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

SOPHIA N. KALANTARIDOU, KATERINA K. NAKA, EVANGELOS PAPANIKOLAOU,
NICOLAOS KAZAKOS, MARIA KRAVARITI, KARIM A. CALIS, EVANGELOS A. PARASKEVAIDIS,
DEMETRIOS A. SIDERIS, AGATHOCLES TSATSOULIS, GEORGE P. CHROUSOS, AND
LAMPROS K. MICHALIS

Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University Hospital (S.N.K., E.P., E.A.P.); Michaeleidion Cardiac Research Center (K.K.N., N.K., D.A.S., L.K.M.); and Department of Endocrinology, University Hospital (M.K., A.T.), University of Ioannina Medical School, 45500 Ioannina, Greece; and Clinical Center Pharmacy Department (K.A.C.) and Pediatric and Reproductive Endocrinology Branch (G.P.C.), National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892

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 ± 4.33%) than in control women (increase by 8.84 ± 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 ± 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-

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 T_4 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 T_4), 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 T_3, free T_4, 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 suprasystolic 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 $0.5 \pm 2.8\%$ for the endothelium-dependent response and $0.7 \pm 1.8\%$ 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 $\pm$ 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 T_4 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	*P*
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 $3.06 \pm 4.33\%$) compared with control women (increase by $8.84 \pm 2.15\%$; $P < 0.0005$; 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 $7.41 \pm 3.86\%$ ($P < 0.005$ 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
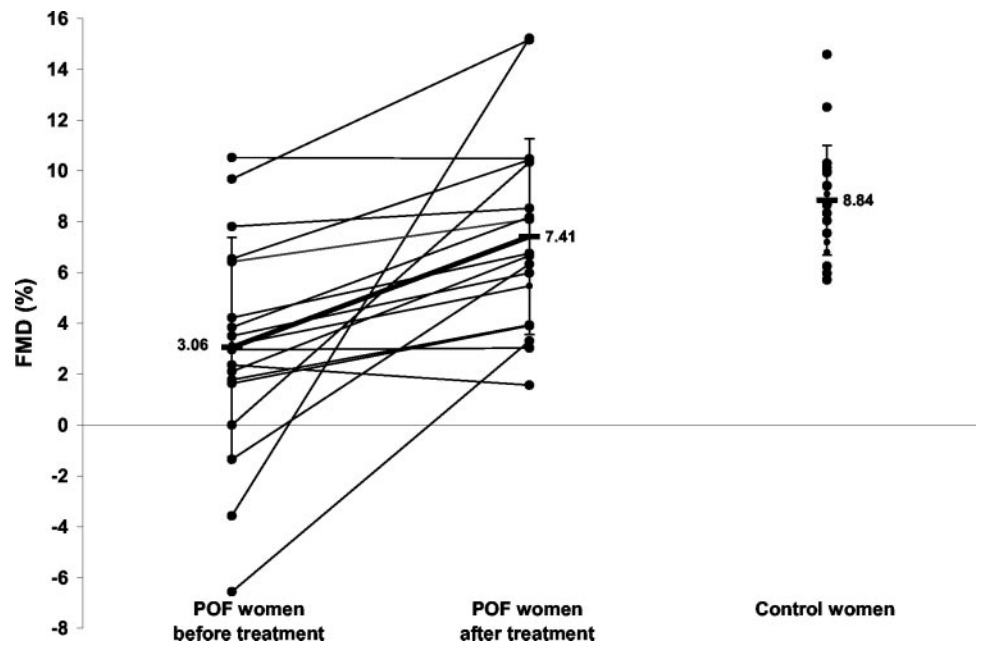
FIG. 1. Flow-mediated dilation (percent change in brachial artery during hand hyperemia; endothelium-dependent response) in women with POF before and after hormone therapy and in control women.

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.

Acknowledgments

References

1. **Colditz GA, Willett WC, Stampfer MJ, Rosner B, Speizer FE, Hennekens CH** 1987 Menopause and the risk of coronary heart disease in women. N Engl J Med 316:1105–1110
2. **Walsh BW, Schiff I, Rosner B, Greenberg L, Ravinkar V, Sacks FM** 1991 Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. N Engl J Med 325:1196–1204
3. **Sack MN, Rader JR, Cannon RO** 1994 Oestrogen and inhibition of oxidation of low-density lipoproteins in postmenopausal women. Lancet 343:269–270
4. **Koh KK, Jin DK, Yang SH, Lee SK, Hwang HY, Kang MH, Kim W, Kim DS, Choi IS, Shin EK** 2001 Vascular effects of synthetic or natural progestagen combined with conjugated equine estrogen in healthy postmenopausal women. Circulation 103:1961–1966
5. **Kalantaridou SN, Davis SR, Nelson LM** 1998 Premature ovarian failure. Endocrinol Metab Clin North Am 27:989–1006
6. **Coulam CB, Adamson SC, Annegers JF** 1986 Incidence of premature ovarian failure. Obstet Gynecol 67:604–606
7. **Ohta H, Sugimoto I, Masuda A, Komukai S, Suda Y, Makita K, Takamatsu K, Horiguchi F, Nazawa S** 1996 Decreased bone mineral density associated with early menopause progresses for at least ten years: cross-sectional comparisons between early and normal menopausal women. Bone 18:227–231
8. **Anasti JN, Kalantaridou SN, Kimzey LM, Defensor RA, Nelson LM** 1998 Bone loss in young women with karyotypically normal spontaneous premature ovarian failure. Obstet Gynecol 91:12–15
9. **Van der Schouw YT, van der Graaf Y, Steyerberg EW, Eijkemans JC, Banga JD** 1996 Age at menopause as a risk factor for cardiovascular mortality. Lancet 347:714–718
10. **Cooper GS, Sandler DP** 1998 Age at natural menopause and mortality. Ann Epidemiol 8:229–235
11. **Snowdon DA, Kane RL, Beeson WL, Burke GL, Sprafka JM, Potter J, Iso H, Jacobs Jr DR, Phillips RL** 1989 Is early natural menopause a biologic marker of health and aging? Am J Public Health 79:709–714
12. **De Kleijn MJJ, van der Schouw YT, Verbeek ALM, Peeters PHM, Banga JD, van der Graaf Y** 2002 Endogenous estrogen exposure and cardiovascular mortality risk in postmenopausal women. Am J Epidemiol 155:339–345
13. **Jacobsen BK, Knutsen SF, Fraser GE** 1999 Age at natural menopause and total mortality and mortality from ischemic heart disease: the Adventist Health Study. J Clin Epidemiol 52:303–307
14. **Hu FB, Grodstein F, Hennekens CH, Colditz GA, Johnson M, Manson JE, Rosner B, Stampfer MJ** 1999 Age at natural menopause and risk of cardiovascular disease. Arch Intern Med 159:1061–1066
15. **Vita JA, Keany Jr JF** 2001 Hormone replacement therapy and endothelial function. The exception that proves the rule? Arterioscler Thromb Vasc Biol 21:1867–1869
16. **Friedewald WT, Levy RI, Fredrickson DS** 1972 Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:409–502
17. **Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Deanfield JE** 1992 Non-invasive detection of endothelial dysfunction in children and adults at risk for atherosclerosis. Lancet 340:1111–1115
18. **Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R, International Brachial Artery Reactivity Task Force** 2002 Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. J Am Coll Cardiol 39:257–265
19. **Ross R** 1993 The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362:801–809
20. **Kaplan JR, Adams MR, Anthony MS, Morgan TM, Manuck SB, Clarkson TB** 1995 Dominant social status and contraceptive hormone treatment inhibit atherogenesis in premenopausal monkeys. Atheroscler Thromb Vasc Biol 15:2094–2100
21. **Clarkson TB, Anthony MS, Klein KP** 1996 Hormone replacement therapy and coronary artery atherosclerosis: the monkey model. Br J Obstet Gynaecol 103(Suppl 13):53–57
22. **Anderson TJ, Uehata A, Gerhard MD, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC, Selwyn AP, Meredith IT** 1995 Close correlation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 26:1235–1241
23. **Schachinger V, Britten MB, Zeiher AM** 2000 Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 101:1899–1906
24. **Pinto S, Virdis A, Ghiadoni L, Bernini G, Lombardo M, Petraglia F, Genazzani AR, Taddei S, Salvetti A** 1997 Endogenous estrogen and acetylcholine-induced vasodilation in normotensive women. Hypertension 29:268–273

25. **Virdis A, Ghiadoni L, Pinto S, Lombardo M, Petraglia F, Gennazzani A, Buralli S, Taddei S, Salvetti A** 2000 Mechanisms responsible for endothelial dysfunction associated with acute estrogen deprivation in normotensive women. Circulation 101:2258–2263

26. **Grodstein F, Stampfer MJ, Manson JE, Coltitz GA, Willett WC, Rosner B, Speizer FE, Hennekens CH** 1996 Postmenopausal estrogen and progestin use and the risk of cardiovascular disease. N Engl J Med 335:453–461

27. **Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Vittinghoff E** 1998 Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. JAMA 280:605–613

28. **Writing Group for the Women's Health Initiative Investigators** 2002 Risk and benefits of estrogen plus progestin in healthy postmenopausal women. Principal results from the Women's Health Initiative randomized controlled trial. JAMA 288:321–333

29. **Manson JE, Hsia J, Johnson KC, Rossouw JE, Assaf AR, Lasser NL, Trevisan M, Black HR, Heckbert SR, Detrano R, Strickland OL, Crouse JR, Stein E, Cushman M, Women's Health Initiative Investigators** 2003 Estrogen plus progestin and the risk of coronary heart disease. N Engl J Med 349:523–534

30. **Cooper GS** 2002 Hormone replacement following early menopause. JAMA 288:2824–2825

31. **Nelson LM, Anasti JN, Kimzey LM, Defensor RA, Lipetz KJ, White BJ, Shawker TH, Merino TJ** 1994 Development of luteinized Graafian follicles in patients with karyotypically normal spontaneous premature ovarian failure. J Clin Endocrinol Metab 79:1470–1475

32. **Poehlman ET, Toth MJ, Gardner AW** 1995 Changes in energy balance and body composition at menopause: a controlled longitudinal study. Ann Intern Med 123:673–675

33. **Kim TJ, Anasti JN, Flack MR, Kimzey LM, Defensor RA, Nelson LM** 1997 Routine endocrine screening for patients with karyotypically normal spontaneous premature ovarian failure. Obstet Gynecol 89:777–779

34. **Hamelin BA, Methot J, Arsenault M, Pilote S, Poirier P, Plante S, Bogaty P** 2003 Influence of the menstrual cycle on the timing of acute coronary events in premenopausal women. Am J Med 114:599–602

35. **Bairey-Merz CN, Johnson BD, Sharaf BL, Bittner V, Berga SL, Braunstein GD, Hodgson TK, Matthews KA, Pepine CJ, Reis SE, Reichek N, Rogers WJ, Pohost GM, Kelsey SF, Sopko G, WISE Study Group** 2003 Hypoestrogenemia of hypothalamic origin and coronary artery disease in premenopausal women: a report from the NHLBI-Sponsored WISE Study. J Am Coll Cardiol 41:413–419

36. **Strong JP, Malcom GT, McMahan CA, Tracy RE, Newman III WP, Herderick EE, Cornhill JF** 1999 Prevalence and extent of atherosclerosis in adolescents and young adults: implications for prevention from the Pathobiological Determinants of Atherosclerosis in Youth Study. JAMA 281:727–735

37. **Herrington DM, Espeland MA, Crouse III JR, Robertson J, Riley WA, McBurnie MA, Burke GL** 2001 Estrogen replacement and brachial artery flow-mediated vasodilation in older women. Arterioscler Thromb Vasc Biol 21:1955–1961

38. **Hodis HN, Mack WJ, Lobo RA, Shoupe D, Sevanian A, Mahrer PR, Selzer RH, Liu CR, Liu CH, Azen SP, Estrogen in the Prevention of Atherosclerosis Trial Research Group** 2001 Estrogen in the prevention of atherosclerosis: a randomized, double-blind, placebo-controlled trial. Ann Intern Med 135:939–953

39. **Hodis HN, Mack WJ, Azen SP, Lobo RA, Shoupe D, Mahrer PR, Faxon DP, Cashin-Hemphill L, Sanmarco ME, French WJ, Shook TL, Gaarder TD, Mehra AO, Rabbani R, Sevanian A, Shil AB, Torres M, Vogelbach KH, Selzer RH, Women's Estrogen-Progestin Lipid-Lowering Hormone Atherosclerosis Regression Trial Research Group** 2003 Hormone therapy and the progression of coronary-artery atherosclerosis in postmenopausal women. N Engl J Med 349:535–545

40. **Post WS, Goldschmidt-Clermont PJ, Wilhide CC, Heldman AW, Sussman MS, Ouyang P, Milliken EE, Issa JP** 1999 Methylation of the estrogen receptor gene is associated with aging and atherosclerosis in the cardiovascular system. Cardiovasc Res 43:985–991

41. **Bush DE, Jones CE, Bass KM, Walters GK, Bruza JM, Ouyang P** 1998 Estrogen replacement reverses endothelial dysfunction in postmenopausal women. Am J Med 104:552–558

42. **De Kleijn MJJ, Wilmink HW, Bots ML, Bak AAA, van der Schouw YT, Planellas J, Engelen S, Banga J-D, Grobbee DE** 2001 Hormone replacement therapy and endothelial function. Results of a randomized controlled trial in healthy postmenopausal women. Atherosclerosis 159:357–365

43. **Grodstein F, Clarkson TB, Manson JE** 2003 Understanding the divergent data on postmenopausal hormone therapy. N Engl J Med 348:645–650