significant.

Results

Eighteen women with premature ovarian failure were enrolled and completed the study. Twenty age- and BMI-matched regularly menstruating women were included as controls.

Study protocol 1

Table 1 summarizes baseline characteristics (age, BMI, age at menarche, smoking habits, family history of cardiovascular disease, and hypothyroidism) of the study patients and controls; the two groups were well matched, with the exception of the incidence of hypothyroidism, which, as expected, was higher in women with premature ovarian failure. However, all patients with hypothyroidism were receiving T₄ replacement and had normal thyroid function tests during the screening evaluation. Table 2 summarizes reproductive and endocrine characteristics in women with premature ovarian failure (duration of ovarian failure, history of menstrual disturbances, family history of premature ovarian failure, and bone mineral density).

At baseline, women with premature ovarian failure had significantly higher gonadotropin levels than controls [FSH, 78.9 ± 22.9 *vs.* 5.7 ± 2.4 mIU/ml (78.9 ± 22.9 *vs.* 5.7 ± 2.4 IU/liter; *P* < 0.0001); LH, 54.6 ± 16.4 *vs.* 3.5 ± 2.3 mIU/ml (54.6 ± 16.4 *vs.* 3.5 ± 2.3 IU/liter; *P* < 0.0001); in women with premature ovarian failure and control women, respectively; Table 3]. No differences were observed in fasting serum levels of total cholesterol, HDL and LDL cholesterol, or triglycerides between the two groups [total cholesterol, 218 ± 44 *vs.* 202 ± 38 mg/dl (5.65 ± 1.14 *vs.* 5.23 ± 0.98 mmol/liter; *P* nonsignificant); HDL cholesterol, 57 ± 17 *vs.* 55 ± 10 mg/dl (1.48 ± 0.44 *vs.* 1.42 ± 0.26 mmol/liter; *P* nonsignificant); LDL cholesterol, 146 ± 33 *vs.* 128 ± 34 mg/dl (3.78 ± 0.85 *vs.* 3.32 ± 0.88 mmol/liter; *P* nonsignificant); triglycerides, 73 ± 27 *vs.* 95 ± 36 mg/dl (0.82 ± 0.31 *vs.* 1.07 ± 0.41 mmol/liter; *P* nonsignificant); in women with premature ovarian failure and control women, respectively; Table 3].

TABLE 1. Demographic and clinical characteristics of women with POF (n = 18) and control women (n = 20)

	POF women	Control women	<i>P</i>
Age (mean, SD), yr	35.4 (5.5)	35.0 (3.6)	NS
Median	38	36	
Range	23–40	29–40	
BMI (mean, SD), kg/m ²	24.2 (3.1)	23.0 (2.6)	NS
Median	23.7	22.8	
Range	19.8–29.4	19.6–29.4	
Age at menarche (mean, SD), yr	12.8 (1.1)	12.6 (0.83)	NS
Median	13	12.5	
Range	11–15	11.5–14	
Current smoking, no. (%)	5/18 (28)	6/20 (30)	NS
Family history of cardiovascular disease, no. (%)	2/18 (11)	3/20 (15)	NS
Hypothyroidism, no. (%)	4/18 (22)	0/20 (0)	<0.05

NS, Not significant.

TABLE 2. Reproductive and endocrine characteristics of women with POF

Characteristics	
Years since ovarian failure (mean, SD)	1.81 (1.56)
Median	1
Range	0.5–5
History of menstrual disturbances, no. (%)	12/18 (67)
Family history of POF, no. (%)	6/18 (33)
BMD, T-score (mean, SD)	−1.24 (0.86)
Median	−1.24
Range	−2.89 to 0.2
Normal BMD (T-score >−1)	8 women
Osteopenia (T-score <−1 & >−2.5)	8 women
Osteoporosis (T-score <−2.5)	2 women

BMD, Bone mineral density.

FMD was significantly lower in women with premature ovarian failure at baseline (percent increase in brachial artery diameter during hand hyperemia by 3.06 ± 4.33%) compared with control women (increase by 8.84 ± 2.15%; *P* < 0.0005; Table 4 and Fig. 1). The percent increase in hyperemic flow was not significantly different between the groups (Table 4). GTN-induced vasodilation did not differ between groups. Heart rate and blood pressure did not change during the study.

Study protocol 2

Table 3 summarizes the comparison of clinical variables (hormone and lipid concentrations and blood pressure) in women with premature ovarian failure before and after 6-month hormone therapy and in the control women. In women with premature ovarian failure, FSH and LH levels decreased significantly after hormone therapy without reaching control values [FSH, 42.1 ± 23.4 mIU/ml (42.1 ± 23.4 IU/liter; *P* < 0.0001 compared with pretreatment and *P* < 0.0005 compared with control women); LH, 36.4 ± 18.3 mIU/ml (36.4 ± 18.3 IU/liter; *P* < 0.005 compared with pretreatment and *P* < 0.0001 compared with control women)]. There were also significant changes in LDL cholesterol and triglycerides; values after hormone therapy were not different from control values. LDL cholesterol decreased [130 ± 25 mg/dl (3.37 ± 0.65 mmol/liter); *P* < 0.05 compared with pretreatment and *P* nonsignificant compared with control women], whereas triglycerides increased after hormone therapy [88 ± 26 mg/dl (0.99 ± 0.29 mmol/liter); *P* < 0.05 compared with pretreatment and *P* nonsignificant compared with control women]. No differences were found in total cholesterol, glucose levels, and blood pressure between women with premature ovarian failure, before and after hormone therapy, and control women.

After hormone therapy for 6 months, FMD improved in women with premature ovarian failure, increasing to 7.41 ± 3.86% (*P* < 0.005 compared with pretreatment), and reaching normal values (*P* nonsignificant compared with control women; Table 4 and Fig. 1). The percent increase in hyperemic flow was not significantly different before and after hormone therapy (Table 4). Heart rate and blood pressure did not change during the study. Endothelium-independent vasodilation did not change posttreatment in women with premature ovarian failure (Table 4).

TABLE 3. Comparison of clinical variables in women with premature ovarian failure at baseline and after hormone therapy and in women with normal ovarian function (controls)

Clinical variables	POF women at baseline	POF women after hormone therapy	Controls	P		
				Baseline POF vs. controls	Baseline POF vs. POF after hormone therapy	POF after hormone therapy vs. controls
FSH (mIU/ml)	78.9 (22.9) ^a	42.1 (23.4)	5.7 (2.4)	<0.0001	<0.0001	<0.0005
LH (mIU/ml)	54.6 (16.4)	36.4 (18.3)	3.5 (2.3)	<0.0001	<0.005	<0.0001
Estradiol (pg/ml)	37 (24)	92 (59)	105 (58)	<0.0005	<0.005	NS
Glucose (mg/dl)	86 (9)	84 (9)	86 (11)	NS	NS	NS
Total cholesterol (mg/dl)	218 (44)	208 (36)	202 (38)	NS	NS	NS
HDL cholesterol (mg/dl)	57 (17)	61 (17)	55 (10)	NS	NS	NS
LDL cholesterol (mg/dl)	146 (33)	130 (25)	128 (34)	NS	<0.05	NS
Triglycerides (mg/dl)	73 (27)	88 (26)	95 (36)	NS	<0.05	NS
BP systolic (mm Hg)	122 (11)	119 (11)	115 (11)	NS	NS	NS
BP diastolic (mm Hg)	76 (11)	72 (9)	76 (7)	NS	NS	NS

BP, Blood pressure; NS, not significant. Systeme International conversion factors: for estradiol (pmol/liter), 3.671; for glucose (mmol/liter), 0.0555; for total, HDL and LDL cholesterol (mmol/liter), 0.0259; for triglycerides (mmol/liter), 0.0113.

^a Mean (±SD).

TABLE 4. Assessment of endothelial function

	POF women at baseline	POF women after 6 months of hormone treatment	Control women	P		
				POF baseline women vs. controls	POF baseline vs. POF after treatment	POF after treatment vs. controls
End-diastolic diameter						
Baseline (mm)	2.96 (0.43) ^a	3.07 (0.43)	3.09 (0.44)	NS	NS	NS
Posthyperemia (mm)	3.05 (0.42)	3.29 (0.43)	3.36 (0.48)	<0.05	<0.005	NS
Absolute increase with hyperemia (mm)	0.08 (0.12)	0.22 (0.11)	0.27 (0.07)	<0.0001	<0.0005	NS
% Increase with hyperemia (FMD)	3.06 (4.33)	7.41 (3.86)	8.84 (2.15)	<0.0005	<0.005	NS
Post-GTN (mm)	3.54 (0.50)	3.68 (0.41)	3.75 (0.45)	NS	NS	NS
Increase with GTN (mm)	0.57 (0.15)	0.61 (0.12)	0.66 (0.15)	NS	NS	NS
% Increase with GTN	19.46 (4.88)	20.42 (5.34)	21.86 (6.12)	NS	NS	NS
Flow						
Baseline (ml/min)	54 (44)	62 (39)	61 (37)	NS	NS	NS
Increase with hyperemia (ml/min)	164 (69)	204 (109)	193 (172)	NS	NS	NS
Increase with hyperemia (%)	433 (218)	416 (329)	356 (250)	NS	NS	NS

NS, Not significant.

^a Mean (±SD).

Discussion

Impairment of vascular endothelial function is an early marker of atherosclerosis (19). Our findings demonstrate, for the first time, that premature ovarian failure is associated with significant endothelial dysfunction. Thus, the process of atherosclerosis starts very early in young women with premature ovarian failure. There is evidence suggesting that women who experience premature ovarian failure are at increased risk for all-cause and cardiovascular-related mortality (9–14). This is particularly important because experimental studies (20, 21) have indicated that effective preventive therapy of atherosclerosis can be achieved using hormone therapy before atherosclerotic plaque formation. We found that hormone therapy restored endothelial function in young women with premature ovarian failure within 6 months.

Assessment of vascular endothelial function may serve as an integrating index of cardiovascular disease risk factor burden (18). Endothelium regulates vascular tone through the release of vasodilators, such as nitric oxide and prostacyclin, and vasoconstrictors, such as endothelin, in response to physical and chemical stimuli. Increased blood flow is an important stimulus for endothelium-mediated vasodilation

(flow-mediated vasodilation), mainly because of endothelial release of nitric oxide. Flow-mediated vasodilatation can be assessed noninvasively in the brachial artery using high frequency ultrasound to measure changes in brachial artery diameter in response to hyperemic flow induced by a 5-min pressure cuff arterial occlusion. Flow-mediated vasodilation in the brachial artery correlates with the assessment of endothelial function in the coronary circulation (22), which has been shown to represent an independent predictor of cardiovascular events (23). Endothelial dysfunction, demonstrated as reduced vasodilation, has been associated with the presence of cardiovascular risk factors (18).

Natural menopause (>50 yr of age) is associated with endothelial dysfunction. Such dysfunction was found also in women more than 40 yr of age, who suffered acute endogenous estrogen deprivation after ovariectomy (24, 25). In the latter studies impaired endothelial function was present within 1 month after ovariectomy, whereas it was restored after 3 months of hormone therapy.

Observational studies suggest that postmenopausal hormone therapy reduces the risk of cardiovascular disease in women (26). Surprisingly, large prospective randomized trials failed to confirm the cardioprotective role of hormone

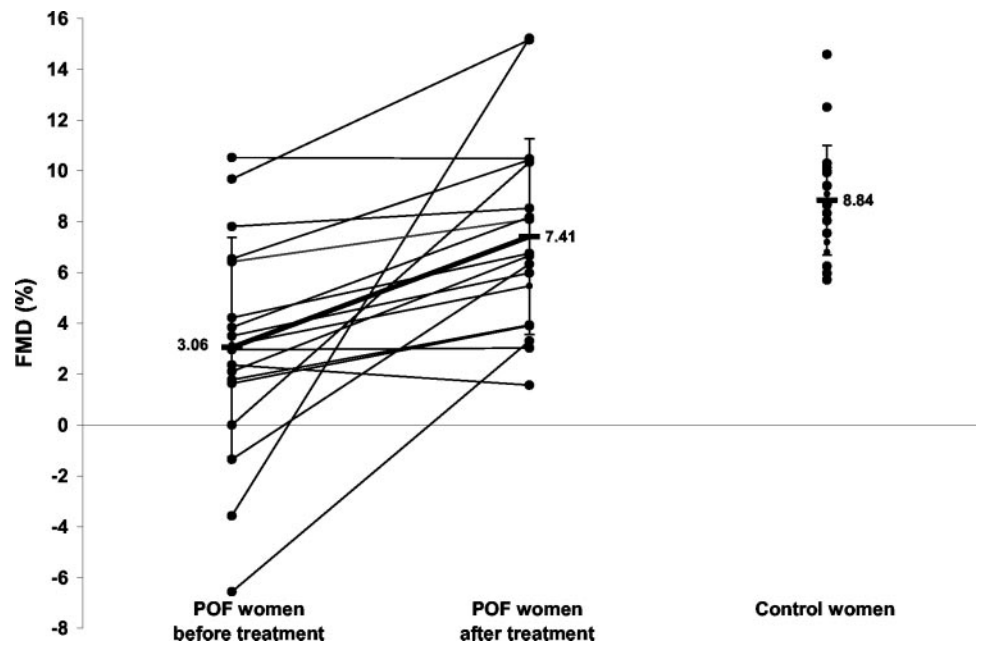


FIG. 1. Flow-mediated dilation (percent change in brachial artery during hand hyperemia; endothelium-dependent response) in women with POF before and after hormone therapy and in control women.

therapy (27–29). The Heart and Estrogen/Progestin Replacement Study trial, found no effect of continuous-combined estrogen plus progestin treatment for secondary prevention of coronary heart disease (27), whereas the Women's Health Initiative trial, a randomized, placebo-controlled, primary prevention trial, found small increases in coronary heart disease in study participants on the above continuous-combined regimen compared with women taking placebo (28, 29).

Despite the large number of studies of normal menopause and cardiovascular disease, remarkably little is known about the cardiovascular effects of ovarian failure in young women. In fact, the Heart and Estrogen/Progestin Replacement Study and Women's Health Initiative findings have led young women with premature ovarian failure to wonder whether they should stop hormone therapy. Nevertheless, studies of hormone therapy in women 50 yr of age or older cannot be applied to this group of young women (30). Premature ovarian failure is not merely early natural menopause, but, in fact, is characterized by intermittent ovarian function and is associated with an increase in several metabolic and heart disease risk factors that are not found in age-matched women with normal ovarian function (31–33).

In normal young women estradiol production varies cyclically during their menstrual cycles, with the highest serum concentrations in the preovulatory phase, whereas these concentrations are lowest during menses. Interestingly, a recent study demonstrated that premenopausal women had an increased vulnerability to coronary events during and immediately after menses, *i.e.* when endogenous estrogen concentrations are very low (34). Furthermore, another study revealed that premenopausal women with low estrogen levels due to hypothalamic hypogonadism had significantly increased incidence of angiographic coronary artery disease compared with premenopausal women with normal ovarian function (35). These findings along with the findings of our study in young women with low estrogen levels due to

ovarian failure suggest that vascular endothelial dysfunction is significantly accelerated in premenopausal women with low estrogen levels.

A protective role of estrogens against atherosclerosis is suggested by the finding that estrogen treatment reduced the progression of coronary artery atherosclerosis in oophorectomized monkeys (20). However, there was no effect on preexisting plaques (21). It is possible that hormone therapy may be beneficial in younger women before atherosclerotic plaque formation (36–38), but it may not inhibit the progression from atherosclerotic plaques to coronary events in older women (37–39). Increased methylation of the estrogen receptor gene, resulting in reduced expression of estrogen receptors with advancing age and atherosclerosis (40), may explain the potentially diminished response to hormone therapy in older postmenopausal women. Thus, a woman's age and the number of years of ovarian failure are potential factors influencing the effects of gonadal hormones on coronary heart disease. It is unclear whether the response of the endothelium to hormone therapy is similar between young women with premature ovarian failure and older healthy postmenopausal women. Several studies have shown that endothelial function improves in older postmenopausal women receiving hormone therapy (37, 41, 42). It is very difficult to compare the results from these studies because of the differences in the methodologies used, the age and characteristics of the population, the regimens, and the doses and duration of hormone therapy. A great variability exists in the degree of endothelial dysfunction reported in postmenopausal women before hormone therapy as well as in the extent of improvement of endothelial function after hormone therapy. Nevertheless, it appears that our findings are in the middle of the range of previously published studies in older postmenopausal women.

Of note, most observational studies of hormone treatment showing that it protects against coronary heart disease involved the use of estrogen given alone or in cyclic regimens,

whereas most randomized studies indicating that there is no effect involved the use of continuous combined hormone therapy (43). Therefore, estrogen alone or cyclic estrogen/progestin, but not continuous-combined, therapy may have a beneficial effect on cardiovascular disease.

In the present study we administered cyclic hormone therapy in women with premature ovarian failure, because women with premature ovarian failure can have spontaneous pregnancies (5). Therefore, the hormone therapy should produce regular predictable menstrual flow patterns. If these young patients missed an expected menses, they would be tested for pregnancy and instructed to discontinue the hormone therapy. Continuous combined hormone therapy results in the absence of menses. We used age-matched women with normal ovarian function as controls for two reasons: 1) to determine whether women with premature ovarian failure have endothelial dysfunction, and 2) to investigate whether hormone therapy can restore their endothelial function to normal levels (*i.e.* similar to the endothelial function of age-matched premenopausal women with normal ovarian function). Several factors, such as exercise, smoking, diet, vitamins, antioxidants, or cardiovascular medications, may affect FMD. In our study, women with premature ovarian failure did not receive any vitamins, antioxidants, or cardiovascular medications and did not undertake any exercise or dietary modifications, and their smoking habits remained unchanged. However, after the 6-month period of hormone therapy, there was an expected change in the lipid profile, which may have contributed to the improvement of endothelial function.

Our study has several limitations. First, it was observational in nature and not a randomized trial; nevertheless, we believe that it would be unethical to deprive young women with premature ovarian failure of hormone therapy. Second, we did not exclude other cardiovascular risk factors, such as smoking; however, these risk factors were well matched between women with premature ovarian failure and the control group and should not influence our findings.

In conclusion, young women with premature ovarian failure have significant vascular endothelial dysfunction, an early stage of atherosclerosis. Early onset of endothelial dysfunction associated with sex steroid deficiency may contribute to the increased risk of cardiovascular disease and mortality in young women with premature ovarian failure. Hormone therapy restores endothelial function within 6 months of treatment. Further research is required with different hormone formulations, different doses of hormones, and different durations of hormone use in young women with premature ovarian failure.

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