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Esterases in inbred strains of mice with differential cholesterolemic responses to a high-cholesterol diet

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Summary

Specific esterase isoenzyme patterns in plasma may be associated with responsiveness of serum cholesterol to dietary cholesterol. In rabbits and rats the presence and absence of a high-mobility, anodal esterase band on electrophoresis have been shown to be associated with hypo- and hyperresponsiveness, respectively. We fed for 28 days male mice of 7 inbred strains either a low-cholesterol, commercial diet or a diet containing 2% (w/w) cholesterol, 0.5% cholic acid and 5% olive oil. Feeding the high-cholesterol diet revealed marked inter-strain differences in the responses of plasma and liver cholesterol; the increases ranged from 21 to 129% and from 10 to 80-fold, respectively. There was no association between esterase isoenzyme patterns in plasma and the sensitivity to the high-cholesterol diet. The mean baseline plasma total esterase activity tended to be positively associated with the absolute response of plasma cholesterol to the high-cholesterol diet ($r = 0.56$; $n = 7$), but the positive relationship between the baseline concentration of the ES-1 component in plasma and the cholesterolemic response was stronger ($r = 0.84$; $n = 7$; $P < 0.05$). The high-cholesterol diet caused a significant increase in plasma total esterase activities in 6 out of the 7 strains. Evidence is presented that the increase in plasma total esterase activity, which was associated with an increase in the activity and concentration of the so-called ES-2 isoenzyme, is the result of an enhanced release of esterases from the intestine, rather than from the liver. A significant, positive correlation was found between the baseline intestinal esterase activity and the cholesterolemic response after cholesterol feeding ($r = 0.83$; $n = 7$; $P < 0.05$).

Key words: Dietary cholesterol; Esterases; Inbred mice; Liver cholesterol; Plasma cholesterol

Introduction

Thompson [1] has demonstrated that feeding a high-fat, high-cholesterol diet to the male inbred

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C57BL/6J mouse consistently produced atheromatous lesions in the wall of the aortic sinus. In a subsequent study Roberts and Thompson [2] compared 13 strains of inbred mice, and found that the strains differed greatly in susceptibility to the atherogenic diet. The most resistant strain (CBA/J) showed a low and the most susceptible strain (C57BR/cdj) a high response of serum cholesterol to the experimental diet. Morriset et al. [3] have also reported that susceptible inbred mice, which developed more atherosclerosis, showed greater increases in serum cholesterol concentrations when challenged with a high-fat, high-cholesterol diet. However, Paigen et al. [4] have recently reported that in 10 inbred strains of mice there was no correlation between the response of serum cholesterol and that of atherosclerotic lesion formation to an atherogenic diet. Thus atherosclerotic lesions can be produced experimentally in mice, and resistent and susceptible strains are available. Inbred mouse strains may prove useful in studying genetic influences on the development of atherosclerosis. Research in this area would make considerable progress if genetic markers for the degree of diet-induced hypercholesterolemia and atherosclerosis could be identified.

The plasma of vertebrate animals contains arylesterases, enzymes that can hydrolyse artificial fatty acid esters of aromatic alcohols. The esterases are generally used as genetic markers [5]. Although the physiological function of plasma esterases is obscure, there is some evidence that they are involved in cholesterol metabolism. Inbred rabbits with a low response of plasma cholesterol to dietary cholesterol were found to display a high-mobility esterase band (coded for by Est-2) on starch-gel electrophoresis, but hyperresponsive strains did not [6]. This association of hyporesponsiveness to dietary cholesterol with the Est-2 phenotype, was also observed when crosses were made between New Zealand White and Vienna White rabbits in order to produce progeny of the known Est-2 genotype [7].

In inbred strains of rats the cholesterolemic response was low in 6 out of 7 strains displaying the high-mobility esterase band, which is coded for by a gene called ES-1 there, and which is assumed to be homologous with Est-2 in the rabbit [8]. Absence of the isoenzyme was associated with hyperresponsiveness to a high-cholesterol diet in 2 out of 3 strains [9]. Okamoto et al. [10] have also reported such an association between responsiveness to dietary cholesterol and plasma esterase pattern in inbred rats.

Further support for a relation between esterases and cholesterol metabolism comes from the observation that cholesterol loading of hypo- and hyperresponsive inbred strains of rats [11] and rabbits [12] caused an increase in plasma total esterase activity. The baseline plasma esterase activity was significantly higher in a hyporesponsive rat and a hyporesponsive rabbit strain, and the activity was still higher after cholesterol feeding [11,12]. One interpretation of these data is that a low esterase activity causes an increased susceptibility to dietary cholesterol, whereas induction of plasma esterase activity is required to compensate for cholesterol loading.

The present study was carried out to investigate whether the evidence for an association between plasma esterases and cholesterol metabolism also extends to mice. For this purpose 7 inbred strains of mice were fed a high-fat, high-cholesterol diet, and their cholesterolemic responses and plasma esterase patterns and activities were determined. In order to obtain clues as to the site of production of plasma esterases, hepatic and intestinal esterases were also studied. Parts of this work have appeared in abstract form [13,14].

Materials and methods

Animals, diets, blood sampling

Male mice of the following strains were used: C57BL/U, R/U(RC/CpbU), V/U(VC/CpbU), C3H/U, H/U(HC/CpbU), S/U(SC/CpbU), and FT/U(FTC/CpbU). All strains are fully inbred and maintained at the Department of Laboratory Animal Science, Utrecht. The animals were kept in wire-topped, plastic cages with a layer of sawdust as bedding. The cages were located in a room with controlled lighting (14 h/day), constant temperature (18–20°C) and constant relative humidity (55–65%). The animals were fed a commercial pelleted mouse diet (RMH-B^R, Hope Farms, Woerden, The Netherlands).

At the beginning of the experiment the mice of

each strain, aged about 5 months, were divided into a control and a diet group of 6 animals each. For each strain, the control and diet group had similar distributions of body weight and litter origin. The control groups of each strain received the low-cholesterol commercial diet (analysed cholesterol content, 23 mg/100 g) for 28 days. The diet groups received the hypercholesterolemic diet which consisted of the commercial diet (92.5%, w/w) to which 2% of cholesterol, 0.5% of cholic acid and 5% of olive oil had been added. Two mice of the R/U strain died in the group fed cholesterol.

At the end of the experiment, blood samples were taken in the non-fasting state by orbital puncture under light diethyl-ether anesthesia. Then, the anesthetized animals were killed by cervical dislocation, and the livers were removed. The small intestine was removed, cut lengthwise, and subsequently rinsed in saline. Plasma, livers and small intestine were stored at − 20°C.

Analytical methods

Analyses of control and diet animals were always performed in 1 batch. Plasma total cholesterol was measured enzymatically using the kit (Monotest) supplied by Boehringer-Mannheim GmbH, FRG. Liver cholesterol was extracted and analysed according to [15].

The activities in plasma of alkaline phosphatase, alanine aminotransferase and aspartate amino transferase were determined according to the recommendations of the German Society of Clinical Chemistry [16]. Reagents were purchased from Boehringer-Mannheim GmbH, FRG.

Total arylesterase activities in plasma and in extracts of liver and small intestine were determined at pH 8 using β-naphthylpropionate as a substrate according to [17]. Reaction conditions were chosen so that the amount of product formed was linear with time and enzyme concentration. Enzyme activity was corrected for spontaneous hydrolysis of the substrate. Plasma and tissue extracts had been stored for about 1 month at −20°C until analysis.

Esterase activity was measured in homogenates of the whole small intestine and of aliquots of the livers. These had been homogenized on ice in 5 volumes of buffer (0.05 M Tris; pH 7.5, containing 0.1% Triton X-100) with a 30-s burst of an Ultraturrax tissue homogenizer at 20000 rpm. The homogenate was frozen at − 20°C, thawed and subsequently centrifuged at 4°C for 20 min at 40000 × *g*. The supernatant was removed, and the sediment was washed with the Tris buffer and centrifuged again. The combined supernatants were then stored at − 20°C for about 2 weeks until esterase determinations.

Esterase phenotypes were determined by 4.5–12% polyacrylamide gradient gel electrophoresis at pH 9. After electrophoresis the gels were stained with Fast Blue BB (Merck, Darmstadt, FRG) and with α-naphthylacetate (pH 7.5) as substrate, and photographed.

The concentrations of esterases ES-1 and ES-2 were determined in plasma of 4 randomly chosen mice of each group. Plasma levels of ES-1 were assessed by rocket immunoelectrophoresis [18], using antiserum which was prepared as described [19]. Purified ES-1F [19] was used as a standard. ES-2 in plasma was determined by crossed immunoelectrophoresis [18,20]. Plasma esterases were first separated by 8.8% polyacrylamide gel electrophoresis; the ES-2 region was removed and subjected to rocket immunoelectrophoresis. Antiserum against ES-2B was prepared as described [21]; purified ES-2B was used as a standard. The combined within-day and between-day coefficients of variation for the ES-1F and ES-2B standards were 18 and 14%, respectively.

Statistics

Diet comparisons within strains were evaluated using Student's 2-tailed *t*-test. Pearson correlation coefficients for group mean values of the different strains and their significance according to a 2-tailed test were calculated [22].

Results

Plasma cholesterol and body weight

Table 1 shows the levels of plasma total cholesterol in mice fed either the commercial or the high-cholesterol diet. Cholesterol feeding revealed marked inter-strain differences in the response of plasma cholesterol; the increase varied from 21% (C57BL/U) to 129% (FT/U). The mean plasma cholesterol level on the low-cholesterol

TABLE 1

PLASMA CHOLESTEROL LEVELS AND BODY WEIGHTS OF INBRED STRAINS OF MICE FED LOW- OR HIGH-CHOLESTEROL DIETS

Results, expressed as mean ± SD for 6 animals per strain per dietary group.

Strain	Plasma cholesterol (mmol/l)		Body weight (g)	
	Low	High	Low	High
C57BL/U	2.67±0.20	3.23±0.52 *	30.2±2.4	28.1±1.7
R/U [a]	2.48±0.67	3.81±0.83 *	29.8±2.2	27.2±3.3
V/U	3.26±0.28	4.66±0.45 *	31.5±4.4	28.4±2.5
C3H/U	2.75±0.09	5.42±0.45 *	31.2±0.7	31.1±1.3
H/U	3.16±0.15	5.62±1.37 *	34.0±2.6	31.1±2.6
S/U	3.29±0.24	6.30±1.23 *	28.0±1.1	29.9±1.5
FT/U	3.56±0.44	8.15±1.77 *	27.6±1.5	27.3±1.9

[a] The group fed the high-cholesterol diet consisted of 4 animals. Data refer to the end of the experimental period, which lasted 28 days.

* Significantly different from group fed the low-cholesterol diet ($P < 0.05$).

diet was positively associated with the mean level on the high-cholesterol diet ($r = 0.83$; $n = 7$; $P < 0.05$).

In all strains, except for the S/U mice, body weight was somewhat lower on the high-cholesterol diet. When compared with the initial body weights (not shown), the cholesterol-fed mice either lost some weight (C57BL/U, V/U, FT/U) or gained weight somewhat slower than the controls (R/U, H/U). However, this effect of the high-cholesterol diet did not reach a level of statistical significance.

Esterase activities

Plasma esterase activities on the low-cholesterol diet are shown in Table 2. The C57BL/U mice had the lowest, and the V/U strain the highest activity. Cholesterol-feeding caused a significant increase in plasma total esterase activity in all strains, except for the V/U and S/U strains. The increase in plasma esterase activity varied from 2% (V/U) to 56% (C57BL/U).

The inter-strain variation in the activity of esterases in the liver on the low-cholesterol diet was relatively small. In 4 out of the 7 strains there was a significant increase in hepatic esterase activity after cholesterol feeding, the increase being about 25%. In the FT/U strain hepatic esterase activity per g liver remained essentially unchanged after feeding the high-cholesterol diet.

Intestinal esterase activities on the low-cholesterol diet differed markedly between the strains, the FT/U strain displaying a more than 2-fold higher activity than the C57BL/U strain. In contrast to the plasma and hepatic esterase activity, the intestinal total activity did not appear to be significantly affected by consumption of the high-cholesterol diet (Table 2).

Correlation of esterase activities and cholesterolemic response

Linear correlation coefficients were calculated

TABLE 2

ESTERASE ACTIVITIES IN PLASMA, LIVER AND SMALL INTESTINE OF INBRED STRAINS OF MICE FED LOW- OR HIGH-CHOLESTEROL DIETS

Enzyme activities were measured with β-naphthylpropionate as a substrate.

Strain	Plasma esterase (μmol/min/ml)		Hepatic esterase (μmol/min/g)		Intestinal esterase (μmol/min/g)	
	Low	High	Low	High	Low	High
C57BL/U	34±4	53± 7 *	620± 73	782±129 *	1114±180	1135± 543
R/U [a]	45±7	55± 4 *	527± 71	651±135	1660±471	2698±1094
V/U	62±6	63±10	464± 61	549± 57 *	1911±412	1975± 821
C3H/U	48±7	67±10 *	463± 63	547±137	1494±262	1749± 109
H/U	60±4	74± 8 *	523± 43	674± 53 *	1750±482	1867± 509
S/U	60±8	72±13	423± 75	555±122 *	2068±568	1498± 311
FT/U	57±6	78±10 *	546±133	517± 53	2532±296	2425± 603

See legend to Table 1.

between group mean basal esterase activities or the absolute change in esterase activities and the absolute increase in plasma cholesterol after cholesterol feeding. There were no significant associations between the basal activities of total plasma esterase ($r = 0.56$; n = 7; $P > 0.10$), hepatic esterases ($r = -0.31$; n = 7; $P > 0.10$) or intestinal esterases ($r = 0.83$; n = 7; $P < 0.05$) and the cholesterolemic response to the high-cholesterol diet. The correlation coefficients between the change in esterase activities and the response of plasma cholesterol were: plasma esterases, $r = 0.42$; hepatic esterases, $r = -0.75$; intestinal esterases, $r = -0.42$.

The highest correlation with the cholesterolemic response was thus found for the basal activity of total intestinal esterases ($r = 0.83$; n = 7; $P < 0.05$), which is illustrated in Fig. 1.

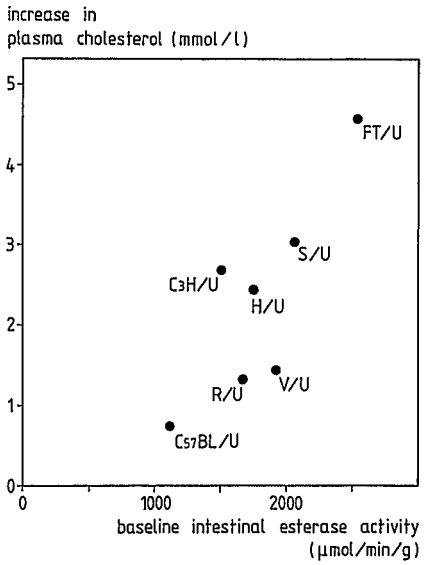

Fig. 1. Relationship between baseline total activity of intestinal esterases and the response of plasma cholesterol to a high-cholesterol diet in 7 inbred strains of mice ($r = 0.83$; n = 7; $P < 0.05$).

Esterase patterns

Plasma total esterase activities (measured with β-naphthylpropionate as a substrate) represent the sum of the activities of a number of different esterases. This is illustrated by the separation of these enzymes on polyacrylamide gradient gel electrophoresis, and visualized with α-naphthylacetate as a substrate (Fig. 2). There are differences between the mouse strains in the esterase zones which are controlled by the *Es-2*, *Es-1* and *Es-5* loci. With regard to the most anodal serum esterase zone (ES-2) all strains, except for the

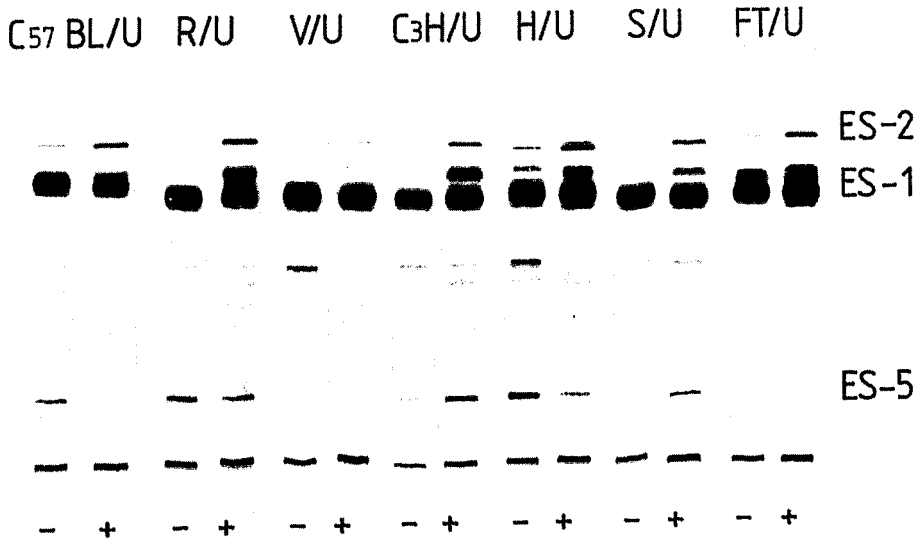

Fig. 2. Electrophoresis on polyacrylamide gradient gels of esterases in plasma (10 µl of plasma, 1:10 diluted with 65 mM Tris, 31 mM boric acid, 10% sucrose, pH 9.2) from 7 inbred strains of mice fed either a low-cholesterol, commercial diet (−) or a high-cholesterol diet (+). α-Naphthylacetate was used as a substrate for visualizing enzyme activity.

H/U strain, display the esterase produced by the *Es-2* ^{b} allele, that is the faster enzyme. The H/U strain shows the somewhat slower zone, which is produced by the *Es-2* ^{c} allele. After cholesterol feeding there was a marked increase in the intensity of the ES-2 zone. This increase was least pronounced in the V/U strain.

As to the ES-1 zone, the C57B1/U strain differed from the other strains. The C57B1/U mice are homozygous for the Es-1 ^{b} allele and show the relatively faster ES-1A isoenzyme, whereas all other strains are homozygous for the Es-1B allele, and thus show the slower Es-1 ^{a} isoenzyme. Another esterase zone migrating between ES-2B and ES-1B was seen in strains C3H/U, H/U, R/U, and S/U (not indicated in Fig. 2). This isozyme, which is known to be closely linked to ES-2 (Ronai et al., unpublished) also showed a marked increase after cholesterol feeding.

The slow ES-5 zone is absent in the V/U and FT/U strains (*Es-5* ^{a} genotype). In the C57BL/U, R/U and H/U strains with *Es-5* ^{b} genotype the high-cholesterol diet somewhat decreased the intensity of the ES-5 band (Fig. 2). In the C3H/U and S/U strains there tended to be an increased activity of ES-5 after cholesterol feeding.

Figure 3 shows that the livers of the mice contain a large number of esterases. In contrast to plasma ES-2, the corresponding esterase in the liver did not respond dramatically to the diet containing cholesterol and cholate. There was only a slight increase in the intensity of the liver ES-2 zone in the R/U and V/U strains (Fig. 3). In the C3H/U and H/U strains there tended to be a decrease in hepatic ES-2.

The intestinal esterases are illustrated in Fig. 4. Cholesterol loading caused an increase in the activity of the ES-2 zone in all strains. The ES-11 band of the small intestine appeared to be increased by the high-cholesterol diet in the C57BL/U and C3H/U, but not in the other strains.

Plasma concentrations of ES-1 and ES-2

Table 3 shows the levels in plasma of ES-1 and ES-2 as determined by rocket immunoelectrophoresis. In all strains there was a dramatic increase in ES-2. The plasma ES-1 concentration

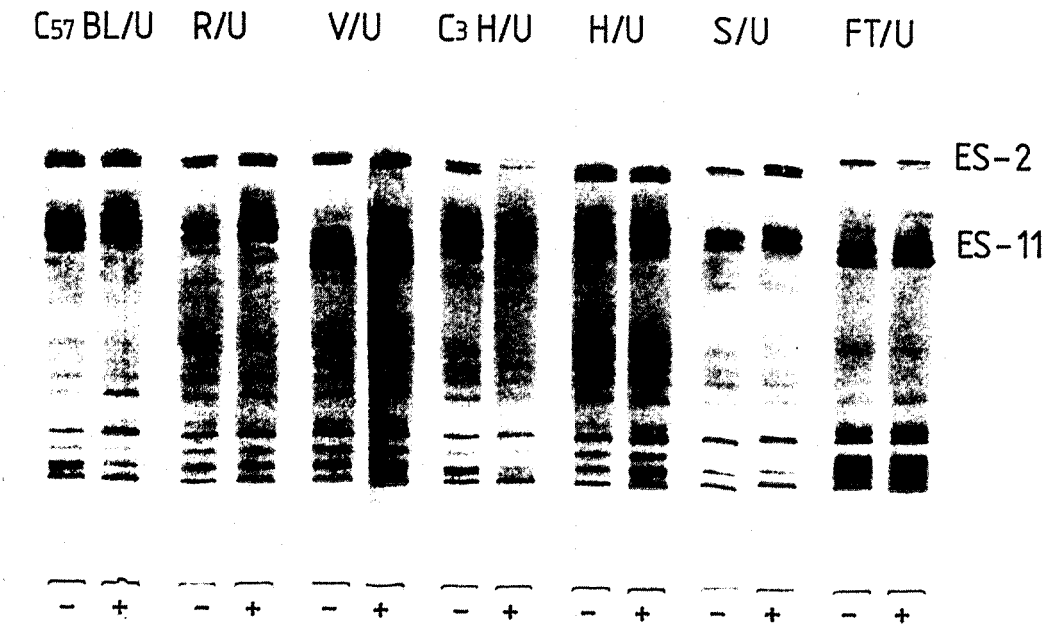

Fig. 3. Electrophoresis on polyacrylamide gradient gels of esterases in liver (10 μl of homogenate, 1 : 50 diluted) from 7 inbred strains of mice. See legend to Fig. 1.

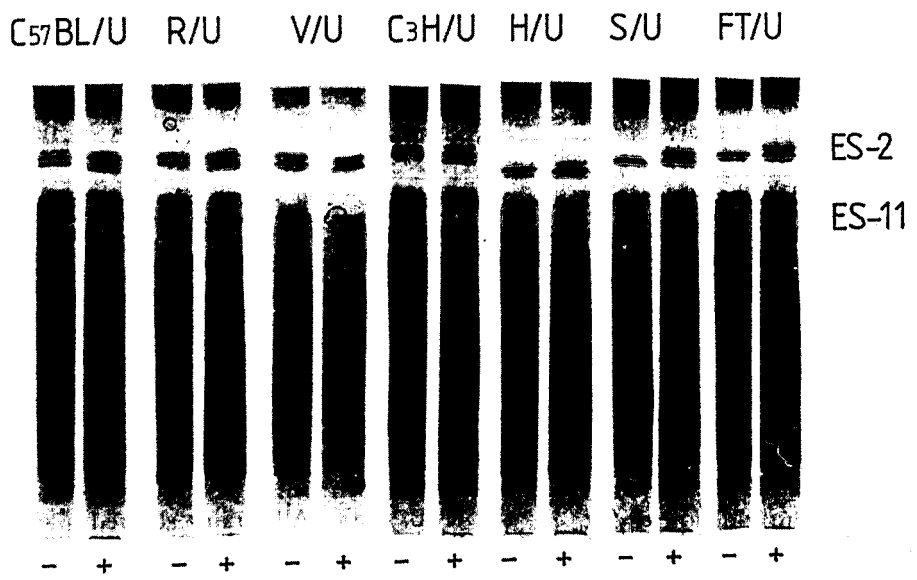

Fig. 4. Electrophoresis on polyacrylamide gradient gels of esterases in small intestine (10 μl of homogenate, 1:50 diluted) from 7 inbred strains of mice. See legend to Fig. 1.

was not significantly influenced by cholesterol feeding. There were no statistically significant associations between the basal group mean levels of ES-2 ($r = 0.44$; n = 7; $P > 0.10$) and the absolute response of plasma cholesterol to cholesterol feeding. Likewise, there were no significant correlations between the change in ES-1 ($r = 0.51$; n = 7; $P > 0.10$) or in ES-2 ($r = -0.28$; n = 7; $P > 0.10$) and the cholesterolemic responses. A statistically significant, positive association was found between the basal concentration of plasma ES-1 and the response of plasma cholesterol ($r = 0.84$; n = 7; $P < 0.05$), as shown in Fig. 5.

TABLE 3

PLASMA LEVELS OF ES-1 AND ES-2 OF INBRED STRAINS OF MICE FED LOW- OR HIGH-CHOLESTEROL DIETS

Results, expressed as mean ± SD for 4 animals per strain per dietary group. Data refer to the end of the experimental period, which lasted 28 days.

Strain	Plasma ES-1 (μg isozyme/ml)		Plasma ES-2 (μg isozyme/ml)	
	Low	High	Low	High
C57BL/U	465±229	536±239	0.60±0.49	2.74±0.41 *
R/U	393±166	270±105	0.09±0.01	1.68±0.66 *
V/U	495± 85	452± 54	0.17±0.04	0.76±0.32 *
C3H/U	603±128	608±124	0.22±0.09	1.79±0.62 *
H/U	491± 90	511± 99	0.71±0.32	2.29±0.57 *
S/U	713±241	760±282	0.25±0.08	2.56±0.81 *
FT/U	711± 66	812± 85	0.85±0.47	1.63±0.59 *

See legend to Table 1.

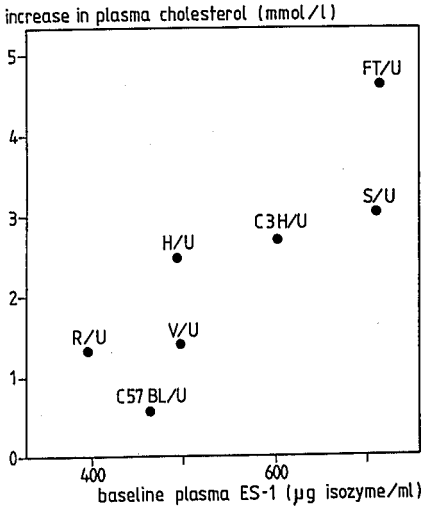

Fig. 5. Relationship between baseline concentration of plasma ES-1 and the response of plasma cholesterol to a high-cholesterol diet in 7 inbred strains of mice ($r = 0.84$; n = 7; $P < 0.05$).

TABLE 4

LIVER WEIGHT AND LIVER CHOLESTEROL IN INBRED STRAINS OF MICE FED LOW- OR HIGH-CHOLESTEROL DIETS

Strain	Liver wet weight		Liver cholesterol			
	(g)		(μmol)		(μmol/g)	
	Low	High	Low	High	Low	High
C57BL/U	1.67±0.13	2.55±0.25 *	11.6±0.9	356±107 *	7.0±0.7	138±34 *
R/U [a]	1.30±0.21	2.64±0.28 *	8.8±1.5	706± 95 *	6.8±0.4	268±22 *
V/U	1.15±0.10	1.95±0.25 *	11.9±2.2	343± 56 *	10.3±1.4	178±28 *
C3H/U	1.56±0.12	2.12±0.09 *	9.4±1.5	91± 47 *	6.0±0.6	42±20 *
H/U	1.46±0.16	2.60±0.16 *	10.8±1.5	565± 59 *	7.4±0.7	217±20 *
S/U	1.39±0.03	2.69±0.16 *	10.1±1.5	552± 96 *	7.3±1.1	205±38 *
FT/U	1.07±0.09	1.62±0.13 *	9.6±0.8	109±102 *	9.0±0.9	67±60 *

See legend to Table 1.

Liver cholesterol

Table 4 shows that the amount of hepatic cholesterol on the low-cholesterol, commercial diet was similar in all strains. The high-cholesterol diet caused a dramatic increase in liver cholesterol in all strains, but there were marked inter-strain differences in the response. The C3H/U and FT/U strains accumulated relatively low amounts of cholesterol in their livers. In contrast, the R/U mice appeared to store extremely large amounts of cholesterol. There was no significant correlation between the absolute increase in liver cholesterol and the cholesterolemic response ($r = -0.43$, n = 7).

The increase in liver cholesterol was associated with an increase in liver weight. Hepatomegaly, expressed as percentual increase in liver wet weight, varied from 36% in the C3H/U to 103% in the R/U strain. All strains showed a significant ($P < 0.05$) liver enlargement after cholesterol feeding, when liver weight was expressed on the basis of body weight.

Indicator enzymes for liver function

The high content of cholesterol in the liver as well as the hepatomegaly after cholesterol feeding would suggest that liver function was impaired. This is supported by the drastically increased activities of the indicator for possible biliary obstruction, alkaline phosphatase, in the plasma of the cholesterol-fed mice (Table 5). The activity of alanine aminotransferase was significantly increased in the C57BL/U, R/U, V/U and S/U strains. The highest activities of alkaline phosphatase were attained by the R/U and V/U mice. With regard to alanine aminotransferase, the

TABLE 5

ACTIVITIES OF INDICATOR ENZYMES IN PLASMA OF INBRED STRAINS OF MICE FED LOW- OR HIGH-CHOLESTEROL DIETS

Strain	Alkaline phosphatase (U/l)		Alanine aminotransferase (U/l)		Aspartate aminotransferase (U/l)	
	Low	High	Low	High	Low	High
C57BL/U	89±10	409±42 *	110±60	330±173 *	138±53	221± 45 *
R/U [a]	173±24	686±73 *	43±16	163± 53 *	104±27	238±119 *
V/U	165±28	691±39 *	58±14	107± 42 *	127±46	110± 19
C3H/U	124±16	390±46 *	102±35	124± 53	117±33	111± 28
H/U	104±32	583±35 *	85±26	89± 21	180±89	140± 30
S/U	96± 9	343±38 *	35± 6	162± 71 *	100±21	155± 59
FT/U	163±17	500±37 *	46±36	51± 19	104±40	112± 55

See legend to Table 1.

C57BL/U strain revealed the highest activity. Cholesterol-induced elevation of aspartate aminotransferase activity was most pronounced in the C57BL/U and R/U strains.

Inter-strain differences were also observed on the low-cholesterol, commercial diet. The C57BL/U strain displayed the lowest activity of alkaline phosphatase, and the R/U strain the highest activity, the activity in the latter strain being almost 2-fold higher. The lowest activities of plasma alanine aminotransferase and aspartate aminotransferase were found in the S/U strain. Compared with the S/U strain, the activity of alanine aminotransferase was more than 3-fold higher in the C57BL/U mice, and that of aspartate aminotransferase was 80% higher in the H/U.

Discussion

The main objective of this study was to see whether esterases are associated with cholesterol metabolism, and particularly, with the response of plasma cholesterol to a high-cholesterol diet. For this purpose we studied 7 inbred strains of mice with differential cholesterolemic responses to a diet enriched with cholesterol.

There was a weak positive association between the baseline plasma total esterase activity and the cholesterolemic response in the inbred strains of mice ($r = 0.56$; $n = 7$). This association probably may partly be a reflection of the positive correlation between the baseline concentration of the ES-1 isozyme in plasma and the cholesterolemic response (Fig. 5). These findings would appear to be in contrast to our previous observations in two inbred strains of either rats or rabbits. Basal plasma total esterase activities were found to be significantly higher in a rabbit strain and in a rat strain hyporesponsive to dietary cholesterol than in the corresponding hyperresponsive strains [11,12]. Thus the rabbit and rat data, in contrast to the mouse data, would suggest that high plasma total esterase activities are associated with a low response of plasma cholesterol to dietary cholesterol.

In the present study we did not observe a relationship between *Es-2* genotype of plasma esterases (Fig. 2) and the sensitivity to the high-cholesterol diet. This may also contradict earlier results in inbred strains of rabbits [6] and rats [9], in which we found evidence for a relationship between the presence of an isoenzyme of high-mobility on starch-gel electrophoresis and hyporesponsiveness to dietary cholesterol. Thus, it can be concluded that there is no general, species-independent association between plasma esterases and sensitivity to high-cholesterol intakes. No satisfactory explanation can be offered to explain these anomalies. One could argue that the species differences point to the absence of a causal relationship between plasma esterases and the increment in plasma cholesterol seen after cholesterol feeding. On the other hand, one could put forward that plasma of the laboratory animals used contains at least 10 different arylesterases, most of them probably unrelated to cholesterol metabolism. In the light of this fact, it would be desirable to study and measure the various plasma esterases separately.

The high-cholesterol diet caused a clear increase in plasma total esterase activities in all strains of mice, except for the V/U and S/U strains (Table 2). Such an increase in esterase activity has also been observed in rats [6] and rabbits [9]. It is not known in what tissue these esterases are produced. The most pronounced increase in plasma esterases may occur in the ES-2 isoenzyme (Fig. 2, Table 3). Cholesterol feeding also caused an increase in the ES-2 isoenzyme of the intestine (Fig. 3). Thus, it could be suggested that the increase in plasma ES-2 esterase after cholesterol feeding is due to enhanced production and release by the intestine. This suggestion is supported by the work of Lewis and Hunter [23], who found that injection of fat into the stomach of rats caused a marked increase in the activity of esterases of high electrophoretic mobility in the intestinal lymph, and later also in the serum.

The combination of dietary cholesterol and cholate may induce impairment of liver function through excessive accumulation in this organ (Table 4). This may cause destruction of hepatocytes and consequently release of enzymes into the circulation. This is indicated by the cholesterol-induced increases in the activities in the plasma of aspartate and alanine aminotransferase in several mice strains (Table 5). Hepatic cholesterol accumulation may also cause biliary obstruction. This

would be supported by the increased activities of plasma alkaline phosphatase (Table 5). The important point here is that the observed increases in plasma esterase activity (ES-2) are most likely not the result of liver damage. Fig. 3 shows that the high-cholesterol diet did not affect the activity of the hepatic ES-2 band. Thus the increase in plasma ES-2 may indeed be the result of a dietary-cholesterol-induced production and release by the intestine. It remains to be determined whether this release is a specific effect of the diet or the result of non-specific cell damage.

Hepatic arylesterase activity may be involved in the sensitivity of mice to the high-cholesterol, high-cholate diet. There was a significant negative association ($r = -0.75$, $n = 7$, $P < 0.10$) between the change in hepatic esterase activity and the increase in plasma cholesterol. Thus, the higher the increase in hepatic esterase activity, the lower the cholesterolemic response. Perhaps, the increase in hepatic esterase activity is a compensatory mechanism to handle increased intakes of cholesterol. At present, this can not be explained in molecular terms.

Figure 1 shows that the baseline intestinal esterase activity is directly related to the response of plasma cholesterol after cholesterol feeding. It could be suggested that the increase in the rapidly exchangeable pool, i.e. cholesterol in plasma plus liver, is more important than that in plasma alone. The increase in this pool is almost completely represented by the increase in liver cholesterol because the mice may not contain more than 0.5 ml of plasma. There was no significant correlation between baseline intestinal esterase activity and the increase in liver cholesterol ($r = -0.17$, $n = 7$). A low intestinal esterase activity may thus specifically protect against cholesterol-induced hypercholesterolemia. If we speculate that intestinal esterases are involved in cholesterol absorption, this would imply that in the strains with a low activity, cholesterol absorption is also relatively low. This would then mean that a low efficiency of cholesterol absorption is associated with hyporesponsiveness to dietary cholesterol. Studies with rhesus monkeys [24], squirrel monkeys [25] and African green monkeys [26] have indeed shown that hyperresponsive animals absorb a higher percentage of intestinal luminal cholesterol than hyporesponders. That manipulation of cholesterol absorption influences plasma cholesterol concentrations is indicated by studies with a copolymer of maleic acid and an 18-carbon alpha-olefin, which blocks cholesterol absorption. When the compound was given to human subjects, a significant correlation was found between percent LDL cholesterol reduction and percent absorption inhibition [27]. Clearly, the possible involvement of intestinal esterases in the efficiency of cholesterol absorption and sensitivity to dietary cholesterol remains to be established.

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