Effect of Dietary Cholesterol on the Activities of Key Enzymes of Cholesterol Metabolism in Hyperlipidemia- and Atherosclerosis-Prone Japanese Quail

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Summary The hyperlipidemia- and atherosclerosis-prone (HAP) Japanese quail is a strain developed for the study of atherosclerosis by genetic selection from the commercially available (CA) Japanese quail. To delineate the characteristics of cholesterol metabolism in this strain, concentrations of serum lipids as well as hepatic enzyme activities were compared between HAP and CA quail. The hepatic enzymes studied are involved in the key step reaction in cholesterol metabolism: HMG-CoA reductase, ACAT, and cholesterol 7α-hydroxylase. The animals were fed ad libitum with either 1% cholesterol or cholesterol-free semipurified diet for 28 days. Although a significant increase (p<0.01) in serum cholesterol was observed in both strains on elapse of cholesterol feeding, formation of atheroma was seen exclusively in HAP quail of the cholesterol-fed group. The serum and liver cholesterol levels of HAP quail fed the cholesterol diet were significantly higher (p<0.01) than those of CA quail. No significant differences were seen in the rate of cholesterol biosynthesis (HMG-CoA reductase activity), cholesterol ester formation (ACAT activity) and cholesterol catabolism (7α-hydroxylase activity) between CA and HAP quail. Furthermore, the fecal excretions of acidic and neutral sterol showed no significant difference between strains. Although the formation of atheroma in HAP quail may be presumably due to the contribution of the marked increase in serum cholesterol level, the rate of cholesterol catabolism and synthesis in HAP quail compared well with those of CA quail. These observations suggest that the retarded rate of cholesterol biosynthesis or catabolism is not responsible for hypercholesterolemia in HAP quail.

Key Words quail, hypercholesterolemia, HMG-CoA reductase, ACAT, cholesterol 7α-hydroxylase, atherosclerosis

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Atherosclerosis is a disease resulting from multiple complex interactions of hyperlipidemia, dyslipoproteinemia, hypertension, and smoking. Hypercholesterolemia is considered to be one of the main causes of atherosclerosis. Investigators have been using many kinds of animals to study the relationship between hyperlipidemia and atherosclerosis. Since Day et al. and Morrissey et al. used Japanese quail as their animal model for research on atherosclerosis (1,2), this animal has become an experimental model of interest for studies on lipid metabolism or atherosclerosis. The beneficial features of the Japanese quail as an animal model are its small body size, short life cycle and low food consumption, and susceptibility to cholesterol loading. For these reasons, studies on the pathogenesis, prevention and nutritional aspects of atherosclerosis in Japanese quail have been carried out in many laboratories. Moreover, Shih et al. developed a strain of Japanese quail susceptible to atherosclerosis (SUS) (i.e., hyperresponder) by genetic selection from CA quail (3). They reported on aspects of general characterization and histological examination of the selected SUS quail. Radcliffe and Liebsch described in detail the relationship between hypercholesterolemia and atherosclerosis in susceptible to atherosclerosis (SEA) Japanese quail (4). They examined the effects of purified atherogenic diets on the development of hypercholesterolemia, distribution of serum lipoprotein cholesterol, and the development of atherosclerotic lesions (4). They showed that the serum concentrations of cholesterol and very low density lipoprotein (VLDL), plus low density lipoprotein (LDL) cholesterol of the SEA strain, were higher than those quail fed a cholesterol-free basal diet. High density lipoprotein (HDL) cholesterol concentration was unaffected by this dietary treatment.

These studies have obviously improved our knowledge on the lipid metabolism in animal models such as the SUS or SEA quail. It is possible that the hypercholesterolemia in this strain could be due to high intestinal absorption of cholesterol, impaired feedback regulation of endogenous cholesterol, and a low rate of conversion of cholesterol to bile acid. However, no mechanism for the induction of hypercholesterolemia and atherosclerosis in these unique strains has been presented.

Thus, the primary purpose of this study is to clarify the mechanism responsible for hypercholesterolemia in this strain. In order to characterize the lipid metabolism of hyperlipidemia- and atherosclerosis-prone (HAP) quail, we focused on the relationship between lipid concentration in serum and activities of some key enzymes of cholesterol metabolism in the liver: HMG-CoA reductase which is the rate limiting enzyme of cholesterol biosynthesis, acyl-CoA cholesterol acyltransferase (ACAT) which converts cholesterol to cholesterol ester, and cholesterol 7α-hydroxylase which catalyzes the rate limiting step for bile acid biosynthesis. Furthermore, we examined acidic and neutral sterol excretion and cholesterol level deposited in the atherosclerotic plaque, and also conducted histological observations in ascending aorta and its large branches by microscopy in order to gain more insight into atheroma formation.
MATERIALS AND METHODS

**Animals and diets.** Male Japanese quail were purchased from Ryukyu Bio-tech (Okinawa, Japan) and were divided into 2 groups. Male HAP Japanese quail developed by genetic selection from the highly atherosclerosis-susceptible CA quail were kindly supplied by Otsuka Pharmaceutical Co., Ltd., and were also divided into 2 groups. Ryukyu University’s guidelines for the care and use of laboratory animals were followed in this study. Animals were housed individually under controlled room temperature (22±2°C) and lighting cycle (lighting 0800 to 2000 h) conditions. Quail were fed a semipurified diet *ad libitum* for 4 weeks. The diet composition is shown in Table 1. The experimental diet contained 1% cholesterol and the control diet was cholesterol-free. In this study, the diet was not supplemented with cholic acid, as this compound inhibits conversion of cholesterol to bile acid and is considered to be a potential modulator of cholesterol metabolism.

**Preparation of microsomes.** Animals were killed by decapitation after overnight fasting. The liver was removed immediately, and rinsed in ice-cold saline. All of the following manipulations were performed at 4°C. Microsomes were prepared as described previously (5). In short, a portion of liver was homogenated in a 50 mM phosphate buffer containing 250 mM sucrose, 75 mM nicotinamide, 2.5 mM EDTA and 20 mM mercaptoethanol. On completion of centrifugation at 10,000× g for 20 min, microsomes were isolated from supernatant by additional centrifugation at 304,000× g for 1 h, and stored at −80°C. Aliquots of thawed microsomes were used for assays of HMG-CoA reductase, ACAT and 7α-hydroxylase activity. Microsomal protein was measured by the method of Lowry et al. (6).

**Measurement of enzyme activity.** Assays for HMG-CoA reductase, ACAT and cholesterol 7α-hydroxylase were carried out essentially as described elsewhere (5, 7, 8), with minor modifications.

For the assay of HMG-CoA reductase, microsomes were dissolved in 100 mM phosphate buffer containing 20 mM EDTA and 10 mM DTT. Fifty microliter of 200 mM KH₂PO₄ substrate solution containing 50 nmol [4-¹⁴C] cholesterol (5,000 dpm/
nmol), and 2 μM NADPH (pH 7.2) was mixed with 50 μl of microsomal suspension (0.25–0.5 mg protein) to start the enzyme reaction. After incubation at 37°C for 30 min, the reaction was terminated by the addition of 50 μl of 6 N HCl. An aliquot of the reaction mixture was applied to a silica gel G plate and the radioactivity of [4-14C]mevalonolactone was measured by Hewlett-Packard scintillation spectrometer.

The ACAT activity was determined by the formation of labeled cholesterol ester from [1-14C]oleoyl-CoA. In a standard assay, 0.48 ml of potassium phosphate buffer (0.2 M, pH 7.4) containing 2.5 mg of bovine serum albumin and about 300 μg of microsome protein was preincubated at 37°C for 5 min. The esterification of cholesterol was initiated by the addition of 17 nmol of [1-14C]oleoyl-CoA (11,658 dpm/nmol) in 20 μl of sodium acetate buffer, and the final incubation volume of 0.5 ml incubated at 37°C for 10 min. The reaction was terminated by addition of 5 ml of chloroform-methanol (2:1, v/v) and the products were extracted by the method of Folch et al. (9). The lipid extracts were dissolved in a small volume of hexane and applied to silica gel G thin-layer plates, that were developed with petroleum ether-diethylether-glacial acetic acid 85:15:3 (by vol). The separated lipid fractions were visualized with iodine vapor. The area containing cholesterol ester was scraped into a counting vial containing 10 ml toluene scintillation fluid, and the radioactivity was measured by a Hewlett-Packard scintillation spectrometer.

For the assay of cholesterol 7α-hydroxylase, reversed-phase high-performance liquid chromatography assay was used (8). Briefly, the microsome pellets were dissolved in 100 mM of KH2PO4 (pH = 7.4) containing 1 mM EDTA, 50 mM NaF, 5 mM DTT and 0.015% CHAPS. After the addition of 10 mM cholesterol, samples were preincubated at 37°C for 15 min. The reaction was initiated by the addition of 100 μl of 10 mM NADPH, and continued for 15 min at 37°C. The reaction was terminated by the addition of 30 μl of 20% sodium cholate. To this reaction mixture, was added 40 μl of cholesterol oxidase to convert 7α-hydroxycholesterol to 7α-hydroxy-4-cholesten-3-one (7α-HCO) and incubated for 10 min at 37°C. On addition of ethanol, the reaction products were extracted 3 times with petroleum ether at 37°C. The extracts were dried under N2 gas, and then dissolved in 40 μl of acetonitrile-methanol (7:3, v/v). Ten microliter of extract was applied to a reversed-phase column (4.6 × 25 cm, 5 μm, Soken Chemical Co., Japan), equilibrated with acetonitrile-methanol (7:3, v/v), and eluted with the same solvent at a flow rate of 1.0 ml/min. Absorbance of the products were monitored at 240 nm. Identification of the reaction products was done by comparison of their retention times with those of authentic samples (7α-hydroxy cholesterol: Steraloids, Wilton, NH, USA). The amount of 7α-HCO is estimated in nanomoles based on a calibration curve constructed with the known concentration of the standard (from 0.01 to 20 nmol).

**Lipoprotein isolation.** Fractionation by cumulative floatation was adopted for the preparation of lipoproteins. Density cutoff for the separation of lipoproteins of normal Japanese quail applies in the present study (10), and were 1.006, 1.02, 1.08...
and 1.21 (g/ml) for separation of chylomicron, VLDL, LDL and HDL, respectively.

**Lipid and lipoprotein analyses.** Serum and lipoprotein cholesterol, free cholesterol, and triglyceride, were analyzed enzymatically using the Cholesterol E-test WAKO, Free Cholesterol test WAKO and Triglyceride E-test WAKO (Wako Pure Chemicals Co., Ltd, Osaka, Japan), respectively. The liver lipids were extracted with chloroform-methanol (2:1, v/v). Cholesterol and triglyceride levels were assayed, as described elsewhere (11).

Aorta was hydrolyzed by 70% ethanol containing 15% KOH, and the nonsaponifiable lipids were extracted with petroleum ether. The concentration of aorta cholesterol was measured by the method previously described (12).

**Analysis of neutral and acidic sterols.** Fecal neutral sterols were extracted with ethanol at 50°C for 2 h. Trimethylsilyl-derivatives of neutral sterols were analyzed by gas-liquid chromatography, as previously described (11).

Total fecal bile acids were extracted with ethanol at 50°C for 2 h, and analyzed enzymatically by the method of Eaton et al. with slight modification. The standard used for this assay was cholic acid (13).

**Histological examination.** The aorta and its large branches with the heart was obtained from each quail, fixed with 10% formaldehyde solution containing phosphate buffered saline (pH=7.4), and embedded in paraffin. Next, these paraffin-embedded arterial specimens were cut into thicknesses of 4 μm, and stained by hematoxylin eosin (H.E.) and elastica van Giesson (E.V.) (14). Intimal thicknesses were measured from three or four representative arteries in each quail including the ascending aorta and its large branches, using an ocular micrometer. The atherosclerotic score was expressed as the average degree of intimal thickness.

**Statistical analysis.** Data are presented as M±SEM (standard error of the mean). The statistical significance of the difference was evaluated by two-way analysis of variance (15).

**RESULTS**

**Growth and lipid profiles of HAP quail**

Final body weight, relative liver weight and food consumption are shown in Table 2. No significant differences were observed in final body weight and food consumption between groups at the end of the feeding period. The liver weight per 100 g body weight was significantly increased by cholesterol feeding in CA and HAP quail (p<0.01). Growth parameters in HAP quail were similar to those of CA quail. These observations also roughly agree with the results reported by Radcliffe and Liebsch (4).

Lipid profiles of serum, liver and microsome are shown in Table 3. Supplementation of cholesterol increased the serum and hepatic cholesterol levels in both strains. Cholesterol levels in serum and liver of HAP quail that were fed the cholesterol diet were significantly (p<0.01) higher than those of CA quail. Thus,
Table 2. Final body weight, liver weight and food intake.

<table>
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<tr>
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<th>CA (−)</th>
<th>CA (+)</th>
<th>HAP (−)</th>
<th>HAP (+)</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>95.7±2.6</td>
<td>98.8±4.2</td>
<td>82.8±4.3</td>
<td>86.4±5.0</td>
</tr>
<tr>
<td>Liver weight (g/100 g B.W.)</td>
<td>1.5±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.4±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.3±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>11.0±0.3</td>
<td>12.4±1.0</td>
<td>8.9±0.5</td>
<td>8.9±0.3</td>
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Data are expressed as M±SEM of 8–10 quail.

Same superscript letters show significant difference at p<0.01.


Table 3. Lipid concentrations of serum, liver and microsome.

<table>
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<tr>
<th></th>
<th>CA (−)</th>
<th>CA (+)</th>
<th>HAP (−)</th>
<th>HAP (+)</th>
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<tr>
<td>Serum (mg/dl)</td>
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<tr>
<td>Total cholesterol</td>
<td>140±9.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1799±7.8&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>371±16.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2956±44.6&lt;sup&gt;BA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>74.5±4.0</td>
<td>163±30</td>
<td>86.7±11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>271±72&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(Chylomicron+VLDL+LDL)-cholesterol</td>
<td>81.2±3.9&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>1680±5.4&lt;sup&gt;BB&lt;/sup&gt;</td>
<td>239±6.6&lt;sup&gt;BA&lt;/sup&gt;</td>
<td>2857±32&lt;sup&gt;BB&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>59.0±2.4&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>119±4.6&lt;sup&gt;BB&lt;/sup&gt;</td>
<td>132±4.5&lt;sup&gt;BA&lt;/sup&gt;</td>
<td>99.2±4.3&lt;sup&gt;BA&lt;/sup&gt;</td>
</tr>
<tr>
<td>(Chylomicron+VLDL+LDL)/HDL</td>
<td>1.4±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.1±0.6&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>1.8±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.0±1.4&lt;sup&gt;bA&lt;/sup&gt;</td>
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<tr>
<td>Liver (mg/g)</td>
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<tr>
<td>Cholesterol</td>
<td>2.4±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.0±6.3&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>2.9±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.9±7.3&lt;sup&gt;BA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>6.1±0.8</td>
<td>5.9±0.8</td>
<td>16.9±10</td>
<td>10.3±1.9</td>
</tr>
<tr>
<td>Microsome (mg/mg prot.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>86.7±13.8</td>
<td>140±30</td>
<td>96.9±17.1</td>
<td>83.3±16.0</td>
</tr>
</tbody>
</table>

Data are expressed as M±SEM of 8–10 quail.

Same superscript capital and small letters show significant difference between strain and diet at p<0.01.


we observed notable hypercholesterolemia and accumulation of cholesterol in the liver of HAP quail in comparison with that of CA quail. These observations were consistently seen throughout this investigation.

The ratio of (chylomicron+VLDL+LDL)-cholesterol to HDL-cholesterol (atherosclerosis index) increased with feeding of cholesterol in both strains: from 1.83 to 29.0 (p<0.01) in HAP quail, and from 1.40 to 14.1 (p<0.01) in CA quail, respectively. Low density fraction (d=1.006–1.080) cholesterol levels of HAP quail fed the cholesterol diet were significantly higher (p<0.01) than those of CA quail. Although HDL cholesterol levels were significantly different statistically with respect to strain and diet, cholesterol levels of cholesterol-fed CA quail were almost the same as those of cholesterol-fed HAP quail. Thus, we confirmed that the HAP quail strain used in this study was a good experimental model for atherosclerosis study.
Fig. 1. The ascending aorta and large branches from HAP Japanese quail. (A): Control diet-fed group. (B): Cholesterol diet-fed group. (×145) Intimal thickness observed in quail fed the cholesterol diet.
Formation of lipid deposition and atheroma in the artery

Histological examination revealed that the ascending aorta and its large branches of CA quail fed either the cholesterol-free or cholesterol diet showed no significant differences in morphology and intimal thickening (Fig. 1A). The tunica media of the HAP quail artery was composed of an alternating layer of foamy cells, smooth muscle cells and fibroblastic cells. Arteries from HAP quail fed the cholesterol diet revealed more lipid rich intimal thickening of varying degrees between individual quail compared to the arteries of CA quail (Fig. 1B and Fig. 2).

Table 4 shows the concentrations of total cholesterol in the abdominal aorta. As most of the cholesterol that accumulates in arteries is esterified cholesterol, the concentration of total cholesterol was used as the indication of cholesterol ester level. Under the conditions where the animals were fed the atherogenic diet, the arteries of HAP quail (3.22 µg/mm²) manifested a higher cholesterol concentration (\( p < 0.01 \)) compared with normal quail (1.48 µg/mm²). The increased concentra-

![Fig. 2. Relationship between serum cholesterol and arterial intimal thickness for male Japanese quail of both strains fed either the control or experimental diet for 4 weeks. ○: CA quail fed the cholesterol-free diet, ●: CA quail fed the 1% cholesterol diet, □: HAP quail fed the cholesterol-free diet, ■: HAP quail fed the 1% cholesterol diet.](image)

Table 4. Cholesterol concentration in the artery.

<table>
<thead>
<tr>
<th></th>
<th>CA (–)</th>
<th>CA (+)</th>
<th>HAP (–)</th>
<th>HAP (+)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1.41±0.08</td>
<td>1.48±0.05(^a)</td>
<td>1.55±0.19(^a)</td>
<td>3.22±0.48(^aa)</td>
</tr>
</tbody>
</table>

M±SEM of 8–10 quail.
Same superscript capital and small letters show significant difference between strain and diet at \( p < 0.01 \).
(–): Cholesterol-free diet, (+): Cholesterol diet.

tion of cholesterol in the artery walls of HAP quail may reflect higher levels of this compound in the circulation.

The relationship between serum cholesterol level and aorta intimal thickness is shown in Fig. 2. The marked increase in serum cholesterol levels observed in HAP quail resulted in the formation of aortic atherosclerotic lesions. From these data, it seems likely that the uptake of cholesterol by macrophage cells into endothelial cells, contributes to the thickening of aorta. Therefore, it seems possible that the

![Liver microsomal enzyme activity](image)

Fig. 3. Liver microsomal enzyme activity. (A): HMG-CoA reductase. (B): Acyl-CoA cholesterol acyltransferase (ACAT). (C): Cholesterol 7α-hydroxylase. Same letters show statistically significant difference at $p<0.01$. 

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serum cholesterol level is the most important factor in atherosclerosis formation in HAP quail.

**Hepatic enzyme activities**

Figure 3 shows the enzyme activities of HMG-CoA reductase (A), ACAT (B) and cholesterol 7α-hydroxylase (C) in quail fed either a 1% cholesterol or cholesterol-free diet. The activity of HMG-CoA reductase, decreased from 1.19 to 0.35 in CA quail and from 1.68 to 0.29 (pmol/min/mg protein) in HAP quail after cholesterol feeding (Fig. 3A). These observations indicate that the addition of cholesterol to the diet causes feedback inhibition of enzymes in both strains. The activity of ACAT tended to increase in both cholesterol-fed quail groups (from 25 to 134 and from 33 to 130 (pmol/min/mg protein), respectively) (Fig. 3B). ACAT is the enzyme responsible for catalyzing ester formation. Cholesterol ester is a well-known atherogenic lipid (16). Supplementation of the diet with cholesterol predictably led to increased formation of cholesterol ester, and hence increased ACAT activity. No significant difference in cholesterol 7α-hydroxylase activity was seen between HAP and CA strains; cholesterol feeding increased this enzyme's activity in CA quail from 6.7 to 10.9 (pmol/min/mg protein) and in HAP quail from 11.6 to 17.0 (pmol/min/mg protein) (Fig. 3C).

Total cholesterol concentrations of microsomes used in the enzyme assay were roughly comparable between dietary groups (Table 3).

**Excretions of acidic and neutral sterols in quail feces**

Table 5 lists the fecal excretions of acidic and neutral sterols and apparent cholesterol absorption. Cholesterol feeding significantly increased the excretion of neutral sterol ($p < 0.01$), but not excretion of acidic sterol. Excretion of bile acids slightly increased from 0.9 to 1.34 (mg/g wet feces) in CA and from 0.32 to 1.19 in HAP quail.

Apparent cholesterol absorption of HAP quail compares well with that of CA quail, indicating the similar input-output of cholesterol between the animal strains.

**Table 5. Excretion of neutral and acidic sterols into feces and apparent cholesterol absorption.**

<table>
<thead>
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<th>CA (−)</th>
<th>CA (+)</th>
<th>HAP (−)</th>
<th>HAP (+)</th>
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<tr>
<td>Neutral sterol (mg/g wet feces)</td>
<td>1.14±0.15a</td>
<td>7.77±1.4a</td>
<td>0.67±0.11b</td>
<td>7.49±0.81b</td>
</tr>
<tr>
<td>Acidic sterol (mg/g wet feces)</td>
<td>0.90±0.15</td>
<td>1.34±0.47</td>
<td>0.32±0.09</td>
<td>1.19±0.46</td>
</tr>
<tr>
<td>Apparent absorption (%)</td>
<td>61.3±5.4</td>
<td>62.5±4.1</td>
<td></td>
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</table>

M±SEM of 9–10 quail per group.

Same superscript letters show significant difference at $p < 0.01$.


*a Calculated as follows; [((cholesterol intake-fecal cholesterol)/cholesterol intake) × 100.

Absorption values were 61.3±5.4 for CA and 62.5±4.1 (%) for HAP quail. This shows that about 60% of the dietary cholesterol was absorbed in both strains.

**DISCUSSION**

This study was designed to address the causes of hyperlipidemia and atherosclerosis in the HAP Japanese quail. We were specifically interested in the liver microsomal enzymes which play a critical role in cholesterol metabolism.

Consistent with previous observations (17), HAP quail manifested considerably increased lipid levels in the present study and developed atherosclerosis rapidly in response to feeding of dietary cholesterol, compared with CA quail. The extent of serum level increase appeared to be rather more pronounced in our animal model than in the previous quail model (4). This feature may potentiate the usefulness of our animal model in the study of cholesterol metabolism. It is, however, essential to isolate the metabolic defect in this strain for the stringent study of cholesterol metabolism. In humans, it is well known that familial hypercholesterolemia is one of the most commonly inherited disorders. It is caused by an abnormality in the receptor for LDL and not by the alteration of enzyme activities.

Hypercholesterolemia has been known to be induced in several animal models such as rabbit (18), monkey (19), rat (20), pigeon (21) and chicken (22) by dietary cholesterol feeding. Impaired cholesterol metabolism explains the reason for hypercholesterolemia in some of these models. Therefore, it is implicated that impaired excretion or catabolism of cholesterol may be responsible in hyperresponsive quail (HAP). In this context, it is noteworthy that hyperresponsive rabbits showed a decrease in bile acid excretion after cholesterol feeding (23). Lofland et al. also observed a similar change in bile acid excretion and higher cholesterol absorption in the squirrel monkey (24). It has also been suggested that the activity of 7α-hydroxylase is considerably lower in hyperresponsive pigeons than in hyporesponsive ones, which reasonably explains the hypercholesterolemia in this strain (25).

However, this view, is not applicable to the HAP quail. The activities of key enzymes in cholesterol metabolism showed no differences between the animal strains, even under atherogenic conditions.

With cholesterol feeding, HMG-CoA reductase activities of CA and HAP quail were predictably down-regulated to the maximal extent. Thus, it is probably true that the major part of serum cholesterol is derived from exogenous supply rather than from endogenous supply by biosynthesis.

The level of ACAT activity is normally dependent on the availability of the cholesterol substrate. In our study, there was actually a good correlation between ACAT activity and microsomal cholesterol content. The enzyme reaction was performed in the presence of excess cholesterol substrate. Thus, the cholesterol content of the microsome had a reasonably negligible effect on enzyme activity. On feeding of the cholesterol-free diet, ACAT activity of HAP quail was slightly higher.
than that of CA quail, with a similar proportion of esterified cholesterol in serum (data not shown). Thus, it seems unlikely that alteration of ACAT activity could influence the proportion of serum cholesterol ester in quail.

Further studies are needed to address this defect of lipid metabolism or catabolism. In this context, the study of cholesterol clearance mediated by LDL or B/E receptors and scavenger pathway by macrophage has become of interest.

According to previous studies (26), cellular accumulation of cholesterol affected the activities of apoprotein receptors B and E. Decreased activity of B/E receptors and an increased rate of lipoprotein cholesterol secretion could account for the rapid accumulation of cholesterol in the blood of hyperresponsive models. It is now an accepted view that apo B-100 is involved in the receptor-mediated incorporation of LDL. We observed the presence of an apolipoprotein corresponding to chicken apo B-100 in the low density lipoprotein fraction ($1.006 < d < 1.08$) of cholesterol-fed quail. In addition, the incubation of LDL fractions with quail liver parenchymal cells resulted in the accumulation of cholesterol in cells (data not shown). From these observations, it seems reasonable to assume that receptor-mediated cholesterol metabolism also occurs in the liver parenchymal cells of quail.

In conclusion, our quail model appeared to differ from other animal models in that the genetic defect in cholesterol catabolism, did not necessarily explain the occurrence of hypercholesterolemia, as was the case for pigeon, rabbit and monkey. There could be a new aspect of the genetic defect distinguishable in our animal model, and this feature could also potentiate the usefulness of our model in the study on the pathogenesis of atherosclerosis or hypercholesterolemia in the future.

The authors thank Otsuka Pharmaceutical Co., Ltd., for their generous supply of HAP Japanese quail. Our thanks also go to G. Maeda and T. Ohmura for technical assistance.

REFERENCES


in inbred strains of rabbits hypo- or hyperresponsive to dietary cholesterol. *Atherosclerosis*, 77, 151-157.

