

Effects of dietary lard and fish oil on the serum lipid level and aortic tissue : a comparison in quail animal model

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SUMMARY : A total of twenty-five 40-day-old, male, Japanese quails were fed with either basal diet, a diet containing 15% butter fat and 2% cholesterol, or a diet containing 15% fish oil and 2% cholesterol for 3 months. The birds which were fed with the diet containing lard and cholesterol showed marked hypercholesterolemia and severe lipid-rich aortic lesions. In accordance to serum cholesterol level, the lard fed group had numerous fibroblasts with or without lipid droplets in the thickened intima of the ascending aorta. The birds fed with the fish oil and cholesterol had no significant increase in their serum cholesterol level or lipid-rich aortic lesions. These data clearly indicate that fish oil is less atherogenic than lard.

INTRODUCTION

Elevated blood lipids have been statistically established as a risk factor in the development of atherosclerosis (9). Therefore, most of the attempts to regulate blood lipids by diet has been centered on the fat in the diet. It has been reported that diets rich in saturated fatty acids are usually associated with increase in serum cholesterol levels and severe atherosclerosis in man (6) and experimental animals (13,19). On the other hand, it has long been considered that polyunsaturated fatty acids lower cholesterol level, and are anti-atherogenic and essential for the growth and proper nutrition of humans and animals (6). We have been working on experimental atherosclerosis using chicken which is susceptible to atherosclerosis as well as economical than the other animal model (16). In the present study, we

chose Japanese quail as animal model because it can thrive in small laboratory cages and is economical and easy to maintain for long-term experiment (1). The first objective of the present study was to determine the degree of atherosclerosis produced by the atherogenic diet rich in saturated fatty acids. We chose lard as a fat rich in saturated fatty acids. A second objective was to compare the atherogenicity of fish oil and lard because fish oil rich in polyunsaturated fatty acids of n-3 family has been reported to be anti-atherogenic (4,21).

MATERIALS AND METHODS

Twenty-five 40-day-old male Japanese quails were used in this study and divided into three groups as shown in Table 2. One group was fed with a basal diet and the other two groups were fed with the test diets which contain 2%

cholesterol and 15% fat of either lard or fish oil. The birds were allowed ad lib. access to both food and water throughout the three-month experiment. The basal diet (Kyoei Co. Ltd. Okinawa) contained 18% protein, 3.8% fat, 6.3% ash, 3.5% fiber, and 2842 Kcal/Kg. The fatty acid composition of test diets was analyzed by gas-liquid chromatography as described elsewhere (15). Serum cholesterol and triglyceride were determined by the enzymatic methods (TC-S 736, Kyowa Tokyo, and TG-V3 Nishin Shoji Osaka). The hearts and the proximal one cm of the ascending aorta and its large branches were collected. These tissues were fixed in 10% formaldehyde solution, embedded in paraffin, sectioned at 4 micrometer, and stained with hematoxylin and eosin, elastica van Gieson, and Mallory Azan. As previously reported (17), intimal thickening was measured on the representative three to four arteries in each quail using an ocular micrometer. The severity of arteriosclerosis was expressed as the average degree of intimal thickening of the arteries in each group. Small pieces of thoracic aortas were fixed in 3% glutaraldehyde solution for electron microscopic examination. These tiny specimens were post-fixed in phosphate-buffered 1% osmium tetroxide, serially dehydrated in increasing concentrations of ethanol, embedded in spur

resin and sectioned with diamond knife. Ultrathin sections were stained with uranyl acetate, and examined with a JEM 2000EX electron microscope.

RESULTS

Biochemistry : The percent composition of fatty acids in the diet of each experimental group is shown in Table 1. All the diets contained enough amounts of linoleic acid to fulfil the minimum requirement of essential fatty acids (3). Table 2 shows body weight and lipid profile in each experimental group. There was no significant difference in final body weight among all the experimental groups. Serum cholesterol levels in quails on the diet containing lard and cholesterol showed a sharp increase. Serum triglyceride level in the lard and fish oil groups were lower than that in the basal group.

Histology : The control birds showed no lipid-rich arterial lesions (Fig. 1), while the quails fed with the lard-containing diet showed moderate to severe lipid-rich intimal lesions in the ascending and proximal thoracic aortas (Fig. 2). The quails fed with the fish oil-containing diet developed slight intimal thickening in the aortas (Fig. 3). Table 3 shows a comparison of the incidence and degree of

Table 1. Percent fatty acid composition of the diet used in each experimental group

| | 12 : 0 | 14 : 0 | 16 : 0 | 16 : 1 | 18 : 0 | 18 : 1 | 18 : 2 | 18 : 3 | 20 : 4 | 20 : 5 | Sat. | Mono. | Poly. |
|----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|------|-------|-------|
| Control | | | 15.9 | 1.4 | 2.6 | 23.1 | 49.6 | 3.6 | 0.9 | 2.9 | 18.5 | 24.5 | 57.0 |
| Lard | | | 22.8 | 3.6 | 13.8 | 40.5 | 16.5 | 2.0 | 0.3 | 0.4 | 36.6 | 44.1 | 19.2 |
| Fish oil | 6.9 | 12.8 | 11.4 | 1.3 | 16.3 | 11.6 | 4.1 | 1.7 | 33.9 | 21.0 | 27.7 | 51.3 | |

Table 2. Dietary groups, number of quails, body weights and mean serum cholesterol and triglycerides concentrations

| | No. of quail | Final body weight (g) | Serum lipids (mg/dl) | |
|----------|--------------|-----------------------|----------------------|----------|
| | | | TG | TC |
| Control | 8 | 113±6 | 173±27 | 182±14 |
| Lard | 8 | 107±4 | 61±7 | 1027±156 |
| Fish oil | 9 | 105±3 | 39±5 | 261±7 |

TG : tryglyceride TC : total cholesterol
Data are expressed as mean ± standard error

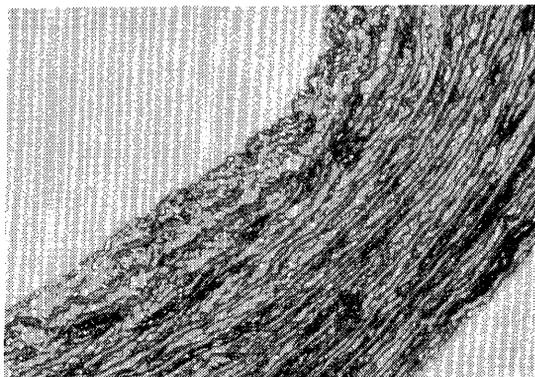


Fig. 1. Aorta from a quail fed with a basal diet. Alternating lamellae of medial smooth muscle cells (SM) are shown (elastica van Gieson $\times 180$).

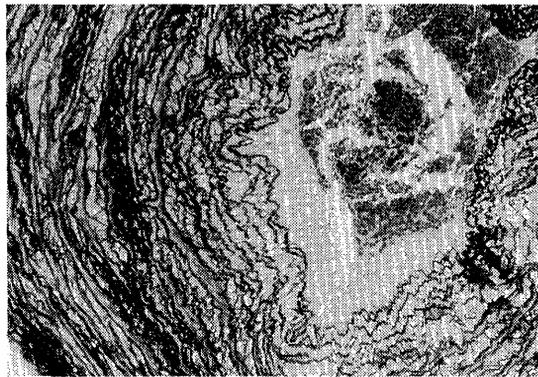


Fig. 3. Photomicrograph of a aorta from a quail fed with a fish oil-containing diet showing slight intimal thickening (elastica van Gieson $\times 180$).

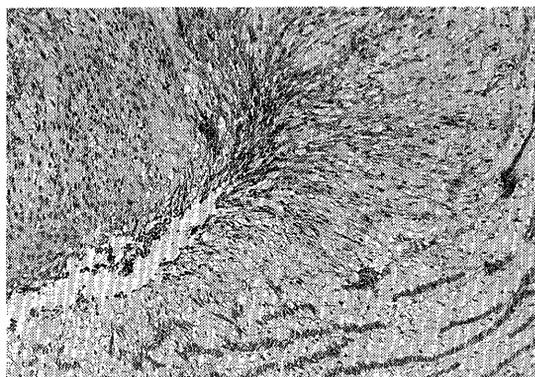


Fig. 2. Photomicrograph of a aorta from a quail fed with a lard-containing diet showing marked intimal thickening (Mallory Azan $\times 180$).

intimal thickening of the ascending aorta and its large branches which were found in birds fed with the control diet, lard-containing diet and fish oil-containing diet. Quails fed with the lard-containing diet had the most fre-

quent and severe intimal thickening of the ascending aorta and its large branches among all the experimental groups.

Electron microscopy : In control quails, the intima consisted of a single layer of elongate endothelial cells. The connective tissue layer beneath the endothelium consisted of a loose reticular network with banded collagen fibers. The media was composed of alternating layer of smooth muscle cells and fibroblastic cells. Intercellular space contained elastic fibers as well as collagen fibers (Fig. 4).

In contrast to the aortas of quails fed with a basal diet, aortas from quails receiving a lard-containing diet had remarkable lipid-rich aortic lesions. The thickened intima was mainly composed of lipid-containing fibroblastic cells (Fig. 5). Deeper portions of the thickened intima contained smooth muscle cells with or without lipid droplets (Fig.6). Smooth muscle cells in the middle layer of the tunica media showed a normal fusiform appearance. Inter-

Table 3. Effects of control, lard-containing diet and fish oil-containing diet on aortic lesions in quails

| Parameter | Groups | | |
|---|---------------|---------------|---------------|
| | Control | Lard | Fish oil |
| Incidence of thickened intima | 5/31 | 9/29 | 4/33 |
| Average of intimal thickness ($\times 10^{-2}$ mm) | 0.8 ± 0.2 | 6.1 ± 1.4 | 1.3 ± 0.4 |

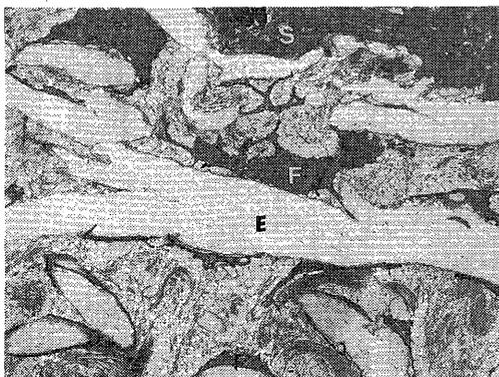


Fig. 4. Fibroblasts (F) and smooth muscle cells (S) are seen in the tunica media ($\times 2,600$). E : elastic fiber.

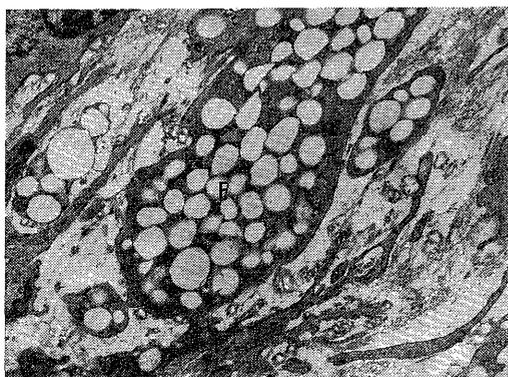


Fig. 5. Lipid-containing fibroblasts (F) are shown. E : elastic fiber ($\times 6,500$).



Fig. 6. Lipid inclusions (I) are more numerous in fibroblasts than in smooth muscle cells (S) ($\times 4,300$).

cellular spaces were slightly widened and contained a small amount of lipid granules.

The lesions observed in quails receiving a fish oil-containing diet were small and mild. The subendothelial space was slightly and focally expanded, and contained a few foam cells and a small amount of extracellular lipid granules.

DISCUSSION

Feeding trial of a diet containing 18% saturated fatty acid-rich lard without cholesterol produced moderate increase in the level of serum cholesterol and the thickness of coronary arterial intima of swine (14). In this experiment, we fed a diet containing 15% lard 2% cholesterol to quails, and found that this diet induces hypercholesterolemia and significant degree of atherosclerotic lesions in the ascending aortic arch, proximal thoracic aorta and their large branches. These results demonstrate that lard enriched with saturated fatty acid is highly atherogenic in quails as observed in other animals (18) and the Japanese quail is as useful as the chicken as an animal model for the study of atherosclerosis (16).

Polyunsaturated fatty acids are categorized into the three major families of unsaturated fatty acids including the oleic acid (n-9) family, the linoleic acid (n-6) family, and the linolenic acid (n-3) family (6). Fish oils contain a large amount of polyunsaturated fatty acids of the n-3 series such as eicosapentaenoic acid (EPA). Many studies have reported various effects upon platelet aggregability (5, 8), blood lipid (12,20), blood pressure (11), and inflammatory or immunological processes (10).

Concerning the effects of fish oils on arteries, several reports of animal experiments (4, 21) have been published. It is speculated that the beneficial, and preventive effect of fish oil to atherosclerosis is achieved through prostaglandin metabolism and platelet function instead of lipid metabolism. In the present study, fish oil fed with group had significantly lower levels of serum cholesterol and less atherosclerosis than the lard fed group. The factors which regulate serum cholesterol level have been known to include : 1) cholesterol absorption ; 2) excretion of neutral and acidic steroids ; 3) cholesterol synthesis ; 4) transfer

of cholesterol from plasma to tissues ; 5) changes in the cholesterol-to-protein ratio in LDL, and 6) changes in membrane fluidity (2, 7). Goodnight *et al.* (6), proposed that changes in the fluidity of lipoproteins and/or cellular plasma membranes are the most promising mechanisms governing the serum cholesterol level. However, the profound hypolipidemic action of fish oil remains as yet unexplained.

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