British Journal of Nutrition (1993), **70**, 721–726

https://doi.org/10.1079/BJN19930167 Published online by Cambridge University Press

Dietary cholesterol lowers the activity of butyrylcholinesterase (*EC* 3.1.1.8), but elevates that of esterase-1 (*EC* 3.1.1.1) in plasma of rats

BY H. A. VAN LITH AND A. C. BEYNEN

Department of Laboratory Animal Science, Faculty of Veterinary Medicine, Utrecht University, PO Box 80.166, NL-3508 TD Utrecht, The Netherlands

(*Received 9 October 1992 – Accepted 5 February 1993*)

The question addressed is whether an increased intake of cholesterol affects esterase-1 (*EC* 3.1.1.1; ES-1) and butyrylcholinesterase (*EC* 3.1.1.8) activity in plasma. Rats were fed on a purified diet either without or with cholesterol (10 g/kg) added at the expense of the carbohydrate source. Dietary cholesterol significantly decreased plasma butyrylcholinesterase activity, but raised plasma ES-1 activity. Evidence is discussed, suggesting that plasma butyrylcholinesterase is involved in plasma cholesterol metabolism, whereas esterase-1 is involved in intestinal cholesterol absorption.

Butyrylcholinesterase: Esterase-1 (ES-1): Dietary cholesterol: Plasma: Liver: Rat

A variety of esterases is present in the blood plasma of vertebrate animals (Augustinsson, 1958). The physiological function of these enzymes is still obscure, but there is evidence that they respond to the amount of fat in the diet. Butyrylcholinesterase (*EC* 3.1.1.8), also referred to as cholinesterase or pseudocholinesterase, represents a large portion of plasma total esterase activity. Osada *et al.* (1989) reported that the feeding of a high-fat diet instead of a low-fat diet lowers plasma butyrylcholinesterase activity in rats. However, this observation may be biased because the high-fat diet was prepared by mixing the low-fat diet with fat so that the two diets had different nutrient:energy values for all nutrients. Moreover, the fat used by Osada *et al.* (1989) to prepare the high-fat diet contained cholesterol so that the high-fat diet contained more cholesterol than the low-fat diet. Thus, it is possible that the observed lowering of butyrylcholinesterase activity after feeding the high-fat diet was caused by the increased intake of cholesterol.

In rats, increasing intakes of maize oil, coconut fat, olive oil or medium-chain triacylglycerols at the expense of isoenergetic amounts of carbohydrates cause a pronounced increment in the plasma activity of the so-called esterase-1 (ES-1) isozyme (*EC* 3.1.1.1), an anodal fast-moving esterase zone in the plasma zymogram (Van Lith *et al.* 1992*b*). Under those conditions there was only a relatively small effect of fat type, suggesting that the amount of fat primarily influences plasma ES-1 activity. It was not known whether the amount of cholesterol in the diet affects plasma ES-1 activity.

The aim of the present work was to determine whether cholesterol in the diet influences plasma ES-1 and butyrylcholinesterase activity. For this purpose rats were given either a cholesterol-free or high-cholesterol diet and esterase activities were measured in blood plasma collected at different time-intervals.

https://doi.org/10.1079/BJN19930167 Published online by Cambridge University Press

MATERIALS AND METHODS

Animals and housing

Female outbred Wistar rats (HsdCpb:WU; Harlan-CPB, Zeist, The Netherlands), aged 3 weeks, were used. All animals possessed the Es-1^a but not the Es-1^c allele and, thus, showed the ES-1A isozyme in the plasma zymogram. The purified, cholesterol-free diet (see below) was fed to all animals for the pre-experimental period of 10 d. During this period the rats were housed in groups of five to six animals in wire-topped Makrolon-3 cages (UNO BV, Zevenaar, The Netherlands) with a layer of sawdust as bedding. The cages were located in a room with controlled lighting (light from 07.00 to 19.00 hours), temperature (20–22°) and relative humidity (55–65%).

At the end of the pre-experimental period (day 0) the rats were divided into a test and control group of eighteen animals each. There was an additional control group of six rats, which were to be killed on day 0 (see below). Control and test animals were subdivided into four and three groups respectively, consisting of six animals each. Rats were selected by a computerized randomization program that ensured that body-weight distributions for each group were comparable. The control animals remained on the cholesterol-free diet; the test animals were transferred to a cholesterol-rich diet (see below). During the experimental period the animals were kept three in a cage with randomized cage position.

Diets

The cholesterol-free, control diet consisted of the following components (g/kg): casein 151, maize oil 25, coconut fat 75, maize starch 304·7, dextrin 304·7, molasses 50, cellulose 30, $CaCO_3$ 12·4, $NaH_2PO_4.2H_2O$ 15·1, $MgCO_3$ 1·4, KCl 1·0, $KHCO_3$ 7·7, mineral premix 10, vitamin premix 12. The composition of the mineral and vitamin premixes has been described elsewhere (Grooten *et al.* 1991). To formulate the cholesterol-rich diet, cholesterol was added to the control diet (10 g cholesterol/kg diet) at the expense of maize starch and dextrin in the proportions 1:1 (w/w). The diets were in pelleted form (diameter 10 mm) and stored at 4° until feeding. During the pre-experimental and experimental periods the rats had free access to food and tap water. Because the animals were housed in cages with bedding, feed spillage could not be determined and, thus, feed utilization was not recorded. However, this may not detract from the interpretation of the present results because in previous experiments using comparable rats and comparable purified diets the addition of 10 g cholesterol/kg diet had not caused a difference in feed intake (Beynen *et al.* 1988*a*, *b*; Beynen, 1989).

Preparation of samples

Blood samples were taken into heparinized tubes between 09.00 and 10.00 hours while the non-fasted rats were under light diethyl ether anaesthesia. Samples were drawn on day 0 from six control rats and on days 7, 14 and 21 from six control and six test rats. Plasma was prepared by low-speed centrifugation (500 ***g***, 10 min) and kept at −20° until analysis. After blood sampling, the rats were killed by the intraperitoneal administration of 15 mg pentobarbital (Nembutal®; Sanofi Sante Animale SA, Paris, France) and the livers were removed. After weighing, the livers were immediately frozen (−20°) until analysis.

Analyses of plasma and liver

Total cholesterol in plasma was estimated according to Siedel *et al.* (1983) using a test combination supplied by Boehringer-Mannheim GmbH (Mannheim, Germany). To determine plasma free cholesterol concentration, cholesterol esterase (*EC* 3.1.1.13) was

Table 1. *Body weight and liver weight of rats fed on either a cholesterol-free or a cholesterol-rich diet*†

(Values are means with their standard errors for six rats per group)

Period on diets (d)...		7		14		21		Statistical significance of effect of‡:	
	Diet	Mean	SE	Mean	SE	Mean	SE	C	T
Body wt (g)	Control	122·5	3·4	149·1	5·1	157·7	5·3		*
	Cholesterol-rich	127·6	3·6	146·6	3·9	158·8	3·7		
Liver wt (g)	Control	5·6	0·3	6·8	0·3	6·5	0·3		
	Cholesterol-rich	6·6	0·3	7·7	0·5	7·8	0·3	*	*

T, time (df 2); C, cholesterol (df 1).
* $P < 0.05$.
† For details of diets and procedures, see pp. 722–723.
‡ Based on two-way analysis of variance (residual df 30).

omitted from the reaction mixture. Pieces of liver were homogenized in distilled water and total cholesterol was extracted and analysed colorimetrically according to Abell *et al.* (1952).

Plasma butyrylcholinesterase activities were determined by the method of Ellman *et al.* (1961) using butyrylthiocholine as substrate. Plasma ES-1 activity was determined by scanning densitometry of gradient polyacrylamide gels after visualization of the esterase pattern (Van Lith *et al.* 1991*a*), and expressed relative to a plasma ES-1 standard. This method of reporting ES-1 activities has been validated (Van Lith *et al.* 1991*a*). Esterase activities were linear with time and enzyme concentration. Enzyme activities were corrected for spontaneous hydrolysis of the substrates.

Statistics

Results are expressed as means with their standard errors. The Kolmogorov–Smirnov one-sample test was used to check normality of the data. All within-group results were found to be normally distributed. Data were analysed by two-way analysis of variance with amount of dietary cholesterol and time as main effects. Homogeneity of variances was tested with Bartlett's test. When necessary, the variances were equalized by ranking of the data. After ranking the within-group data were still normally distributed. Two-side probabilities were estimated throughout, and the level of statistical significance was pre-set at $P < 0.05$. All statistical analyses were carried out using a computer program (SPSS/PC+, 1988).

RESULTS

Initial (day −2) body weight of the rats was 96·4 (SE 0·9) g (*n* 42). Body weight increased with time at similar rates in the control and test group (Table 1). In keeping with earlier work (Herman *et al.* 1991), dietary cholesterol as the only dietary variable caused increased liver weight (Table 1).

Initial (day 0) plasma and liver total cholesterol concentrations were 2·66 (SE 0·08) mM and 5·5 (SE 0·2) μmol/g, respectively (*n* 6). Feeding the cholesterol-rich diet caused increased concentrations of total and free cholesterol in plasma (Table 2). The consumption of cholesterol drastically raised liver total cholesterol concentrations. This corroborates

https://doi.org/10.1079/BJN19930167 Published online by Cambridge University Press

Table 2. *Liver and plasma cholesterol concentrations of rats fed on either a cholesterol-free or a cholesterol-rich diet*†

(Values are means with their standard errors for six rats per group)

Periods on diets (d)...		7		14		21		Statistical significance of effect of‡:
	Diet	Mean	SE	Mean	SE	Mean	SE	C
Liver total cholesterol (μmol/g)	Control	5·7	0·2	5·5	0·2	5·4	0·1	*§
	Cholesterol-rich	55·1	10·5	54·4	5·2	60·8	3·0	
Plasma cholesterol (mM)								
Total	Control	2·70	0·15	2·18	0·16	2·43	0·10	
	Cholesterol-rich	3·20	0·19	3·44	0·23	3·34	0·35	*
Free	Control	0·65	0·02	0·53	0·03	0·52	0·03	
	Cholesterol-rich	0·70	0·06	0·68	0·06	0·60	0·06	*

C, cholesterol (df 1).
* $P < 0·05$.
† For details of diets and procedures, see pp. 722–723.
‡ Based on two-way analysis of variance (residual df 30).
§ Two-way analysis of variance after ranking of the data.

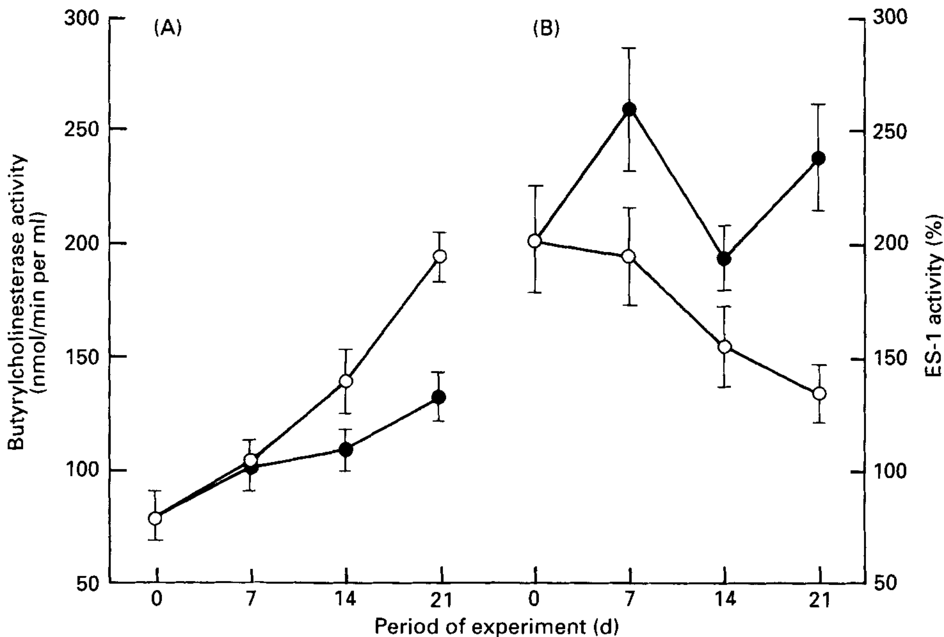

Fig. 1. (A) Plasma butyrylcholinesterase (*EC* 3.1.1.8) and (B) esterase-1 (*EC* 3.1.1.1; ES-1) activity of rats fed on either a cholesterol-free (○) or cholesterol-rich diet (●). Results are expressed as means with their standard errors represented by vertical bars for six rats/group. Statistical significance ($P < 0·05$) is based on two-way analysis of variance (residual df 30): T, effect of time (df 2); C, effect of cholesterol (df 1); T × C, interaction (df 2). Statistically significant effects on butyrylcholinesterase were: T, C, T × C; statistically significant effects on ES-1 were: T, C. For details of diets and procedures, see pp. 722–723.

https://doi.org/10.1079/BJN19930167 Published online by Cambridge University Press

previous work (Herman *et al.* 1991; Zhang *et al.* 1992). Plasma and liver cholesterol concentrations in the rats fed on cholesterol did not rise further after 7 d (Table 2).

During the course of the experiment plasma butyrylcholinesterase activity in control rats increased, whereas ES-1 activity dropped (Fig. 1). Cholesterol in the diet in part counteracted the rise of plasma butyrylcholinesterase activity with time. On the other hand, cholesterol consumption elevated plasma ES-1 activity.

DISCUSSION

The present study shows that an increased intake of cholesterol as the only dietary variable lowered plasma butyrylcholinesterase activity in rats. Thus, it is now possible to explain the apparent discrepancy between our work (Van Lith *et al.* 1990, 1991*b*) and that of Osada *et al.* (1989). Increased intakes of maize oil at the expense of isoenergetic amounts of carbohydrates slightly, but significantly, raise plasma butyrylcholinesterase activity in rats (Van Lith *et al.* 1991*b*), whereas an increased consumption of coconut fat does not influence butyrylcholinesterase activity (Van Lith *et al.* 1990). Osada *et al.* (1989) found that a diet enriched with a saturated margarine lowers plasma butyrylcholinesterase activity. However, the high-fat diet contained more cholesterol than the control diet, and, thus, the effect observed by Osada *et al.* (1989) can be explained by the increased intake of cholesterol with the high-margarine diet. The present study supports this reasoning. Butyrylcholinesterase activity can be influenced by the type of fat in the diet. Fish oil added to the diet at the expense of either olive oil, coconut fat or maize oil raises butyrylcholinesterase activity in blood plasma of rats (Van Lith *et al.* 1992*a*).

The present study may support the idea of Kutty (1980) that plasma butyrylcholinesterase activity is involved in plasma cholesterol metabolism. During the course of the experiment group mean plasma butyrylcholinesterase activity was significantly associated with group mean plasma free-cholesterol level from pooled control and test rats (r −0·863; n 6; $P < 0{\cdot}03$). This relationship could be spurious rather than causative. There was no significant association between group mean plasma butyrylcholinesterase activity and total cholesterol concentration in either plasma or liver.

There was a significant increment in plasma ES-1 activity after cholesterol loading. This observation is compatible with our view (Van Lith *et al.* 1992*b*) that ES-1 is either involved in the uptake of lipids, including cholesterol, by the intestinal brush-border membranes or in the transport of lipids across the membrane of the mucosal endoplasmic reticulum. During either process ES-1 might be released from the intestine into intestinal lymph. There is indirect evidence for this suggestion. Increased fat intakes at the expense of isoenergetic amounts of carbohydrate have been found to raise both the activity of plasma ES-1 and jejunal ES-1 in rats (Van Lith *et al.* 1992*b*). An increased intake of cholesterol by rats might also elevate ES-1 activity in jejunum. Beynen *et al.* (1987) showed that the activity and concentration of mouse plasma ES-2, which is the mouse homologue of rat ES-1 (Van Zutphen & Den Bieman, 1988), are raised after the feeding of a high-cholesterol diet which was associated with an increase in ES-2 in the small intestine. Rat ES-1 and human esterase ESB_2 (*EC* 3.1.1.1) have the same evolutionary origin (Van Lith *et al.* 1993). Thus, it is possible that in humans ESB_2 is involved in intestinal cholesterol metabolism.

REFERENCES

Abell, L. L., Levy, B. B., Brodie, B. B. & Kendall, F. E. (1952). A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *Journal of Biological Chemistry* **195**, 357–366.

Augustinsson, K. B. (1958). Electrophoretic separation and classification of blood plasma esterases. *Nature* **181**, 1786–1789.

https://doi.org/10.1079/BJN19930167 Published online by Cambridge University Press

Beynen, A. C. (1989). Increased concentrations of liver cholesterol in rats fed lactulose. *Die Nahrung* **33**, 89–90.

Beynen, A. C., Fielmich-Bouman, A. M. & Lemmens, A. G. (1988*a*). Dietary lactitol elevates liver cholesterol in rats. *International Journal for Vitamin and Nutrition Research* **58**, 471–472.

Beynen, A. C., Lemmens, A. G., De Bruijne, J. J., Ronai, A., Wassmer, B., Von Deimling, O., Katan, M. B. & Van Zutphen, L. F. M. (1987). Esterases in inbred strains of mice with differential cholesterolemic responses to a high-cholesterol diet. *Atherosclerosis* **63**, 239–249.

Beynen, A. C., Lemmens, A. G., De Vries, H. & Van Der Meer, R. (1988*b*). Differential metabolic basis for the hypocholesterolemic effects of cholestyramine and pectin in rats. *Atherosclerosis* **73**, 87–88.

Ellman, G. L., Courtney, K. D., Andres, V. & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology* **7**, 88–95.

Grooten, H. N. A., Ritskes-Hoitinga, J., Mathot, J. N. J. J., Lemmens, A. G. & Beynen, A. C. (1991). Dietary fluoride prevents phosphorus-induced nephrocalcinosis. *Biological Trace Element Research* **29**, 147–155.

Herman, S., Sediaoetama, A. D., Karayadi, D. & Beynen, A. C. (1991). Influence of background composition of the diet on the lipemic effect of fish oil versus corn oil in rats. *Journal of Nutrition* **121**, 622–630.

Kutty, K. M. (1980). Biological function of cholinesterase. *Clinical Biochemistry* **13**, 239–243.

Osada, J., Hortensia, A., Gonzalo, C., Miro-Obradors, M. J. & Palacios-Alaiz, E. (1989). Changes in serum cholinesterase (*EC* 3.1.1.8) activity in rats consuming a high-fat diet. *British Journal of Nutrition* **62**, 343–348.

Siedel, J., Hagelle, E. O., Ziegenhorn, J. & Wahlefeld, A. W. (1983). Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. *Clinical Chemistry* **29**, 1075–1080.

SPSS/PC+ (1988). *Statistical Package for the Social Sciences: Base Manual* V 2.0. Chicago, IL: SPSS Inc.

Van Lith, H. A., Haller, M., Van Hoof, I. J. M., Van Der Wouw, M. J. A., Van Zutphen, L. F. M. & Beynen, A. C. (1993). Characterization of rat plasma esterase ES-1A concerning its molecular and catalytic properties. *Archives of Biochemistry and Biophysics* **301**, 265–274.

Van Lith, H. A., Haller, M., Van Tintelen, G., Van Zutphen, L. F. M. & Beynen, A. C. (1992*a*). Plasma esterase-1 (ES-1) activity in rats is influenced by the amount and type of dietary fat and butyrylcholinesterase activity by the type of dietary fat. *Journal of Nutrition* **122**, 2109–2120.

Van Lith, H. A., Haller, M., Van Zutphen, L. F. M. & Beynen, A. C. (1991*a*). Determination of rat-plasma esterase-1 (ES-1) activity by scanning densitometry of gradient polyacrylamide gels with zymogram detection. *Electrophoresis* **12**, 1045–1050.

Van Lith, H. A., Herman, S., Zhang, X., Van Der Palen, J. G. P., Van Zutphen, L. F. M. & Beynen, A. C. (1990). Influence of dietary fats on butyrylcholinesterase and esterase-1 (ES-1) activity in plasma of rats. *Lipids* **25**, 779–786.

Van Lith, H. A., Meijer, G. W., Van Der Wouw, M. J. A., Den Bieman, M., Van Tintelen, G., Van Zutphen, L. F. M. & Beynen, A. C. (1992*b*). Influence of amount of dietary fat and protein on esterase-1 (ES-1) activities of plasma and small intestine in rats. *British Journal of Nutrition* **67**, 379–390.

Van Lith, H. A., Van Zutphen, L. F. M. & Beynen, A. C. (1991*b*). Butyrylcholinesterase activity in plasma of rats and rabbits fed high-fat diets. *Comparative Biochemistry and Physiology* **98A**, 339–342.

Van Zutphen, L. F. M. & Den Bieman, M. G. C. W. (1988). Gene mapping and linkage homology. In *New Developments in Biosciences: Their Implications for Laboratory Animal Science*, pp. 197–201 [A. C. Beynen and H. A. Solleveld, editors]. Dordrecht, The Netherlands: Martinus Nijhoff Publishers.

Zhang, X., Joles, J. A., Koomans, H. A., Van Tol, A. & Beynen, A. C. (1992). Excessive cholesterolemic response in analbuminemic rats fed a cholesterol-rich diet containing casein. *Journal of Nutrition* **122**, 520–527.

https://doi.org/10.1079/BJN19930167 Published online by Cambridge University Press