ESTROGEN ATTENUATES FATTY STREAK FORMATION IN CORONARY ARTERIES OF CHOLESTEROL-FED DIABETIC MALE RABBITS

M. KHAZAEI*
M. NEMATBAKHSH**
G.H. DASHTI***

SUMMARY: In this study we investigated the histological role of estrogen on Fatty Streak (FS) formation in some branches of coronary arteries (CA) in cholesterol-fed diabetic male rabbits. Seventeen rabbits were obtained from Pasteur Institute of Iran and diabetes was induced by intravenous injection of Alloxan (200 mg/kg). After induction of diabetes, they were randomly divided into three groups: (i) control, (ii) low-dose estrogen treatment (1mg/animal) and (iii) high-dose estrogen treatment (5mg/animal). Estrogen was injected i.m, weekly. Control group received normal saline injection. All groups were fed with cholesterol rich diet (1%) during the experiment. After six weeks, the animals were sacrificed. Hearts were isolated and four branches of CA (Right and Left CA; RCA and LCA, Anterior and Posterior interventricular arteries; AIVA and PIVA) were dissected and evaluated for the relative size of FS and the percentage of arterial lumen narrowing by FS using scale micrometer. The average grades of pathological evaluation for branches of coronary arteries showed that in animals receiving high dose estrogen, the FS formations in RCA, LCA, AIVA and PIVA (0.33 ± 0.21, 0.17 ± 0.17, 0.0 ± 0.0, 0.0 ± 0.0, respectively) were significantly lesser than control group (1.40 ± 0.24, 2.2 ± 0.37, 0.80 ± 0.2, 1.20 ± 0.37, respectively). We conclude that estrogen has a beneficial role in histological changes of coronary arteries, it can attenuate progression of FS formation administration diabetic rabbits.

Key words: Estrogen, Diabetes, Fatty streaks, Coronary arteries.

INTRODUCTION

Cardiovascular diseases (CVD) including coronary artery afflictios are the leading causes of morbidity and mortality in developed countries (1), especially in diabetic subjects (2). Recent studies have indicated that pre-menopausal women and women who received Estrogen Replacement Therapy (ERT), are relatively protected from coronary heart diseases (3-6). It is documented that menopause and consequent estrogen deprivation increase the risk of CVD in women (7, 8). The disparity between CVD in premenopausal women and men of the same age suggests that endogenous sex hormones such as estrogen have a major impact on atherosclerotic processes as recently reviewed by Luft (9). One potential mechanism for cardioprotective effect of estrogen may be its beneficial effects on plasma lipoprotein profiles (10, 11). However, other studies demonstrated that only 25-

*From Department of Physiology, Medical University of Birjand, Birjand, Iran.
**From Department of Physiology, Medical University of Isfahan, Isfahan, Iran.
***From Department of Anatomy, Medical University of Isfahan, Isfahan, Iran.

50% of antiatherogenic effects of estrogen are attributable to its effects on lipids metabolism (12, 13). Recent observations suggest that estrogen may act directly on vascular tissues (14) and it may contribute significantly to the cardioprotective influences (15).

The accumulation of atherogenic lipoproteins in the arterial wall intima constitutes a fundamental event in atherogenesis (16), and it has been suggested that estrogens could be involved in this process (17). Previous studies showed the beneficial effects of estrogen on endothelial function, vascular tone and reactivity, intimal cell proliferation, expression of adhesion molecules, and LDL oxidation [as reviewed by MC Cid *et al.* (18) and GM Rubaney *et al.* (19)]. Therefore, it is possible that estrogen may inhibit atherosclerotic process by direct effects on the arterial wall.

Atherosclerosis is a chronic inflammatory condition of the vascular wall (1). The lesions of atherosclerosis are divided into three categories: the Fatty Streak (FS), the intermediate or fibrofatty lesion, and the fibrous plaque (20). The FS is the earliest visible lesion, and, although not clinically significant, it is the precursor to clinically significant disease (20). In this study, we investigated the histological role of ERT on FS formation in coronary arteries of cholesterol-fed diabetic male rabbits.

MATERIALS AND METHODS

Animals

This study was carried out on 17 male rabbits (2-2.5 kg). The animals were purchased from the Pasteur Institute of Iran. They were housed four per cage in 12 h light/dark cycles with free access to drinking water.

Table 1: The pathological evaluation for part A coronary arteries (FS: Fatty Streak).

Pathology condition	Evaluation	Grade
No FS or fatty dots in the artery	-	0
Less FS existence in the artery	+	1
Moderate FS existence in the artery	++	2
Existence of FS in most parts of artery	+++	3
Existence of FS in all parts of artery	++++	4

Table 2: The pathological evaluation for part A coronary arteries (RSFS: Relative Size of FS; PFSAL: Percentage of FS in Arterial Lumen).

RSFS	0	0.1-.99	1 - 1.99	2 - 2.99	3 - 4
PFSAL	0	1-25 %	26-50%	51-75%	76-100%
Grade	0	1	2	3	4

Experimental design

After one week of habituation to the laboratory, Alloxan (200 mg/kg) was injected through the marginal ear vein, to induce diabetes as previously described (21-24). After two weeks, rabbits with mean blood glucose above 300 mg/dl were defined as being diabetic and selected for the study (25). Then, they were randomized into three groups. All groups received cholesterol-rich diet (1%) prepared by adding one gram of pure cholesterol (Merck, Germany) in 4 ml of olive oil to 0.1 kg of commercial rabbit chow. All groups were treated for six weeks as follows:

Group 1 (n = 5): cholesterol-rich diet + weekly intramuscular injection of distilled water.

Group 2 (n = 6): cholesterol-rich diet + weekly intramuscular injection of 1 mg estradiol valerate (Abureihan Co, Iran).

Group 3 (n = 6): cholesterol-rich diet + weekly intramuscular injection of 5 mg estradiol valerate.

Evaluation of fatty streak formation

After five weeks, the animals were sacrificed. Their chests were opened and the hearts were removed rapidly. Four branches of coronary arteries were isolated: Right coronary artery (RCA), left coronary artery (LCA), anterior and posterior interventricular arteries (AIVA, PIVA, respectively) with least tissue around them. The samples were fixed in formalin for pathological processes. Totally, 272 microscopic slides, including 1249 tissue sections, were obtained.

For pathological evaluation, the coronary branches were divided into two parts. Part A includes LCA and RCA, and part B includes AIVA and PIVA. First, the coronary arteries of part A were evaluated according to Table 1. Second, two variables (variable 1: relative size of FS; RSFS and variable 2: the percentage of FS in arterial lumen; PFSAL) were defined for evaluation of FS existence in CA as follows:

$$RSFS = \frac{\text{Size of FS } (\mu)}{\text{Arterial wall thickness } (\mu)}$$

The grades of zero, 1, 2, 3 and 4 were considered for variable 1, when RSFS was 0, (0.1-0.99), (1-1.99), (2-2.99) and (3-4), respectively.

Table 3: The pathological evaluation for part B coronary arteries.

Number of lesion section existence	0	1-3	1-6	1-9	1-12
Grade	0	1	2	3	4

$$PFSAL = \frac{\text{Size of FS } (\mu)}{\text{Size of arterial lumen } (\mu)} \times 100$$

The grades of zero, 1, 2, 3, and 4 were considered for variable 2, when PFSAL was 0, (1-25%), (26-50%), (51-75%) and (76-100%), respectively. All measurements were obtained by longitudinal scale micrometer. According to these two variables, the evaluation grades were obtained from Table 2. The mean final evaluation grades were calculated based on Tables 1 and 2. In coronary arteries of part B, the lesions were small and distributed in all parts of arteries. Therefore they were evaluated based on the number of lesion section (number of section with FS, Table 3).

Statistical analysis

The data were reported as mean ± SE and were analyzed with One-Way ANOVA using tukey's test for determination of any differences between the groups. The P value less than 0.05 was considered as significant.

RESULTS

The average grades of pathological evaluation for branches of coronary arteries are indicated in Table 4. The results showed that in animals that received high dose estrogen, the FS formations in RCA, LCA, AIVA and PIVA (0.33 ± 0.21, 0.17 ± 0.17, 0.0 ± 0.0, 0.0 ± 0.0, respectively) were significantly lesser than control group (1.40 ± 0.24, 2.20 ± 0.37, 0.80 ± 0.20, 1.20 ± 0.37) and low dose estrogen-treated group (0.83 ± 0.17, 2.00 ± 0.0, 0.33 ± 0.21, 0.50 ± 0.22) (P<0.05). Samples of these arteries are shown in Figure 1.

DISCUSSION

The aim of this study was to evaluate the effect of estrogen of histological changes in some branches of coronary arteries in diabetic male rabbits. Results showed that in estrogen-treated animals, the FS formation was lesser than control.

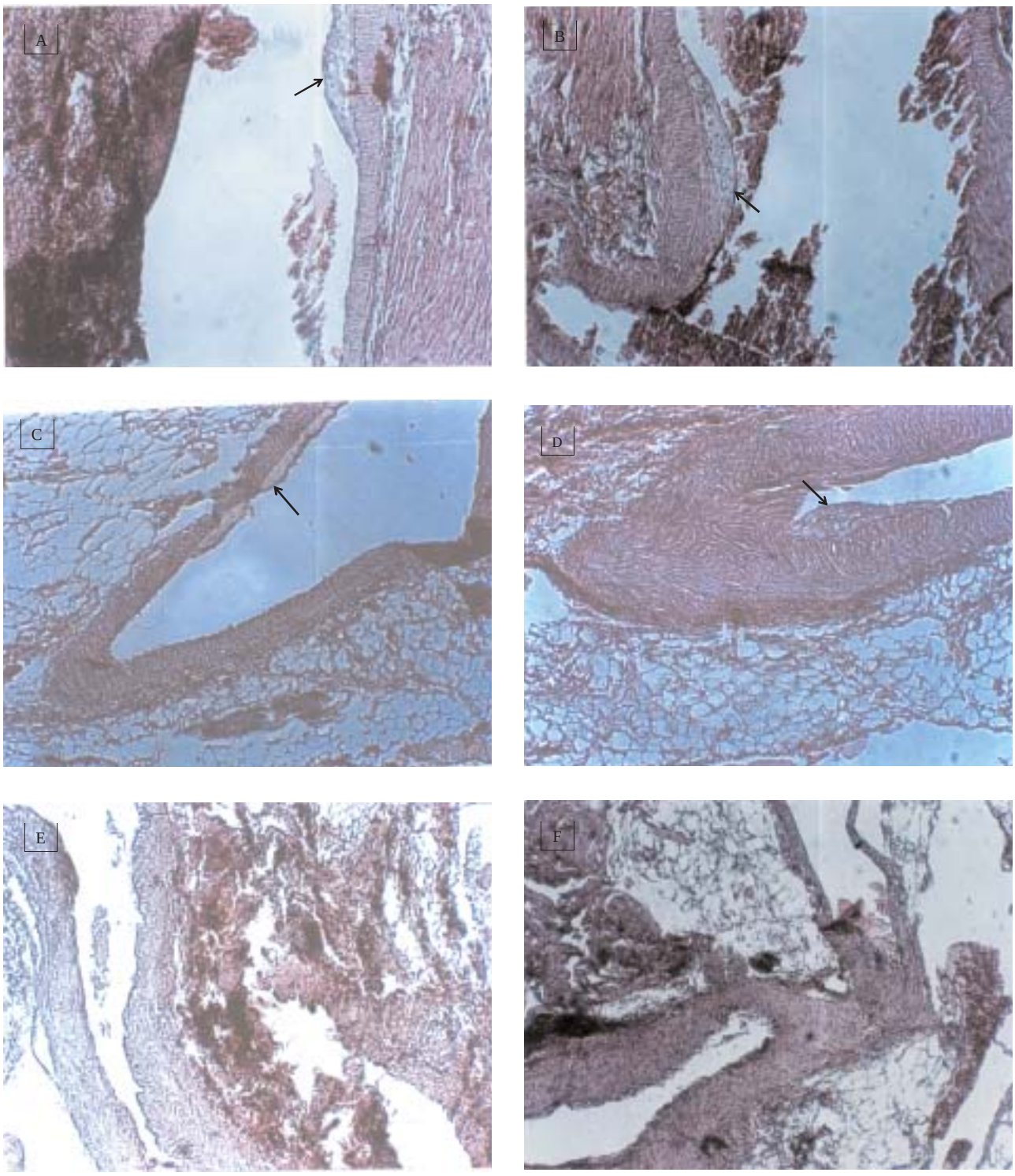
Previous studies have shown that the lower incidence of cardiovascular diseases in female than male may be related to female sex hormone and ERT had the beneficial effects on reducing CVD (7, 26-30). The mechanisms responsible for these observations are incompletely understood, although favorable effects on lipid profiles have been thought to account for a portion of estrogen's protective action (31). Disruption of the anatomic and functional integrity of the endothelium has been postulated for the initiation of atherosclerosis (1). The accumulation of atherogenic lipoproteins in the arterial wall intima constitutes a fundamental event in atherogenesis, and it has been suggested that estrogens could be involved in this process (32). According to our results, there was significant decrease in FS formation in group 3 (high-dose estrogen-treated) compared to the other groups. In addition, FS formation in coronary arteries of group 2 (low dose estrogen-treated animals) was lesser than group 1, although the results were not statistically significant. These results agree with some recent studies in hypercholesterolemic subjects (32-35). There are a few studies in hypercholesterolemic diabetic animals. In one study Tse *et al.* (36) reported that accelerated atherosclerosis in the aorta of diabetic male Apo-E knockout mice can be prevented by chronic estradiol treatment, however, in contrast to our results, a recent study in postmenopausal women with abnormal glucose tolerance test has demonstrated that hormone replacement therapy was associated with a worsening of coronary atherosclerosis (37). Although, estrogen may act at any stage of development of atherosclerosis, most studies have concentrated on the effects of estrogen on the early events of atherosclerosis (20). The FS is the earliest visible lesion of atherosclerosis (20). The first step of the development of FS

Table 4: The grade of pathological evaluation for coronary arteries (RCA: Right Coronary Artery; LCA: Left Coronary Artery; AIVA: Anterior Interventricular Artery; PIVA: Posterior Interventricular Artery).

Group	RCA	LCA	AIVA	PIVA
1	1.40 ± 0.24	2.20 ± 0.37	0.80 ± 0.20	1.20 ± 0.37
2	0.83 ± 0.17	2.0 ± 0.0 #	0.33 ± 0.21	0.50 ± 0.22
3	0.33 ± 0.21*	0.17 ± 0.17*	0.0 ± 0.0*	0.0 ± 0.0*

significant difference from group 3 ($p < 0.05$).
* significant difference from groups 1 and 2 ($p < 0.05$).

Figure 1: The accumulation of FS in Coronary Arteries (CA). Estrogen attenuates FS formation in CA of hypercholesterolemic diabetic rabbits. A: Left CA (LCA) of group 1; B: LCA of group 1; C: LCA of group 2; D: Right CA (RCA) of group 2; E: LCA of group 3; F: RCA of group 3.



is lipoprotein transport into the artery wall (38) and estrogen deficiency causes increases in arterial LDL degradation and accumulation at all arterial sites, especially in coronary arteries (39). Estrogen may also inhibit atherogenesis by decreasing adhesion of monocytes to endothelial cells, which is important in formation of FS (1) either by directly inhibiting vascular cell adhesion molecule-1 expression (40) or by increasing nitric oxide synthesis by endothelial cells (41) or both. Therefore, it seems that estrogen is responsible for the histological changes in coronary arteries of groups 2 and 3.

CONCLUSION

This study demonstrated that estrogen has a beneficial role in histological changes of coronary arteries and can attenuate FS formation in hypercholesterolemic diabetic animals.

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Correspondence:
M. Khazaei
No:23, Modarress 22,
Birjand, Khorasan Jonoobi,
IRAN.
e-mail: majkhazaei@yahoo.com