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Brief Report

Gender Difference in Endothelial Permeability of Aorta in Rabbits Consuming Normal or High-Cholesterol Diets

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Abstract

The endothelial permeability of aorta (EPA) is influenced by atherosclerosis. Whether the physiological difference in EPA is a sex-dependent phenomenon remains to be determined. The objective of this study was to determine the EPA difference between male and female rabbits. An experimental model was designed to obtain 4 groups of animals. The ovariectomized rabbits were fed with normal (group I) and 1% cholesterol-rich diets (group III). The male rabbits also received normal (group II) and cholesterol-rich diets (group IV). After 5 wks, the EPA was measured by Evan Blue (EB) dye uptake method. The EB uptake was significantly ($p<0.05$) lower in females, regardless of the diet they used. In groups I to IV, the mean±SD EB uptake was 4.83±2.06, 15.15±2.91, 7.01±2.40, and 20.58±3.62 μg/g weight of aorta, respectively. The lower EPA in females may play a role in reducing the risk of development of atherosclerosis.

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Keywords • permeability • aorta •sex

Due to the presence of estrogen, the risk of atherosclerosis and coronary artery disease in females is less than males[1-7] Atherosclerosis, itself, increases the vascular endothelial permeability.[8] It was also shown that the endothelial permeability of aorta (EPA) is increased in cholesterol-fed animals.[9] Estrogen, on the other hand, attenuates EPA and aorta fatty streaks in high cholesterol-fed male animals.[10] Collis *et al*, have demonstrated that estrogen can attenuate acetylcholine-induced coronary arterial constriction in women with coronary artery disease, but could not show such an effect in men's coronary artery.[11] Estrogen-related vaso-relaxation is similar in aorta of both genders[12.] The feedback differences in males and females are mostly estrogen-dependent.[13,14] Regardless of the effect of estrogen on atherosclerosis and coronary artery disease process; a question to be addressed is whether EPA is originally and physiologically different in males and females. The purpose of the present study is therefore, to determine the EPA in animals with different sex and diets.

Nineteen white rabbits (10 males and 9 ovariectomized females) weighing 1-2 kg were obtained from the Pasteur Institute of Iran. The ovariectomized rabbits were chosen to avoid the probable role of estrogen on vessel permeability.

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Table 1: Mean ± SD endothelial permeability of aorta (μg/gram weight of tissue) in male and female rabbits on different diets.

Diet	Male	Female*
Normal	15.15±2.91	4.83±2.06
Cholesterol-rich	20.58±3.62	7.01±2.40

*Significantly ($p<0.05$) different from that of males

After one week of habituation to the laboratory, they were divided into four groups. The groups were fed for five wks according to the following schedule: Group I (n=5); ovariectomized animals on normal diet. Group II (n=5); male rabbits on normal diet. Group III (n=4): ovariectomized animals on diet enriched with 1% cholesterol. And, group IV (n=5); male rabbits on diet enriched with 1% cholesterol.

Cholesterol-enriched diet was prepared by adding 1 g of pure cholesterol (Merck, Germany) to 4 ml of olive oil per 0.1 kg of commercial food. To rule out the effect of olive oil, normal diet supplemented with 4 ml olive oil per 1 kg of rabbit diet was used.

The Evans Blue (EB) method described elsewhere,[9,10] was used to measure EPA. Briefly, after an overnight fasting, the 10 mg/kg of EB solution was injected into the vein of the ear. After three hrs, the animals were anesthetized using 50 mg/kg of sodium pentobarbital and sacrificed. The whole aorta was removed, cleansed and washed with saline for two hrs. To extract the EB from the tissue, the aorta was kept in 5 ml of formamide at 80 °C for two hrs. After standing overnight, the concentration of EB (μg/g weight of tissue) in formamide solution was determined photometrically at a wavelength of 623 nm using a standard curve.

The EPA (the EB uptake) measured in the four groups of experimental animals are shown in Table 1. Clearly, the EPA increased significantly ($p<0.05$) by a diet rich in cholesterol. Moreover, regardless of the diet consumed, EPA was significantly ($p<0.05$) lower in female than male animals.

Two aspects should be considered: Firstly, high cholesterol diet will increase the EPA which is proven to be due to the aggregation of fatty dots and fatty streaks in aortic wall.[9,10] Secondly, atherosclerosis is characterized by increasing EPA, and therefore, the physiological advantage of less EPA may explain the low risk for aortic atherosclerosis in females. At the present time, the reason for the difference in EPA between males and females is not clearly understood. Enhanced pinocytotic activity of the endothelial cells and opening of inter-endothelial tight-junctions may account for the increasing EPA in males.[15] In fact, besides the protective effects of estrogen in reducing the chance of development of coronary artery disease,[1,4,5,16,17] the low EPA, per se, plays an important role in prevention of the disease in females.

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