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ARYLESTERASE ACTIVITIES IN THE PLASMA OF RATS, RABBITS AND HUMANS ON LOW- AND HIGH-CHOLESTEROL DIETS

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Abstract—1. Arylesterase activities were measured with β-naphthylpropionate and/or α-naphthylacetate as substrate in the plasma of rats, rabbits and humans on low- and high-cholesterol diets.

2. The plasma esterase activities measured with α-naphthylacetate were similar in rats, rabbits and humans. With β-naphthylpropionate as a substrate, rabbits were found to have a markedly higher esterase activity than rats and humans.

3. Basal plasma esterase activity was significantly higher in an inbred rat strain which is hyporesponsive to dietary cholesterol than in a hyperresponsive strain.

4. In rats, but not in humans and rabbits, plasma esterase activity was significantly increased by a high-cholesterol diet.

5. In individual humans and random-bred rabbits and rats there was no association between initial plasma total esterase activity and the subsequent plasma cholesterol response to cholesterol feeding.

6. We suggest that arylesterases are associated with cholesterol metabolism and with the response to dietary cholesterol in rats; evidence for such a role in rabbits and humans is, however, inconclusive.

INTRODUCTION

The plasmata of various vertebrates contain a number of different arylesterases (Augustinsson, 1958) which are defined by their capability to hydrolyse artificial fatty acid esters of aromatic alcohols. In human serum at least 13 (Pilz, 1964) and in rabbit serum (Pilz *et al.*, 1968) 12 different arylesterases have been identified. The enzymes have pH optima ranging from 7 to 9.5 and differ in substrate specificity, including the chain length of the esterified fatty acid. The genetics of serum esterases in the rat have been described in detail (Van Zutphen, 1983).

The physiological function of the arylesterases is obscure. It has been suggested (Pilz *et al.*, 1966; Pilz, 1968) that they catalyse the covalent coupling of fatty acids to tyrosine groups in plasma albumin. Plasma esterases may also be involved in cholesterol metabolism. In inbred strains of rabbits (Van Zutphen and Fox, 1977) and rats (Okamoto *et al.*, 1972; Van Zutphen and Den Bierman, 1981) it was found that variation in the response of plasma cholesterol level to dietary cholesterol was associated with a genetically determined variation in plasma esterases. A fast moving esterase zone was present in the plasma zymogram of hyporesponsive strains, but not in that of hyperresponsive strains.

The esterase pattern after gel electrophoresis (Van Zutphen and Fox, 1977; Van Zutphen and Den Bieman, 1981) only gives qualitative information. Therefore, the present study was carried out in an attempt to correlate absolute levels of plasma total esterase activities and the serum cholesterol responses to dietary cholesterol in rats, rabbits and humans. In addition, the effect of a high-cholesterol diet on plasma total esterase activity was measured. Part of this work has appeared in abstract form (Beynen *et al.*, 1983).

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MATERIALS AND METHODS

Analytical methods

Plasma esterase activities were determined at pH 8.6 using α-naphthylacetate (Merck, Darmstadt, FRG) or β-naphthylpropionate (Sigma Chemical Co., St. Louis, MO, USA) as a substrate according to Pilz and Johann (1965) and Pilz (1961), respectively. Reaction conditions were chosen so that enzyme activity was proportional to time and enzyme concentration. Enzyme activity was corrected for spontaneous hydrolysis of the substrate. Plasma samples were stored at −20°C for periods up to 4 weeks until analysis. All samples of one experiment were analysed within one batch. Reproducibility (coefficient of variation) was routinely less than 1.5%.

Rat plasma esterase phenotypes were determined by starch gel electrophoresis (pH 8.75) and staining with Fast Blue BB (Merck, Darmstadt, FRG) and with β-naphthylpropionate (pH 7.3) as substrate (Okamoto *et al.*, 1972).

Plasma total cholesterol was measured enzymatically according to Röschlau *et al.* (1974) using the kit (Monotest) supplied by Boehringer-Mannheim GmbH, FRG.

Experiments with rats

Male rats of two fully inbred strains and male and female random-bred Wistar rats were used. The inbred strains, SD/Cpb and SHR/Cpb, were purchased at the age of about 3 weeks from the Central Institute for the Breeding of Laboratory Animals (CPB-TNO), Zeist, The Netherlands. The strains had previously been shown to be hypo- and hyperresponsive to dietary cholesterol (Van Zutphen and

Den Bieman, 1981). The Wistar rats were maintained at the Small Animal Centre (CKP) of the Agricultural University.

The inbred and random-bred rats were fed a commercial rat diet (Hope Farms, Woerden, The Netherlands) for 3 and 10 weeks, respectively; they were then divided into an experimental and a control group so that both groups had similar mean body weights and serum cholesterol concentrations. The control animals remained on the low-cholesterol, commercial diet, whereas the other animals were transferred to the high-cholesterol diet. The high-cholesterol diet consisted of 92.5% (w/w) commercial diet, 5% olive oil (Puget, Marseille, France), 2% cholesterol (Duphar BV, Veenendaal, The Netherlands) and 0.5% sodium cholate (Merck, Darmstadt, FRG).

Blood samples were taken after a 24-hr fast before and at the end of the experimental period. The samples were drawn into heparinized tubes by orbital puncture under anesthesia with diethyl ether. Plasma was collected by low speed centrifugation.

Experiment with rabbits

Female random-bred rabbits of the New Zealand White strain were obtained from the Broekman Institute, Helmond, The Netherlands. The rabbits, aged about 11 weeks, were fed a cholesterol-free, semipurified diet for 4 weeks. Then, 9 animals were allocated to a high-cholesterol, semipurified diet, while 7 other rabbits remained on the cholesterol-free control diet. The composition of the semipurified diets was as follows (g/kg): soybean protein isolate, 208; methionine, 2; corn starch, 170; dextrose, 210; molasses, 50; coconut fat, 90; soybean oil, 10; cholesterol, 0 (in the high-cholesterol diet, 2); sawdust, 180 (in the high-cholesterol diet, 178); dicalcium phosphate, 29; sodium chloride, 6; magnesium carbonate, 3; magnesium oxide, 2; potassium bicarbonate, 18; vitamin premix, 12; mineral premix, 10. The composition of the vitamin and mineral premixes have been described (West *et al.*, 1982).

Blood samples were taken from a marginal ear vein between 8 and 10 am, after the removal of any remaining food at 4 pm the previous day.

Experiment with humans

Five healthy subjects (three men and two women, aged 21–36 yr), including G.J.B.W., participated in the experiment. During the study the subjects consumed their habitual diets, with the exception that during the first 10 days cholesterol-rich products were forbidden; during the second 10 days of the study the subjects daily added six egg yolks (equivalent to about 1500 mg of cholesterol) to their diet. During the low-cholesterol period the subjects were asked not to eat eggs or egg-containing products, shell fish, organ meats and butter, and to limit their intake of meat and fish to 100 g/day. In our experience such a diet results in a cholesterol intake of about 200 mg/day. At the end of this period body weight of the subjects was 69.5 ± 9.8 kg ($\pm$ SD). During the cholesterol-rich period fresh egg yolks were supplied daily as fried or boiled whole eggs, as raw yolks homogenized with orange juice, or worked into salads and desserts. The subjects generally avoided monotony by varying between different items.

Blood was drawn into evacuated, heparinized tubes from an antecubital vein after an overnight fast on the last two days of both the low- and high-cholesterol periods. The mean values of these two days were the initial and final values, respectively.

RESULTS

Figure 1 shows the zymogram of esterases in plasma from the two inbred rat strains. An anodal fast moving zone, Es-1 esterase (Van Zutphen, 1983) was clearly present in the SHR/Cpb strain, whereas in the SD/Cpb strain this zone was absent or stained only very faintly. This difference in zymotype between these rat strains has been reported earlier (Van Zutphen and Den Bieman, 1981). The presence or absence of the Es-1 esterase often predicts the serum cholesterol response to dietary cholesterol in inbred rat and rabbit strains. The cholesterolemic response was low in 6 out of 7 inbred rat strains displaying the Es-1 zone, whereas absence of the enzyme was associated with the development of high degrees of hypercholesterolemia after cholesterol feeding in 2 out of 3 inbred rat strains (Van Zutphen and Den Bieman, 1981). Similar results were obtained in a study with six inbred strains of rabbits (Van Zutphen and Fox, 1977) where the cholesterolemic response to dietary cholesterol correlated with the Est-2 genotype. The Est-2 locus of the rabbit is assumed to be homologous with the Es-1 locus in the rat (Fox and Van Zutphen, 1979).

Esterase zymograms (cf. Fig. 1) only give qualitative information. Therefore, we addressed the question of whether plasma total esterase activity would be associated with the plasma cholesterol response to dietary cholesterol. At the same time the effect of dietary cholesterol on plasma total esterase activity was investigated.

Table 1 shows that the SD/Cpb and SHR/Cpb inbred rat strains are indeed hyper- and hyporesponsive to dietary cholesterol. Cholesterol feeding for 23 days induced plasma cholesterol levels of about 8 mmole/l in the SHR/Cpb strain (hyporesponder) and of about 22 mmole/l in the SD/Cpb strain (hyperresponder). The high-cholesterol diet did not significantly affect body-weight gain of the rats (Table 1).

Plasma esterase activity on the low-cholesterol control diet was significantly lower in the hyperresponsive strain, irrespective of whether α-naphthylacetate or β-naphthylpropionate was the substrate (Table 1). Cholesterol feeding significantly increased plasma esterase activities in both strains, the activity still being higher in the hyporesponder rats than in the hyperresponders. These results could mean that plasma esterase activity is involved in cholesterol metabolism. A low esterase activity may cause an increased susceptibility to dietary cholesterol, whereas induction of plasma esterase activity may be required to compensate for cholesterol loading.

In a preliminary attempt to further substantiate our observations we performed a similar experiment with female and male rats of a random-bred Wistar strain. Female rats are known to be more sensitive to dietary cholesterol than their male counterparts (Terpstra *et al.*, 1982). Indeed, Table 2 documents that the female Wistar rats developed very high plasma cholesterol concentrations after cholesterol feeding, whereas the male rats hardly responded. Dietary cholesterol did not influence body weights of the animals (Table 2).

Initial plasma esterase activities, measured with β-naphthylpropionate, were not significantly different in male and female rats. During the course of the experiment plasma esterase activities decreased somewhat (Table 2). In the male rats cholesterol loading caused a rise in esterase activity of

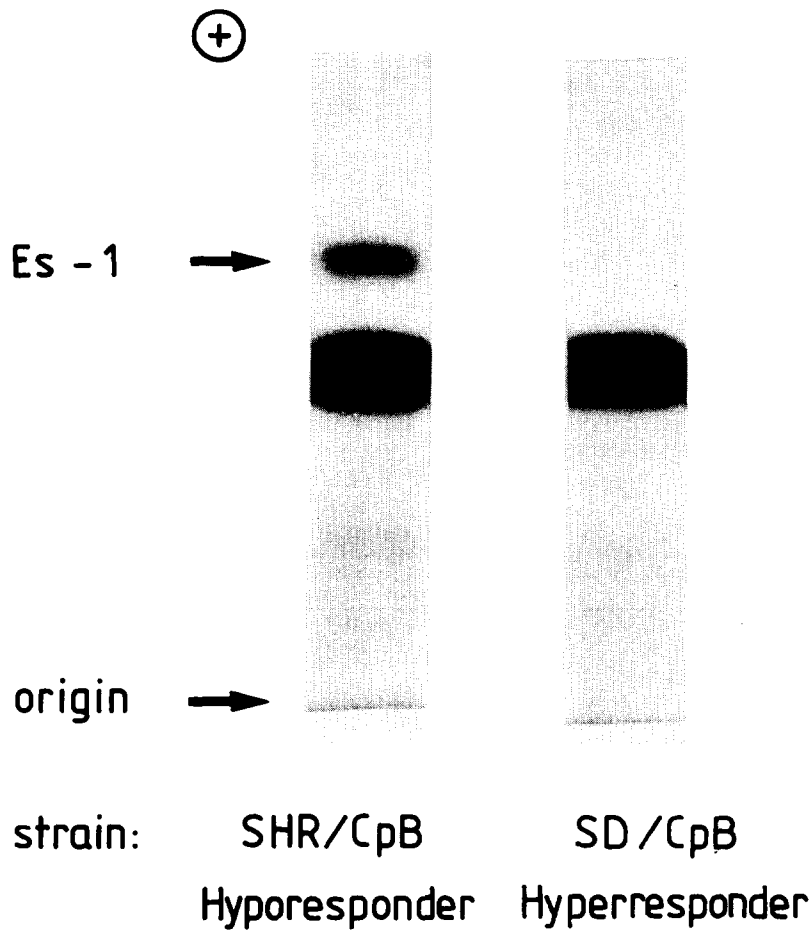

Fig. 1. Electrophoresis on starch gels of esterases in the plasma from two inbred strains of rats on the low-cholesterol control diet. β-Naphthylpropionate was used as substrate for visualizing enzyme activity.

0.87 μmole/min/ml compared with the control animals, and in the females the increase was 0.56 μmole/min/ml (Table 2).

Rabbits are very sensitive to dietary cholesterol; the feeding of a diet containing 0.2% (w, w) cholesterol caused an 8-fold increase in the level of plasma cholesterol within 4 weeks (Table 3). Dietary cholesterol did not affect plasma esterase activity measured with either α-naphthylacetate or β-naphthylpropionate as a substrate. On the cholesterol-free diet there was a marked inter-rabbit variation in the plasma esterase activities, the ranges being 0.77–12.00 and 8.30–19.86 μmole/min/ml for the substrates α-naphthylacetate and β-naphthylpropionate, respectively. For the individual animals ($n = 9$) there was no correlation between the initial plasma esterase

Table 1. Body weight, plasma cholesterol concentrations and plasma esterase activities in two inbred strains of male rats with low or high plasma cholesterol response to a high-cholesterol diet.

	Hypo-responder		Hyper-responder	
	Low-cholesterol control diet	High-cholesterol diet	Low-cholesterol control diet	High-cholesterol diet
Body weight (g)				
initial	97 ± 11	101 ± 13	97 ± 11	96 ± 11
final	166 ± 11	171 ± 15	169 ± 27	153 ± 6
Plasma cholesterol (mmole/l)				
initial	2.48 ± 0.14	2.52 ± 0.17	2.55 ± 0.13	2.62 ± 0.22
final	2.55 ± 0.12	8.53 ± 3.90	3.27 ± 0.07	22.28 ± 8.60
Plasma esterase activity* (μmole/min/ml)				
α-naphthylacetate	6.72 ± 0.27	9.34 ± 1.08	4.70 ± 0.34	7.56 ± 1.18
β-naphthylpropionate	5.91 ± 0.60	9.23 ± 0.38	3.44 ± 0.42	5.96 ± 0.34

Results are expressed as means ± SD for 6 animals in each group. All rats were fed the low-cholesterol diet for 3 weeks; on day 0 half of them were then switched to the experimental high-cholesterol diet. Initial and final values refer to days −5 and 23 of the experiment, respectively.

*Esterase activity was measured using the substrates indicated; values refer to day 23 of the experiment.

Table 2. Body weight, plasma cholesterol concentrations and plasma esterase activities in male and female random-bred Wistar rats fed a low- or high-cholesterol diet

	Male rats		Female rats	
	Low-cholesterol control diet	High-cholesterol diet	Low-cholesterol control diet	High-cholesterol diet
Body weight (g)				
initial	295 ± 40	287 ± 30	189 ± 16	193 ± 20
final	304 ± 43	324 ± 50	184 ± 14	189 ± 20
Plasma cholesterol (mmole/l)				
initial	2.10 ± 0.35	2.10 ± 0.16	1.77 ± 0.22	1.70 ± 0.17
final	1.95 ± 0.18	2.35 ± 0.41	1.53 ± 0.29	11.72 ± 6.57
Plasma esterase activity (μmole/min/ml)*				
initial	3.88 ± 0.31	4.07 ± 0.56	3.85 ± 0.22	3.67 ± 0.48
final	3.56 ± 0.61	4.63 ± 0.55	3.11 ± 0.14	3.49 ± 0.50
change	−0.32 ± 0.36	0.55 ± 0.73	−0.73 ± 0.08	−0.17 ± 0.30

Results are expressed as means ± SD for 5 animals in each group. All animals were fed the low-cholesterol diet for 10 weeks up until day 0 of the experiment; half of the rats were then switched to the experimental high-cholesterol diet. Initial and final values refer to days −2 and 19, respectively.
*Measured with α-naphthylpropionate as a substrate.

Table 3. Body weight, plasma cholesterol concentrations and plasma esterase activities in female, random-bred New Zealand White rabbits fed a low-cholesterol or high-cholesterol semipurified diet

	Cholesterol-free control diet (n = 7)	High-cholesterol diet (n = 9)
Body weight (g)		
initial	2169 ± 151	2089 ± 154
final	2643 ± 191	2552 ± 140
Plasma cholesterol (mmole/l)		
initial	3.02 ± 0.41	2.54 ± 0.75
final	3.08 ± 0.43	16.27 ± 6.81
Plasma esterase activity (μmole/min/ml)*		
α-naphthylacetate		
initial	4.61 ± 2.88	4.92 ± 3.99
final	4.59 ± 3.63	4.97 ± 4.98
change	0.00 ± 0.82	0.08 ± 1.19
β-naphthylpropionate		
initial	14.88 ± 1.67	15.60 ± 4.97
final	14.85 ± 1.44	15.66 ± 4.27
change	−0.03 ± 1.72	0.06 ± 1.86

Results are expressed as means ± SD. All rabbits were fed the low-cholesterol diet for 4 weeks up until day 0; 9 animals were then switched to the high-cholesterol diet. Initial and final values refer to days −5 and 28 of the experiment, respectively.
*Measured using the indicated substrates.

activity and subsequent plasma cholesterol response to cholesterol feeding ($r = -0.23$ for α-naphthylacetate and $r = +0.35$ for β-naphthylpropionate). Likewise there was no correlation ($n = 16$) between the initial plasma esterase activity and the initial plasma cholesterol concentration ($r = -0.14$ for α-naphthylacetate and $r = +0.08$ for β-naphthylpropionate).

In the human volunteers the consumption of six egg yolks per day caused a mean increase of 20% in the concentration of serum cholesterol (Table 4). The individual response varied considerably, the range being −3 to +44%. Similar observations were reported earlier (Katan and Beynen, 1983). On the low-cholesterol diet plasma esterase activities varied from 2.81 to 5.87 μmole/min/ml with α-naphthylacetate and from 3.29 to 5.14 μmole/min/ml with β-naphthylpropionate. Dietary cholesterol did not significantly affect plasma total esterase activities (Table 4). There was no association between initial plasma esterase activity and serum cholesterol response to egg yolk consumption in man.

DISCUSSION

Our results strengthen the evidence that one or more of the esterases found in the plasma of rats are involved in the metabolism of cholesterol. In the two inbred strains and also in the random-bred Wistar rats plasma total arylesterase activity was increased after loading with dietary cholesterol.

In the inbred rat strains plasma esterase activity on the low-cholesterol control diet was significantly lower in the hyperresponsive strain than in the hyporesponsive animals. This substantiates earlier reports (Van Zutphen and Fox, 1977; Van Zutphen and Den Bieman, 1981) which suggest that plasma esterase patterns predict the susceptibility to dietary cholesterol. However, the results obtained with the Wistar rats are not completely in line with those found in the inbred strains. The difference in cholesterolemic response to cholesterol feeding between male and female Wistar rats was not associated with different plasma esterase activities on the low-cholesterol control diet. In addition, comparison of Tables 1 and 2

Table 4. Serum cholesterol concentrations and plasma esterase activities in healthy humans consuming a high-cholesterol diet

Serum cholesterol (mmole/l)	
initial	4.18 ± 0.63
final	5.03 ± 0.82
change	0.85 ± 0.71
Plasma esterase activity (μmole/min/ml)	
α-naphthylacetate	
initial	4.41 ± 1.14
final	4.59 ± 0.85
change	0.19 ± 0.44
β-naphthylpropionate	
initial	4.23 ± 0.66
final	4.31 ± 0.74
change	0.08 ± 0.18

Results are expressed as means ± SD for 5 subjects. The subjects abstained from cholesterol-rich products for 10 days (day −9 to 0) and then starting with day 1 they added six egg yolks per day to their diet for another 10 days. The initial values in the table are the mean values of days −1 and 0 and the final values are the means of days 9 and 10.

shows that on the low-cholesterol diet the inbred hyporesponsive rats have a higher plasma esterase activity with β-naphthylpropionate as substrate than the male Wistar rats, although the inbred rats were more sensitive to dietary cholesterol. Thus plasma total esterase activity is not a consistent predictor of the susceptibility to dietary cholesterol in all rat strains.

No evidence for a role of plasma arylesterases in cholesterol metabolism was obtained in our studies with random-bred rabbits and in humans. However, it should be realized that human and rabbit plasma contain at least 12 different arylesterases (Pilz, 1964; Pilz *et al.*, 1968), most of them probably not related to cholesterol metabolism. As only one of the esterases (Est-2) in the zymogram of rabbit plasma has been found to predict the serum cholesterol response to dietary cholesterol, it would be desirable to study and measure the plasma esterases separately. Such studies are now in progress, and it is anticipated that they will shed more light on the physiological function(s) of plasma arylesterases.

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