

# Effect of menopause on plaque morphologic characteristics in coronary atherosclerosis

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**Background** Coronary artery disease in women appears 10 to 15 years later than in men. To test the hypothesis that the effects of estrogen may manifest themselves as histologic differences in coronary plaques, we examined the hearts of premenopausal and postmenopausal women who died suddenly from coronary artery disease.

**Methods** We studied 51 cases of sudden coronary death and 47 deaths in women who died from noncoronary causes. Coronary deaths were classified on the basis of histologic features. The number of acute plaque ruptures, healed plaque ruptures, vulnerable plaques, and acute plaque erosions were compared between groups. Postmortem values of serum total cholesterol, HDL cholesterol, and thiocyanate were measured, and menopausal status was confirmed by calculating body mass index.

**Results** Women older than 50 years of age were much more likely to have a ruptured plaque than were younger, premenopausal women. Plaque rupture was significantly associated with elevated total cholesterol level. In the 51 women who died of coronary disease, the mean number of vulnerable plaques increased significantly as women advanced into the postmenopausal years.

**Conclusions** Our data suggest that estrogen has an anti-inflammatory effect on atherosclerotic plaques, resulting in plaque stabilization. Plaque erosion, the major substrate for thrombosis in premenopausal women, does not appear to be inhibited by estrogen. Because plaque progression may result both from repeated rupture and repeated erosion, a better understanding of the effect of estrogen on atherosclerosis may yield insights into the nature of coronary artery disease. (Am Heart J 2001;141:S58-62.)

Coronary artery disease in women is delayed by 10 to 15 years compared with men. For several reasons, this delay is believed to be caused, in part, by the so-called protective effect of estrogen on coronary atherosclerosis and a lesser prevalence of traditional risk factors in younger women.<sup>1-7</sup> Epidemiologic, clinical, animal, and in vitro studies have supported the existence of a protective effect of estrogen, which is mediated by changes in LDL cholesterol, HDL cholesterol, lipoprotein(a), fibrinogen, and homocysteine and by direct effects on the vessels themselves.<sup>8</sup> The latter effects may include an influence on vasomotor tone, inhibitory effects on smooth muscle cell proliferation, inhibition of the elaboration of growth factors and inflammatory cytokines,

and effects on vascular cellular adhesion molecules.<sup>9,10</sup> A better understanding of the effect of estrogen on coronary atherosclerosis is crucial to better direct estrogen replacement therapy and lipid lowering treatment in postmenopausal women. Meta-analyses of postmenopausal women treated with hormone replacement therapy (HRT) for osteoporosis report a 25% to 45% decrease in acute coronary events, and HRT complements statin treatment in postmenopausal women in lowering lipid levels. However, a recent randomized, secondary prevention trial has not shown clear-cut results in decreasing coronary events.<sup>11</sup> Although the results from this large cohort trial (Heart and Estrogen/Progestin Replacement Study [HERS]) did not show benefits in the short term, the need for long-term randomized trials to assess the effects of HRT on heart disease remains great. The Women's Health Initiative Study Group, begun in 1992 with planned completion in 2007, is structured to assess the effects of dietary modification, HRT, vitamin D, and calcium supplementation in the long-term development of heart disease and osteoporosis in postmenopausal women.<sup>12</sup>

To test the hypothesis that the effects of estrogen may manifest themselves as histologic differences in coronary plaques between premenopausal women and postmenopausal women and between premenopausal

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**Table I.** Mechanism of death and risk factors in 51 women who died suddenly from severe coronary artery disease

Mechanism of death (n)	Age >50 y (%)	TC (mg/dL)	Body mass index	Glycohemoglobin (mean %)	Cigarette smokers (n)
Plaque erosion (n = 18)	3 (17%)	191 ± 68	27 ± 4.3	6.7 ± 0.7	14 (78%)
Plaque rupture (n = 8)	7 (87%)	272 ± 61	31 ± 4.4	8.8 ± 4.4	4 (50%)
Stable plaque, no infarct (n = 7)	2 (29%)	178 ± 57	30 ± 10.6	8.0 ± 4.5	2 (29%)
Stable plaque, healed infarct (n = 18)	9 (50%)	198 ± 61	28 ± 9.0	10.2 ± 5.0	9 (50%)
P value	.001, rupture vs erosion;	.01, rupture vs stable plaque;	.02, rupture vs eroded plaque	.001, stable plaque, healed infarct vs eroded plaque	
	.03, rupture vs stable plaque, no infarct	.01, rupture vs stable plaque, healed infarct;			
		.02, rupture vs erosion			

TC, Total cholesterol.

**Table II.** Multivariate association between risk factors and morphologic characteristics of culprit plaque in 51 women compared with 47 noncoronary deaths (trauma and cardiac noncoronary)

Risk factor	Plaque morphology	P value (univariate)	Multivariate		
			z score	P value	Odds ratio
Total cholesterol(mg/dL)	Rupture	<.0001	3.0	.002	31
Cigarette smoking	Erosion	.003	2.5	.01	16
Glycosylated hemoglobin >8%	Stable plaque	.001	2.8	.006	7.1
Hypertension	Stable plaque	.006	2.3	.02	4.0

women and men, we examined the hearts of premenopausal and postmenopausal women who died suddenly from coronary artery disease. Coronary arteries in these patients were examined for types of culprit plaques, extent of coronary artery disease, and histologic characteristics of nonculprit lesions.

## Methods

### Case selection

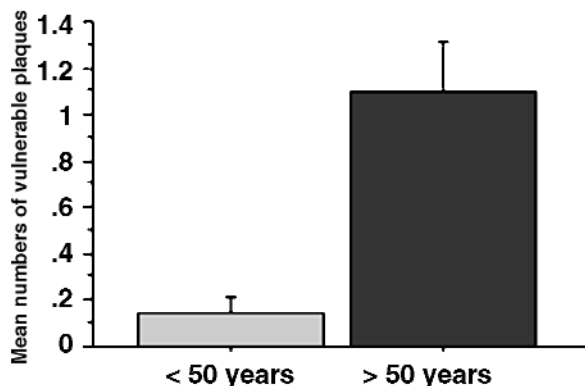
Women who died suddenly were evaluated prospectively in consultation, as previously reported.<sup>13</sup> This study included 51 previously reported cases of sudden coronary death (Table I)<sup>13</sup> and 47 deaths (control group) in women who died from noncoronary causes studied during the same period (Table II). In addition, nonculprit coronary plaque morphologic characteristics were compared between women <50 years and women >50 years.

### Examination of the heart and determination of type of death

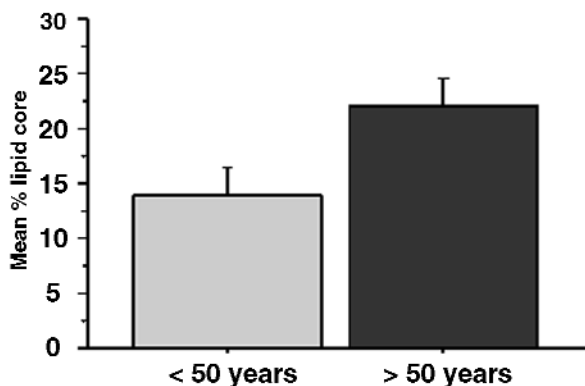
Hearts were perfusion-fixed in buffered formalin, and epicardial arteries were serially sectioned at 2- to 3-mm intervals. Each segment with luminal narrowing of >50% of the cross-sectional area (estimated grossly) was submitted for histologic analysis, which included Movat pentachrome staining. Coronary deaths were defined as natural deaths

without evidence of an extracardiac cause of death in which at least 1 epicardial coronary artery showed >75% luminal narrowing in cross-sectional area from atherosclerotic plaque or plaque with a superimposed thrombus. Sudden death was defined as death that occurred within 6 hours of the appearance of symptoms (witnessed arrest) or death within 24 hours of the time when the victim was last seen alive in her normal state of health. Coronary deaths with acute thrombus were further categorized as plaque rupture if serial sectioning showed a connection between the thrombus and the necrotic core or plaque erosion if there was an intact fibrous cap rich in proteoglycans, with loss of surface endothelial cells and an attached fibrin-platelet thrombus. In coronary deaths without acute thrombus, the category of death was considered stable plaque with or without healed myocardial infarct.

All segments with >50% luminal narrowing in cross-section were histologically analyzed. The number of acute plaque ruptures, healed plaque ruptures, vulnerable plaques, and acute plaque erosions were compared among the 4 groups. Vulnerable plaques (thin-capped atheromas) were defined as a fibrous cap thinner than 65 µm infiltrated by macrophages overlying a necrotic core, as previously defined. The degree of coronary calcification in each segment was determined on a 6-point scale (0, 1+ representing microcalcifications <20 µm in diameter; 2+ representing foci of calcification >20 µm involving 1

**Figure 1**

Mean number of vulnerable plaques in 51 women who died suddenly from severe coronary disease. *Left bar* shows mean numbers in younger women; *right bar* shows mean number in older women. Difference was significant ( $P < .0001$ , Student *t* test).

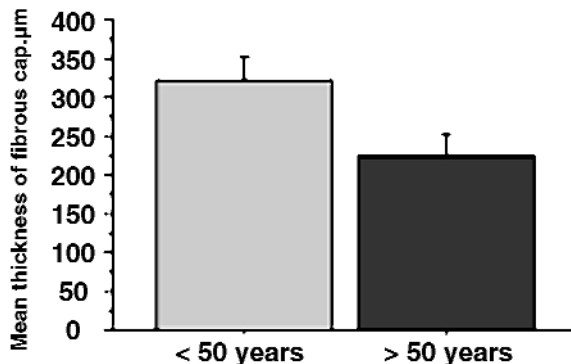
**Figure 2**

Mean proportion (%) of plaques with lipid core in 51 women who died suddenly from severe coronary disease. *Left bar* shows mean proportion of such lesions in younger women; *right bar* shows proportion in older women. Difference was significant ( $P = .02$ , Student *t* test).

quadrant of the plaque; 3+ involving 2 quadrants; 4+ involving 3 quadrants; and 5+ involving the entire circumference of the coronary artery). A mean calcification score was calculated for each heart, and the mean of the means was compared among the 4 groups. The percent lipid composition of the core for each plaque was measured, and the mean was calculated for all plaques in each heart.

#### Determination of risk factors and menopausal status

Postmortem values of serum total cholesterol, HDL cholesterol, and thiocyanate were measured as previously

**Figure 3**

Mean fibrous cap thickness ( $\mu\text{m}$ ) in 51 women who died suddenly from severe coronary disease. *Left bar* shows mean thickness in younger women; *right bar* shows thickness in older women. Difference was significant ( $P = .02$ , Student *t* test).

described.<sup>14</sup> The body mass index was estimated by the formula  $\text{Body weight}/(\text{Body height})^2$  (in kilograms per square meter).

## Results

### Correlation of risk factors with culprit plaque morphologic characteristics

This dataset was composed of 51 women who died suddenly with acute coronary artery disease and 47 control subjects. Women older than 50 years were much more likely to have a ruptured plaque than were younger, premenopausal women (Table I). Plaque rupture was associated with elevated total cholesterol, body mass index, and elevated glycosylated hemoglobin by univariate analysis (Table I). Plaque erosions, which occurred most frequently in premenopausal women, were associated with cigarette smoking by univariate and multivariate analysis (Tables I and II). The association between plaque rupture and elevated total cholesterol level was significant by multivariate analysis (Table II).

### Comparison of coronary plaques in women younger than and older than 50 years who died from coronary disease

In the coronary death dataset of 51 women, the mean number of vulnerable plaques significantly increased as women advanced into their postmenopausal years (Figure 1). The mean lipid core index was higher in postmenopausal women who died suddenly compared with younger women (Figure 2), and the older women had a much thinner fibrous cap (Figure 3). There was a striking increase in calcification in older women versus younger women who died of severe coronary disease (Figure 4).

## Discussion

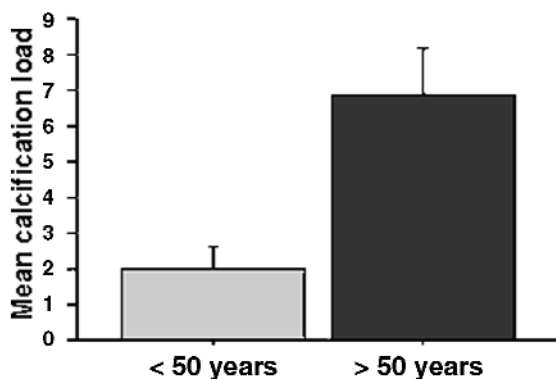
We have demonstrated significant differences in the plaque morphologic characteristics of premenopausal women versus older women who died suddenly from coronary artery disease. The culprit plaque in premenopausal women rarely appeared ruptured; in those few young women with plaque rupture, serum total cholesterol level was severely elevated. Because the major cause of coronary thrombosis in men and postmenopausal women is plaque rupture, the protective effects of estrogen may lie largely in its ability to stabilize the fibrous cap.

Our histologic findings of nonculprit plaques in sudden coronary death revealed several differences between premenopausal women and older women. Previous autopsy studies have shown relatively little calcification in the coronary arteries of women who died suddenly.<sup>15,16</sup> The relatively minimal amount of plaque calcification in premenopausal women who died suddenly with severe coronary disease has two implications. First, screening for coronary artery calcium may not be particularly effective in young women. Second, hypothetical mechanisms of plaque progression, such as repeated plaque rupture, may result in calcified plaques in older women and in men, but those plaques that grow by plaque erosion may not result in a similar degree of calcification.<sup>17</sup> We have shown that the number of vulnerable plaques is decreased in premenopausal women compared with postmenopausal women and that the fibrous cap in younger women is generally thicker than in older women. These findings corroborate the concept that estrogen prevents, in part, processes within the plaque that can lead to future or remote rupture.

Our data suggest that estrogen has an anti-inflammatory effect on atherosclerotic plaques, resulting in plaque stabilization, which is manifested as smaller lipid cores, less calcium, and fewer thin-capped atheromas. These are all features associated with plaques prone to rupture. The other major substrate for coronary thrombosis is plaque erosion, which is characterized by fewer macrophages and inflammatory cells compared with ruptured plaques and is not associated with thin fibrous caps.<sup>18,19</sup> Plaque erosion does not appear to be inhibited by estrogen. It is the major thrombotic substrate in premenopausal women, accounting for more than 75% of all thrombi in these patients.

Further studies are necessary to elucidate the mechanism of plaque erosion, from which premenopausal women are not protected. Because plaque progression may result both from repeated rupture and repeated erosion, a better understanding of the effect of estrogen on atherosclerosis may yield insights into the nature of coronary artery disease. Anecdotal evidence that birth control pills and combined estrogens in premenopausal women have been associated with plaque erosion illus-

Figure 4



Mean calcification score in 51 women who died suddenly from severe coronary disease. *Left bar* shows mean calcification score in younger women; *right bar* shows mean score in older women. Difference was significant ( $P = .0007$ , Student *t* test).

trates a potential paradox in the effect of estrogen on atherosclerosis: generally positive, based on its ability to stabilize the fibrous cap, but possibly also deleterious, based on its prothrombotic effects.

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