PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 1050–1057 (1974)

Mechanisms of the Genetic Control of Plasma Cholesterol in Selected Lines of Show Racer Pigeons[1] (37951)

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The importance in determining genetically controlled mechanisms of plasma cholesterol regulation becomes very apparent in the discussion of increased plasma cholesterol as a risk factor in atherosclerosis. Of particular interest are genetic influences on the response to dietary cholesterol. Several workers have shown that inherited factors are responsible for the majority of the accountable variation seen in plasma cholesterol levels and have reported heritability estimates of over 60% in squirrel monkeys (1), 68–99% in cattle (2), 56% in mice (3), and 26% in chickens (4). Genetic factors controlling plasma cholesterol become increasingly important at times when large amounts of cholesterol are available exogenously to the animal. In species such as man (5), the squirrel monkey (1), and the White Carneau pigeon (6), the response of plasma cholesterol to increased dietary levels has been highly variable between individuals within a species. Certain animals termed "hyporesponders" are able to maintain low levels of plasma cholesterol in response to the dietary challenge, whereas others termed "hyperresponders" do not (1, 6).

Through selective breeding we have produced several strains of pigeons that possess specific atherosclerosis characteristics (7). Two selected lines of Show Racer pigeons, one characterized as a hyporesponder and the other as a hyperresponder, markedly illustrate the significance of heritable factors in the regulation of the plasma cholesterol. Because the interactions of absorption, synthesis, and excretion regulate body cholesterol, we have investigated these factors as mechanisms by which the genetic control of plasma cholesterol may be manifested in the selected lines of Show Racer pigeons.

Materials and Methods. The pigeons used in this study were produced through selective breeding at the Bowman Gray School of Medicine research farm. Strain SR-39 was selected for a characteristically low aorta cholesterol concentration in comparison to random-bred Show Racer pigeons, while strain SR-37 was selected for an increased prevalence of myocardial infarction in comparison to the incidence usually seen for the breed (7). It was evident after further evaluation of these strains that the distinguishing atherosclerosis characteristics probably were influenced by levels of plasma cholesterol. From populations, each numbering 30–35 pigeons, we chose five SR-37 and five SR-39 pigeons which showed pronounced hypercholesterolemia and hypocholesterolemia respectively after a 6-month feeding of Purina pigeon pellets with a 10% lard and 0.5% cholesterol additive. The ten pigeons studied were 7–8 months of age, of either sex, and housed in chicken finishing batteries under conditions described by Clarkson *et al.* (8).

Cholesterol absorption, synthesis, and turnover were quantitated by using the isotopic methods described by Grundy and Ahrens (9). The method involved feeding a constant amount of [^{3}H]cholesterol daily until plasma cholesterol specific activities plateaued, during which the animals were in

[1] This work was supported by Grants HL14164 and HL05883 from the National Heart and Lung Institute, U. S. Public Health Services.

isotopic steady states and, theoretically, all readily miscible cholesterol pools were in equilibrium with plasma cholesterol. The relative contribution of cholesterol absorption (%) to the plasma cholesterol was calculated by dividing the mean plasma cholesterol specific activity by the diet specific activity and multiplying by 100. The relative contribution of synthesis to plasma cholesterol was calculated by subtracting absorption from 100%. In order to reach the isotopic steady state in a shorter time interval, all pigeons were injected intravenously with a priming dose of [^{3}H]-cholesterol as an ethanol:saline (3:7; v/v) suspension before the ^{3}H-labeled diet was begun (10).

After the isotopic steady state was reached, each bird was injected intravenously with 11.5 μCi of [^{14}C]cholesterol-labeled serum in a total volume of 1.2 cc. A labeled serum solution was used to equate the injected solution more closely to *in vivo* conditions, since Nilsson and Zilversmit (11) have shown that injected saline–ethanol–cholesterol suspensions are immediately taken up and released at a later time by the reticuloendothelial system.

Blood samples were drawn at days 1, 3, 5, and 7, and weekly thereafter until day 56 for serum cholesterol and radioactivity measurements to compute plasma cholesterol specific activity. Time curves were obtained by plotting the log of the plasma cholesterol specific activities against the days postinjection. The time curves were analyzed mathematically according to a two-pool model as described by Goodman and Noble (12). The pool concept as formulated and discussed in detail by Gurpide, Mann, and Sandberg (13) is mathematical and does not imply physically distinct compartments *in vivo*. Treating body cholesterol as a two-pool system, one can characterize pool A as being composed of cholesterol in blood, liver, and other tissues which rapidly exchange with blood cholesterol; and pool B as being composed of tissues such as muscle and skin, in which cholesterol exchanges more slowly with blood cholesterol. The data obtained include the cholesterol turnover rate, the size of pool A, and the kinetics of cholesterol transfer between the tissue pools. The size of pool B, however, is not derivable from the data. Exogenous cholesterol absorption in milligrams per day was calculated by multiplying daily cholesterol turnover by the fraction of plasma cholesterol derived from absorption. Synthesis was calculated from the difference between the daily turnover rate and absorption.

Preparation of [^{3}H]cholesterol-labeled feed. [1,2-^{3}H]Cholesterol (New England Nuclear Corp., Boston, MA) was checked for purity, added to melted lard and cholesterol, and coated on Purina pigeon pellets. The diet contained 10% lard and 0.5% cholesterol by weight. A Soxhlet apparatus was used to analyze the diet cholesterol specific activity on each batch of diet by extracting an aliquot with hot isopropanol.

Preparation of [^{14}C]cholesterol-labeled serum. [4-^{14}C]Cholesterol (New England Nuclear Corp., Boston, MA) was checked for purity and dissolved in 0.1 ml absolute ethanol. Pooled serum from four normocholesterolemic Show Racer pigeons was heated at 56° for 30 min to inactivate lecithin:cholesterol acyltransferase and added to the dissolved [^{14}C]cholesterol. The serum was incubated at 42° for 1 hr at 20 oscillations per minute, then dialyzed against 0.9% NaCl for 2 hr. An aliquot of serum was extracted with isopropanol and analyzed for cholesterol and esterified cholesterol radioactivity. Essentially all of the radioactivity was localized in the non-esterified cholesterol fraction.

Plasma cholesterol measurements. Serum cholesterol concentrations were measured on isopropanol extracts by an autoanalyzer adaptation of the method of Block, Jarrett, and Levine (14). A Beckman DPM-100 liquid scintillation counter was used to measure radioactivity on an aliquot of the extract.

Statistical procedures. Programs of a Model 1620 IBM computer in the computer center at the Bowman Gray School of Medicine and a Student's *t* test (15) were used to analyze the data.

Results. The mean plasma cholesterol concentrations for five pigeons from each

of the two selected lines are shown in Fig. 1. At time 0, during which the birds were consuming a cholesterol-free grain diet, no significant differences for mean plasma cholesterol concentrations existed between the strains. However, after being fed the diet containing 10% lard and 0.5% cholesterol, the hyperresponder strain had increased plasma cholesterol to concentrations of 1903 ± 157 mg/dl (mean ± SEM) after only 1 month, whereas the hyporesponders remained unchanged at a plasma cholesterol concentration of 260 ± 42 mg/dl (Fig. 1). Distinctly elevated plasma cholesterol levels were seen for the hyperresponders from 2 through 6 months, during which they had plasma cholesterol levels of 2386 ± 233 mg/dl, while the hyporesponder strain remained low at 385 ± 44 mg/dl. Throughout the experimental period of 15 weeks during which cholesterol absorption, synthesis, and turnover were studied, the mean plasma cholesterol levels for the hyperresponders were consistently higher and more variable from week to week in comparison to the hyporesponders (Fig. 2).

Diet and plasma cholesterol specific activities for the hypo- and hyperresponders are shown in Fig. 3. The diet cholesterol specific activity was measured as 24,660 ± 300 (mean ± SEM). An overall mean plasma cholesterol specific activity taken from the specific activities of 5 through 15 weeks of the diet was used to calculate the percent contribution of diet and synthesis to plasma cholesterol levels. It can be seen from Fig. 3 that the two strains differed significantly in the absorption and synthesis of cholesterol. Calculations showed that 83% of the plasma cholesterol in the hyperresponder strain originated from absorption, while 17% of the plasma cholesterol was synthesized. In contrast, 63% of the plasma cholesterol in the hyporesponders originated exogenously, while 37% was synthesized.

The semilogarithmic plots of the decreasing specific activities of [^{14}C]cholesterol for the two groups of pigeons are shown in Fig. 4. The specific activity of cholesterol decayed rapidly at a decreasing exponential rate during the initial 2 weeks after isotope injection. After 14 days, the curves became linear and differences in slopes illustrated that the hyporesponder group had a more rapid disappearance of [^{14}C]cholesterol. In order to calculate the kinetic data describing cholesterol turnover for the two groups (Tables I and II), individual isotope dilution curves were analyzed for each animal. The mean specific activity of plasma choles-

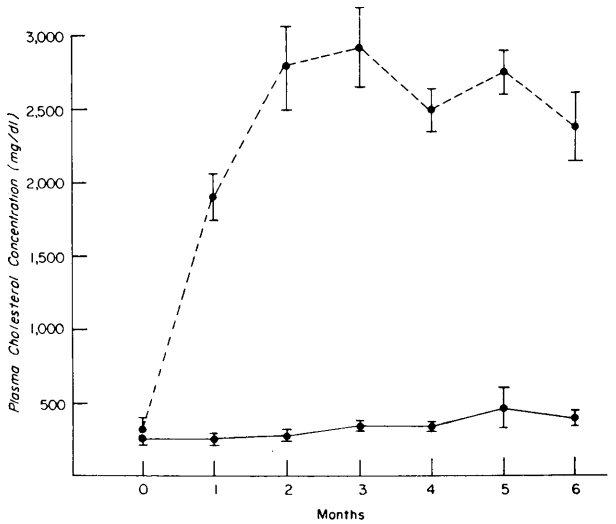

FIG. 1. Plasma cholesterol concentrations for hypo- (•——•) and hyperresponder (•- - -•) Show Racer pigeons fed a cholesterol-containing diet. Each point is the mean ± SEM of five observations.

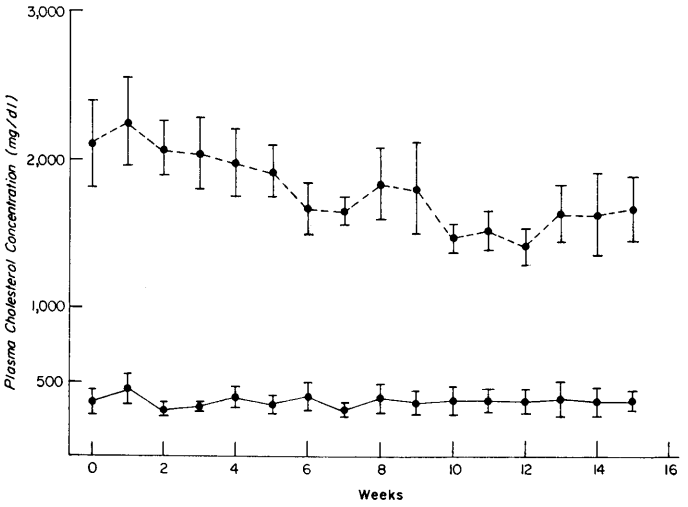

FIG. 2. Plasma cholesterol levels of hypo- (•——•) and hyperresponder (• - - -•) Show Racer pigeons during metabolic study. Each point is the mean ± SEM of five observations.

terol at zero time (C_A) for the first exponential was significantly lower in the hyperresponder group (18,460 ± 1,020, mean ± SEM), in comparison to the hyporesponder group (55,780 ± 8,970) (Table I). In addition, the half-life of the second exponential was only 11.4 days in the hyporesponders, compared to 16.1 days in the hyperresponders. The calculations for the

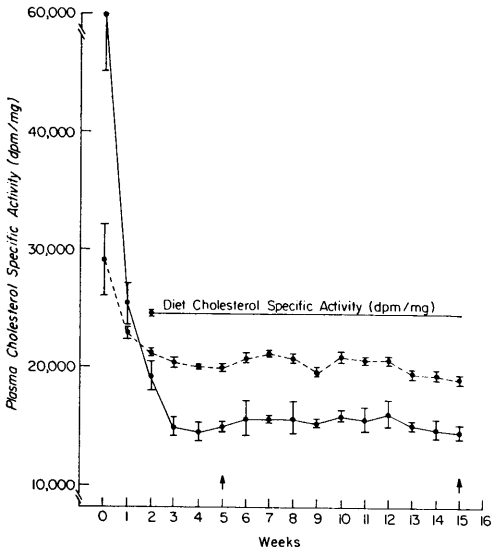

FIG. 3. Diet and plasma cholesterol specific activities for hypo- (•——•) and hyperresponder (• - - -•) Show Racer pigeons fed [^{3}H]cholesterol-containing diets. Each point is the mean ± SEM of five observations. Arrows show the 11 week period used in the calculation of cholesterol absorption and synthesis.

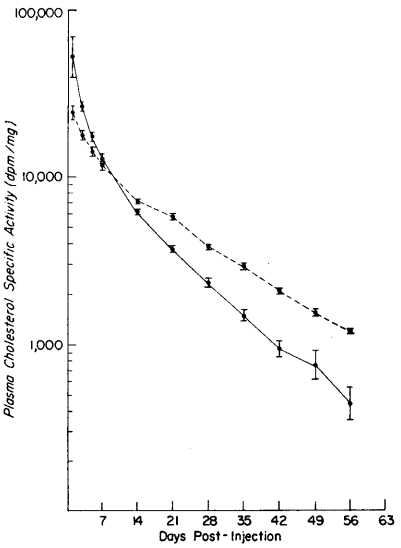

FIG. 4. Isotope dilution curves for hypo- (•——•) and hyperresponder (• - - -•) Show Racer pigeons given a single injection of 11.5 μCi [^{14}C]cholesterol. Each point is the mean ± SEM of five observations.

TABLE I. Kinetic Data Describing Cholesterol Turnover in Hyper- and Hyporesponder Show Racer Pigeons.[a]

	C_A (dpm/mg)	α	C_B (dpm/mg)	β	$t_{1/2}$ first exponential (days)	$t_{1/2}$ second exponential (days)
Hyperresponders						
Mean ± SEM	18,460[b] ± 1,020	0.306 ± 0.044	13,480 ± 900	0.0431[b] ± 0.0013	2.5 ± 0.4	16.1[b] ± 0.5
Hyporesponders						
Mean ± SEM	55,780 ± 8.970	0.403 ± 0.031	14,000 ± 1,230	0.0621 ± 0.0046	1.8 ± 0.1	11.4 ± 0.9

[a] C_A = Plasma cholesterol specific activity at zero time of the first exponential. C_B = Plasma cholesterol specific activity at zero time of the second exponential. α = Slope of the first exponential. β = Slope of the second exponential. $t_{1/2}$ = Exponential half-lives.

[b] Difference between means significant ($P < 0.01$).

TABLE II. Kinetic Data Describing Cholesterol Turnover in Hyper- and Hyporesponder Show Racer Pigeons.[a]

	M_A (mg)	PR_A (mg/day)	k_{AA}	k_{BB}	k_{BA}	k_{AB}	k_A
Hyperresponders							
Mean ± SEM	841[b] ± 51	70.3 ± 1.4	−0.194[c] ± 0.026	−0.156 ± 0.023	0.155 ± 0.023	0.109 ± 0.022	0.084[c] ± 0.004
Hyporesponders							
Mean ± SEM	413 ± 70	75.5 ± 6.4	−0.331 ± 0.032	−0.134 ± 0.016	0.134 ± 0.016	0.141 ± 0.018	0.190 ± 0.015

[a] M_A = size of pool A. PR_A = daily cholesterol turnover. k_{AA}, k_{BB} = rate constants for removal of cholesterol from pools A and B. k_{BA}, k_{AB} = rate constants describing cholesterol transfer from pools B to A and A to B. k_A = rate constant describing excretion from pool A.

[b] Difference between means significant ($P < 0.01$).

[c] Difference between means significant ($P < 0.05$).

slopes of both the first and second exponentials were greater for the hyporesponder group, although only the latter difference was significant ($P < 0.01$).

Additional kinetic data describing cholesterol metabolism are shown in Table II. The size of the readily miscible cholesterol pool was 413 mg in the hyporesponder group and twice as large or 841 mg in the hyperresponder group. Since blood is one of the tissues comprising pool A, it is not surprising that such differences, attributed mainly to plasma cholesterol, exist in M_A. The production rate of pool A (PR_A), which is equivalent to the cholesterol turnover rate since the pigeons were in a cholesterol steady state, was not different between the pigeon groups. The rate constant (k_{AA}) describing the removal of cholesterol from pool A (into pool B and excretion) as well as the rate constant (k_A) describing removal or excretion from pool A were higher for the hyporesponders than the hyperresponders.

No differences were seen between the two groups for the rate constants describing the removal of cholesterol from pool B, the transfer of cholesterol from pool B to pool A, or the transfer from pool A to B.

Calculations for exogenous cholesterol absorption ($PR_A \times$ % absorption) expressed in mg/day for both strains of pigeons are given in Table III. The hyperresponders daily derived a mean level of 58 mg of their plasma cholesterol from absorption and 12 mg from synthesis, while the hyporesponders derived 48 mg of their plasma cholesterol from absorption and 28 mg from synthesis. Although the calculated mean absorption level was higher in the hyperresponder group, the difference was not significant from the hyporesponder group.

Discussion. The importance of genetic factors in determining the response of animals to environmental factors such as diet is emphasized in this study by the facts that one line of pigeons had increased plasma cholesterol levels of 1903 mg/dl and another line, fed a similar diet for the same period, had plasma cholesterol levels of only 260 mg/dl. We have examined certain aspects of cholesterol metabolism whereby genetic control governing differences in the responses of the two described strains of pigeons could be manifested. Apparently, the hyporesponder Show Racer pigeon does not shut back cholesterol synthesis to maintain low plasma levels in response to the high dietary levels. In fact, synthesis contributed significantly less cholesterol to the plasma in the hyperresponders than in the hyporesponders. In squirrel monkeys of two genotypes, Lofland *et al.* (16) found similar rates of cholesterol synthesis in both groups. Their data indicate no evidence of a feedback inhibition due to the diet containing 0.5 mg of cholesterol/kcal which was fed. Since the hyperresponder pigeons used in this study were extremely hypercholesterolemic, perhaps the lower rates of synthesis in the group simply reflect a negative feedback on synthesis, possibly at the level of 3-hydroxy-3-methylglutaryl coenzyme A reductase.

The contribution of diet to plasma cholesterol was higher in the hyperresponder pigeons, but was not significantly different from the hyporesponders. Although food consumption was not quantitated, no evidence was collected to conclude that the

TABLE III. Contributions of Diet and Endogenous Synthesis to Plasma Cholesterol.

	Absorption		Synthesis	
	(%)	(mg/day)	(%)	(mg/day)
Hyperresponders				
Mean ± SEM	83[a] ± 1	58 ± 1	17[a] ± 1	12[a] ± 1
Hyporesponders				
Mean ± SEM	63 ± 2	48 ± 5	37 ± 2	28 ± 1

[a] Difference between means significant ($P < 0.001$).

groups consumed different amounts of diet. In regard to this, Lofland *et al.* (16) reported that hyporesponder squirrel monkeys ingested more food per day than did hyperresponder squirrel monkeys; however, the hyperresponders absorbed a larger percentage of the amount ingested, resulting in no difference in absorption.

The possibility exists that the hyporesponder pigeons might have maintained low plasma cholesterol levels by shifting cholesterol to other tissue pools. This assumption can be negated in part by the fact that the readily miscible pool, which would include tissues such as the liver, kidney, spleen, lung, and intestine, was smaller in the hyporesponder pigeons. The size of the slowly miscible cholesterol pool could not be measured; therefore some accumulation of cholesterol in tissue such as muscle, skin, and adipose tissue cannot be negated. The effect of unsaturated fatty acid diets producing lower plasma cholesterol levels through a redistribution among the body pools indicates that in squirrel monkeys the redistribution of cholesterol is real (17). It is very unlikely in view of the major differences in plasma cholesterol levels that a shift in cholesterol from the plasma to tissues of pool B accounts for the lowered plasma cholesterol levels in the hyporesponder pigeons.

Of particular interest is the finding of similar daily cholesterol turnover rates in both lines of pigeons, indicating that both had similar rates at which new cholesterol appeared in pool A. Lofland *et al.* (16) found production rates in hypo- and hyperresponder squirrel monkeys to be 57 and 62 mg/day, respectively; neither significantly different from the other. In another study, Bell *et al.* (6) found a slightly higher mean production rate of 133 mg/day in hyporesponder White Carneau pigeons, compared to a production rate of 120 mg/day in hyperresponders.

The hyporesponder Show Racers had larger rate constants describing excretion of cholesterol from pool A, implicating a site of genetic control which possibly involved the enzyme systems controlling bile acid–neutral steroid metabolism. Species such as rats (18) and squirrel monkeys (16) are capable of increasing bile acid excretion as a protective mechanism against hypercholesterolemia. Others, such as man, implicate mechanisms initiating increased endogenous neutral steroid excretion when faced with increased dietary levels of cholesterol (5). Another possible genetic control mechanism that may result in decreased plasma cholesterol levels in the hyporesponder Show Racer may be an increased metabolism of plasma lipoproteins. In addition to the hereditary patterns of hyperlipemia and hypercholesterolemia in man, it has been postulated that differences may exist between individuals in their ability to catabolize lipoproteins (19).

In this report both groups of pigeons were studied while in a cholesterol steady state, and the hyporesponder, once in that steady state, maintained lower plasma cholesterol levels than the hyperresponders by increasing excretion. Similar findings also have been reported for the hyporesponder squirrel monkeys (16). We should emphasize that the genetically controlled metabolic differences remaining to be studied are those which enable the hyporesponder to plateau at a low plasma cholesterol level. These important differences probably are manifested very early during the initial adjustment to increased dietary cholesterol.

Summary. Cholesterol absorption, synthesis, and turnover were studied in selected lines of hypo- and hyperresponder Show Racer pigeons by feeding a diet containing [^{3}H]cholesterol and injecting a [^{14}C]cholesterol-labeled serum. The decline in plasma [^{14}C]cholesterol specific activity with time was analyzed according to a two-pool model.

The mass of cholesterol in pool A was higher in the hyperresponder line than in the hyporesponders, reflecting mainly plasma cholesterol differences of 1417–2253 mg/dl in the former compared with 319–457 mg/dl in the latter. No strain differences were found for the rates at which new cholesterol appeared in pool A, but the rate constant describing removal of cholesterol from that pool was higher for the hyporesponder line, signifying genetic control of

plasma cholesterol at the level of excretion. Cholesterol absorption was calculated as 58 mg/day in the hyperresponders and 48 mg/day in the hyporesponders, while endogenous synthesis was 12 and 28 mg/day, respectively.

The authors would like to thank Dr. L. Rhyne for assistance in the statistical procedures and Mr. J. Rodden and Mrs. C. Hine for excellent technical assistance.

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Received Nov. 1, 1973. P.S.E.B.M., 1974, Vol. 145