EXPERIMENTAL HYPERLIPIDEMIA AND ATHEROSCLEROSIS INDUCED BY CHOLESTEROL DIET IN SPF JAPANESE WHITE RABBITS

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Abstract—Experimental hyperlipidemia and atherosclerosis induced by a cholesterol diet in SPF male and female rabbits (JW/KBL) were investigated by the determination of the lipid contents of the plasma, liver and thoracic aorta; determination of morphological changes of the aortic arch by head angiography; and computer tomography of the brain. Rabbits were fed the diet that contained 1% cholesterol for eight weeks. The plasma lipid levels began to rise from two weeks after the cholesterol diet was started, reached the peak four to six weeks later, and then fell in both males and females at eight weeks. The cholesterol of the high density lipoprotein in male rabbit plasma was slightly increased by the cholesterol diet, but not in female rabbits. An increase in the total cholesterol (TC) and triglyceride contents of the liver and an increase in the TC and phospholipid contents of the thoracic aorta were observed at the eighth week. Histological examination of the aortic arch showed marked lipid vacuoles under the endothelial cells, noticeable lipid inclusions in the smooth muscle cells of the intima and granular prominences on the internal surface of the aorta. Head angiography of rabbits fed the cholesterol diet revealed a constriction of the lumina of several arteries due to the lipid depositions. These results suggest that hyperlipidemia and atherosclerosis can be produced at the eighth week using SPF rabbits fed on a cholesterol diet.

Since Anitschkow's first report (1), experimental hyperlipidemia and atherosclerosis in rabbits induced by cholesterol diets have been widely investigated (2–7). The variation of serum lipid levels on hyperlipidemia in rabbits induced by cholesterol diets were reported by Kato (8), Nishimura (9) and others (5–7). The morphology of arterial lesions in this animal model have been studied using a light microscope (LM) (10), a transmission electron microscope (TEM) (2) and a scanning electron microscope (SEM) (3, 4, 8, 9, 11). In recent studies, the importance of hyperlipidemia and atherosclerosis in animals as experimental models for human disease was reevaluated because of the increase in ischemic disease in humans and the new findings about the relationship between atherosclerosis and lipid metabolism in the body. Especially, the role of high density lipoprotein (HDL) on cholesterol metabolism in the blood and the aorta has attracted interest as a protective factor in atherosclerosis (12). We reported that the variation of HDL and other lipids affected hyperlipidemia in rats induced by cholesterol diet (13). In the present report, variation of lipid levels in plasma, liver and aorta and morphological observations in SPF Japanese white rabbits (JW/KBL) induced by a cholesterol diet are described.
Materials and Methods

Male and female rabbits of the SPF JW/KBL strain of the Kitayama Laboratories (Ina, Japan), aged two months and weighing 1.8-2.2 kg, were used. Both sexes were separately divided into two groups, control and treatment, each group consisting of seven to eight rabbits. The control group was fed a ground diet (RC-5, Nippon Clea Co. Ltd., Tokyo, Japan). The treatment group was fed laboratory chow pellets of a cholesterol diet containing 1% cholesterol in the ground diet.

The plasma lipid levels in rabbits fed on the ground and cholesterol diets were examined every two weeks for eight weeks. Rabbits were sacrificed at the end of eight weeks. The lipid contents of the liver and the thoracic aorta were determined. Lipid fractions of the liver and aorta were extracted by the method of Bragdon (14). Total lipids (TL) in plasma, liver, and aorta were determined by the method of Bragdon (14). Total cholesterol (TC) in plasma, liver, and aorta was determined by the modified method of Rosenthal (15). Phospholipid (PL) in plasma was determined by the method of enzymatic spectrophotometry (16). PL in the liver and aorta was determined by the method of Wako (17). Triglyceride (TG) in the plasma and liver was determined by the method of enzymatic spectrophotometry (18). Free cholesterol (FC) in the plasma was determined by the method of enzymatic spectrophotometry (19). HDL-cholesterol (HDL-C) in the plasma was separated by the method of dextran sulfate-magnesium precipitation (20) and determined by the method of enzymatic spectrophotometry (19).

Morphological observations examined only male rabbits that were fed on the ground and cholesterol diets. Macroscopical changes in the aorta were examined using Sudan III stain, and microscopical observation of the aortic arch using LM was performed with hematoxylineosin, Sudan III and Azan-Mallory's stain. The fine structure of the aortic arch was examined using the TEM according to the following procedure: The aortic arch was removed and placed in 2% glutaraldehyde. Small sections of these samples were post-fixed in 1% osmium tetroxide and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and observed with the TEM (Hitachi 7A, Japan). The internal surface of the aortic arch using SEM was examined by the following procedure: Small cross-sections of the aortic arch after fixing in 2% glutaraldehyde and 1% osmium tetroxide were dehydrated in acetone. Dehydrated sections of these samples were dried by the rapid critical point drying method. Then these sections were observed with the SEM (JE-Nippon Denshi, Japan) after coating with platinum in vacuo. An angiograph of the head was by injecting of the contrast medium diatrizoate sodium into the carotid artery under pento-barbital (30 mg/kg, i.v.) anesthesia. CT of the brain was performed using the CT scanner (Hitachi CT-H, Japan) under anesthesia.

Results

Variation of plasma lipid levels: Figures 1-3 show the changes with time of the plasma lipid levels in male and female rabbits fed a cholesterol diet for eight weeks. The TL and TC levels in male rabbits reached a peak six weeks after the feeding of the cholesterol diet and dropped in both at eight weeks. In female rabbits, plasma TL and TC were elevated earlier than in the males and reached peak levels after four weeks of treatment (Fig. 1). HDL-C in the plasma of male rabbits was slightly increased by the feeding of a cholesterol diet, but not in female rabbits (Fig. 2). The HDL-C levels in the plasma of normal female rabbits was about 1.5 times higher than in the males (Fig. 2). The FC levels in
plasma reached their maximum at two to six weeks in the males and at four weeks in the females, and then decreased in both groups. TG and PL levels in the plasma of male rabbits peaked after four weeks of treatment, but in females, at six weeks (Fig. 3).

Liver and aorta lipid contents: Figure 4 shows the lipid contents of the liver and
thoracic aorta in rabbits fed a cholesterol diet for eight weeks. In liver lipids, the TL content of normal female rabbits was significantly lower than that of the males. TL and TC in both male and female rabbits fed a cholesterol diet significantly increased against the normal, but TG increased only in the females. In thoracic aorta lipids, the TC and PL contents significantly increased in both male and female rabbits fed the cholesterol diet.

![Plasma-HDL-C](image1.png)

**Plasma-HDL-C**

![Plasma-FC](image2.png)

**Plasma-FC**

**Fig. 2.** Changes in cholesterol in plasma high density lipoprotein and plasma free cholesterol in rabbits fed a cholesterol diet. Conditions are the same as Fig. 1. HDL-C: cholesterol in high density lipoprotein, FC: free cholesterol. *: Significantly different from the control (P<0.01), †: Significantly different from the male rabbit control (P<0.01).
Morphological examinations: Abnormal posture was seen in male rabbits on the cholesterol diet (Fig. 5). Macrographs of the thoracic aorta revealed marked deposition of lipids on the lumen surface in animals on a cholesterol diet (Fig. 6). Light microscopy of the aortic arches of male rabbits on a cholesterol diet showed thick fatty streaks in the intima. Disturbances of arrangement in the elastica of the tunica media accompanied the

![Graph](image)

**Fig. 3.** Changes in plasma triglyceride and phospholipid in rabbits fed a cholesterol diet. Conditions are the same as Fig. 1. TG: triglyceride, PL: phospholipid. *: Significantly different from the control (P<0.001), t: Significantly different from the male rabbit control (P<0.001).
cholesterol diet (Fig. 7). In the fine structure of the aortic arch in male rabbits fed a normal diet, the TEM showed that the intima was formed by endothelial cells and that crescentic internal elastic laminae and smooth muscle cells in the internal elastic lamina lie between the endothelial cells and the elastica of the tunica media (Fig. 8a). In contrast, the intimal structure of the aortic arch of male rabbits fed a cholesterol diet showed

Fig. 4. Changes of lipids in liver and thoracic aorta in rabbits fed a cholesterol diet. Each column represents the mean±S.E. of five rabbits. * and **: Significantly different from the control at P<0.01 and P<0.001, respectively. †: Significantly different from the male rabbit control (P<0.001).
Fig. 5. Abnormal posture in male rabbits fed a cholesterol diet for 8 weeks.

Fig. 6. Macrograph of the thoracic aorta in male rabbits fed a cholesterol diet for 8 weeks. Sudan III stain.

Fig. 7. Micrograph of the thoracic aorta in male rabbits fed a cholesterol diet for 8 weeks. Azan-Mallory's stain. ×80

Fig. 8. Transmission electron micrograph of the aortic arch in normal and cholesterol diet-fed male rabbits for 8 weeks. a: normal, b: cholesterol diet-fed, CF: collagen fiber, ec: endothelial cell, EF: elastic fiber, L: lumen, smc: smooth muscle cell, fc: foam cell.
predominantly foam cells, frequently including large vacuoles. Endothelial cells were pressed aside by swelling foam cells and had altered nuclei (Fig. 8b).

When the internal surface of the aortic arch in male rabbits fed a normal diet was observed under the SEM, the endothelial cells were arranged along the longitudinal axis of the aorta, and slightly swollen, elliptical nuclei were present in their midsections (Fig. 9a). On the other hand, the internal surface of the aortic arch of rabbits fed on the cholesterol diet showed fissures in the endothelial cell boundaries, expansion of the endothelial nuclei and an appearance of granular prominences (Fig. 9b).

Head angiographs in rabbits fed on the cholesterol diet showed reduction in the caliber of the maxillary, lingual and facial arteries, but basilar arteries and the arterial circle were not revealed by the contrast medium (Figs. 10, 11). Brain CT showed areas of low density in rabbits fed on the cholesterol diet (Fig. 12).

Discussion

Kato (8), in his description of cholesterol-cotton seed oil-induced hyperlipidemia in rabbits, indicated that TL, TC, TG, PL and \( \beta \)-lipoprotein levels in the serum lipids were markedly increased and reached peak levels from the eighth to the tenth week of treatment; and TC, PL and \( \beta \)-lipoprotein continued at
their maximal levels. Nishimura (9), in a description of cholesterol-induced hyperlipidemia and atherosclerosis in rabbits, proved that serum lipid levels reached a peak after ten to twelve weeks of treatment and then decreased.

The plasma lipid levels in this study reached a peak after four to six weeks of treatment with a cholesterol diet. This indicates that hyperlipidemia induced by the cholesterol diet in SPF rabbits occurs earlier than that in mongrel rabbits.

In recent studies, interest has been shown to the HDL level in the blood as a protective factor in atherosclerosis in experimental animals and humans. In the previous paper (13), we reported that hyperlipidemia in rats induced by a cholesterol diet decreased the content of HDL-C in serum, but nevertheless, lipid deposition were not seen in the aorta. In male SPF rabbits, the HDL-C levels in the plasma on the feeding of a cholesterol diet were slightly increased, but not in females. Some roles of the anti-atherosclerotic effect of HDL have been recognized. HDL may modulate the uptake of cholesterol-rich low density lipoprotein (LDL) by peripheral tissues (12, 21–23) and function as a vehicle for the transport of cholesterol to the liver (24, 25). HDL may also have a role in the clearance of cholesterol from the arterial wall (26).

Abraham et al. (5) reported that feeding
with a 1% cholesterol resulted in a drastic fall in HDL with a compensatory rise in both LDL and very low density lipoprotein in Leowinstein male rabbits. Tomikawa et al. (7) reported that 0.5% cholesterol diets caused a significant decrease in HDL2 and HDL3 masses, without significant changes in HDL-C values in Japanese albino male rabbits. In our study, it was observed that the change of HDL levels in rabbits fed a cholesterol diet differed from that in rats, but the arterial lesions and increased lipid contents of the aorta were produced only in rabbits. A difference between the rabbit and rat in the development of atherosclerosis may be related to the ratio of HDL in lipoprotein. Indeed, the atherogenic index (TC−HDL-C/HDL-C) in rabbits showed a higher value than in rats.

Morphological findings in the aorta in rabbits fed a cholesterol diet were marked by lipid vacuoles under the endothelial cells under the LM, by noticeable lipid inclusions in the smooth muscle cells of the intima under the TEM, and by the appearance of granular prominences on the internal surface of the aorta under the SEM. These morphological changes in the aorta in SPF rabbits fed a cholesterol diet for eight weeks were more severe those in mongrel rabbits similarly fed for sixteen weeks showing edematous changes of endothelial cells and expansion of the endothelial nuclei (9). Furthermore, the luminal surface of the aortae of Chinchilla rabbits fed cholesterol (200 mg/kg) in sunflower oil for eight months (4) was similar to that of our SPF rabbits fed a cholesterol diet for eight weeks.

From results of CT and head angiography, the abnormal posture in male rabbits fed on a cholesterol diet may be due to damage to the brain by lipid depositions in the basilar artery and the arterial circle.

Experimental atherosclerosis in SPF rabbits induced by the cholesterol diet was produced earlier than in mongrel rabbits. These findings suggest that the SPF Japanese white rabbit (JW/KBL) is an interesting model for both prevention and regression studies on experimental atherosclerosis.

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