Influence of amount of dietary fat and protein on esterase-1 (ES-1) activities of plasma and small intestine in rats

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The objective of the present study was to characterize nutritionally esterase-1 (ES-1). For this purpose, the effects of replacement of dietary carbohydrates by isoenergetic amounts of either fat or protein on ES-1 activities of plasma and small intestine were studied in male rats. Purified diets differing in the amounts of maize starch plus dextrose, casein and various types of fat were used. Plasma and jejunal ES-1 activities were found to be increased with increasing fat intakes. As to the type of fat, increasing plasma ES-1 activities were induced by coconut fat, olive oil, maize oil and medium-chain triacylglycerols, in this order. Maize oil induced higher jejunal ES-1 activities than coconut fat and olive oil, but had similar effects to medium-chain triacylglycerols. Maize oil was more powerful in increasing plasma ES-1 activity than isoenergetic amounts of casein, but with respect to jejunal ES-1 activity these dietary components were equally effective. It is concluded that the amounts of fat and protein in the diet are important determinants of ES-1 activities in plasma and jejunum.

Dietary fat: Esterase-1: Plasma: Intestine: Rat

Esterases (*EC* 3.1.1.) form a complex system of non-specific enzymes present in most living organisms (Krisch, 1971). They can be detected in most tissues, but are most abundant in liver, intestine, kidney and plasma. The esterases catalyse the hydrolysis of artificial organic esters such as α-naphthylacetate and *p*-nitrophenylacetate. Isozymes of esterase catalyse the hydrolysis of a wide range of xenobiotic carboxylesters and aromatic amides, including various anaesthetics (Mentlein & Heymann, 1984).

The function of the esterases with respect to natural substrates is still obscure, but there is some evidence that they are involved in lipid metabolism. In vitro studies have shown that rat liver esterases can hydrolyse various classes of lipids, including monoacylglycerols (Mentlein *et al.* 1985). Involvement of esterases in lipid metabolism is also indicated by in vivo studies. Replacement of isoenergetic amounts of carbohydrates by either maize oil or coconut fat in the diet of rats caused a slight increase in plasma total esterase activity, as measured with *p*-nitrophenylacetate as substrate (Van Lith *et al.* 1989). This increase was associated with a pronounced increase of the so-called ES-1 isozyme, an anodal, fast-moving esterase zone in the plasma zymogram. Lewis & Hunter (1966) reported that administration of fat by stomach tube caused an increase in the activity of esterase of high electrophoretic mobility in intestinal lymph of rats. It could be suggested that ES-1 is involved in fat absorption and that it is released from the intestine during this process. Three dietary trials with rats have been carried out in order to obtain clues as to this

suggestion and to characterize plasma and jejunal ES-1 activities nutritionally. The amount and type of fat and the amount of protein in the diet have been focussed on as possible determinants of ES-1.

MATERIALS AND METHODS

Animals

Male rats aged 4–6 weeks, of an outbred Wistar colony (Cpb:WU), were used throughout. All animals had been found to possess the Es-1^a allele. The rats had been fed *ad lib.* on a commercial pelleted non-purified diet (RMH-B®; Hope Farms B. V., Woerden). The animals were housed individually exactly as described previously (Beynen, 1987). On day 0 of each experiment the rats were divided into four to eight dietary groups of six animals each. Within each experiment the groups had similar distributions of body-weight. The mean values were: Expt 1 72 g, Expt 2 78 g, Expt 3 95 g.

General characteristics of the experimental diets

Rats were fed on purified diets in meal form. All diets contained at least 2·75 energy % as maize oil in order to provide sufficient linoleic acid. This implies that the diets to which either coconut fat, olive oil or medium-chain triacylglycerols had been added actually contained 2·75 of energy % less in the form of these fat sources than indicated. Separate batches of diet were made for each experiment. The diets were stored at 4° until feeding. The rats had free access to food and tap water.

Background to the experimental design

The objectives of the three trials were as follows. In the first experiment we addressed the question of whether increased intakes of fat, in the form of either coconut fat, maize oil or olive oil, would influence ES-1 activity of plasma and various sections of the small intestine. In the second experiment rats were fed on diets containing various concentrations of either maize oil or medium-chain triacylglycerols in order to compare the effects of these fat sources on ES-1 in plasma and intestine. The information obtained could provide clues as to the physiological function of ES-1 because the long-chain fatty acids in maize oil and the medium-chain fatty acids are absorbed through different mechanisms. In Expts 1 and 2 the fats were added to the purified diet at the expense of isoenergetic amounts of maize starch plus dextrose. It could be argued that the omission of carbohydrates from the diet as well as the addition of fat to the diet is responsible for the observed effects on ES-1 in plasma and intestine. This prompted us to perform a third experiment in which dietary maize oil in a high-protein diet was substituted for isoenergetic amounts of either carbohydrates or casein.

Diets

Expt 1. The diets contained various concentrations of either coconut fat (Croklaan NV, Wormerveer) maize oil (Mazola®; Knorr Caterplan Gmbh, Heilbronn/Neckar, Germany) or olive oil (Levant®; Huilerie L'Abeille, Marseille, France). Table 1 shows the composition of the diets. The constant components consisted of (g/kg diet): casein 160, molasses 100, cellulose 150, dicalcium phosphate 6·1, calcium carbonate 6·2, magnesium carbonate 0·7, magnesium oxide 0·3, potassium bicarbonate 18, sodium chloride 5, vitamin premix 12, mineral premix 10. The mineral premix consisted of the following (mg/kg diet): sodium citrate ($Na_3C_6H_5O_7.2H_2O$) 1535, $FeSO_4.7H_2O$ 900, MnO_2 140, $KAl(SO_4)_2.12H_2O$ 200, $ZnSO_4.H_2O$ 125, KBr 20, $NiSO_4.6H_2O$ 8·5, NaF 8·5, $CuSO_4.5H_2O$ 100, $CoSO_4.7H_2O$ 5, $Na_2Mo_4.2H_2O$ 5, KI 5, As_2O_3 0·2, $Na_2B_4O_7.10H_2O$ 5, $Na_2SeO_3.5H_2O$ 0·15, maize meal

Table 1. *Expt 1. Composition of the diets*

	Dietary fat concentrations*							
	Coconut fat		Maize oil		Olive oil			
	5·5	44·0	5·5	44·0	5·5	11·0	22·0	44·0
Ingredient (g)								
Maize starch	255·85	98·35	255·85	98·35	255·85	233·35	188·35	98·35
Dextrose	255·85	98·35	255·85	98·35	255·85	233·35	188·35	98·35
Coconut fat	10	150	0	0	0	0	0	0
Maize oil	10	10	20	160	10	10	10	10
Olive oil	0	0	0	0	10	30	70	150
Constant components	468·3	468·3	468·3	468·3	468·3	468·3	468·3	468·3
Total (g)	1000	825	1000	825	1000	975	925	825
Chemical analysis (g/kg diet)								
Crude fat	21	194	21	195	21	43	87	191
Fatty acids (g/kg total fatty acids)								
8:0	34	68	0	0	0	0	0	1
10:0	25	48	0	0	0	0	0	0
12:0	200	409	2	1	3	1	1	0
14:0	82	175	2	1	2	1	1	0
16:0	105	103	104	103	111	111	115	121
18:0	27	33	20	20	25	27	28	29
18:1	196	73	292	292	504	609	664	687
18:2	312	64	555	562	325	218	161	131
Total Sat	481	835	137	133	150	149	152	158
Total Mono	198	99	296	296	512	619	675	700
Total Poly	317	65	564	571	333	226	168	136

Sat, saturated; Mono, monounsaturated; Poly, polyunsaturated.
* Expressed as calculated percentage of energy.

6942·65. The vitamin premix consisted of the following (mg/kg diet): thiamin 60, riboflavin 22·5, nicotinamide 152, DL-calcium panthothenate 56, pyridoxine 22·5, cyanocobalamin 0·015, choline chloride 2000, inositol 1000, pteroylmonoglutamic acid 8·5, biotin 1, *p*-aminobenzoic acid 500, menadione 4, DL-α-tocopheryl acetate 50, retinyl acetate and retinyl palmitate 30 (15000 IU), cholecalciferol 30 (3000 IU), L-ascorbic acid 400, maize meal 7663·485. The calculated fat concentrations expressed as g/kg (energy %), were 20 (5·5), 41 (11·0), 86 (22·0) and 194 (44·0). Fat was added to the diets at the expense of isoenergetic amounts of maize starch and dextrose in proportions 1:1 (w/w).

Expt 2. In this experiment, either maize oil or medium-chain triacylglycerols (Ceres®, Van den Bergh & Jurgens, Rotterdam) were added to the diet at the expense of maize starch and dextrose. Table 2 shows the composition of the diets. The composition of the constant components and the diets containing 5·5 and 44·0 energy % as maize oil were identical to those of Expt 1.

Expt 3. For this experiment, diet composition other than those used in Expts 1 and 2 were used. In order to study the effect of substitution of fat for protein, a high-protein, low-fat diet had to be used to avoid reduced growth by protein deficiency after replacement of protein by fat. The low-fat diets contained 4·9 energy % from maize oil and either 17·5 or 51·9 energy % from casein (Table 3). Extra maize oil (34·4 energy %) was added at the

Table 2. *Expt 2. Composition of the diets*

	Dietary fat concentrations*							
	Maize oil				Medium-chain triacylglycerols			
	5·5	11·0	22·0	44·0	5·5	11·0	22·0	44·0
Ingredient (g)								
Maize starch	255·85	233·35	188·35	98·35	255·85	233·35	188·35	98·35
Dextrose	255·85	233·35	188·35	98·35	255·85	233·35	188·35	98·35
Maize oil	20	40	80	160	10	10	10	10
Medium chain triacylglycerols	0	0	0	0	10	30	70	150
Constant components	468·3	468·3	468·3	468·3	468·3	468·3	468·3	468·3
Total (g)	1000	975	925	825	1000	975	925	825
Chemical analysis (g/kg diet)								
Crude fat	23	43	89	190	22	44	89	185
Fatty acids (g/kg total fatty acids)								
8:0	0	0	0	0	265	393	485	527
10:0	0	0	0	0	175	289	345	375
12:0	1	0	0	0	8	12	13	14
14:0	4	1	0	0	3	2	0	1
16:0	107	104	103	103	61	33	18	9
18:0	21	20	20	13	12	6	3	1
18:1	297	303	303	302	162	88	46	25
18:2	534	546	550	555	298	164	84	42
Total Sat	142	135	132	129	526	736	864	927
Total Mono	306	308	307	305	165	89	46	25
Total Poly	542	553	553	563	303	167	85	42

Sat, saturated; Mono, monounsaturated; Poly, polyunsaturated.
* Expressed as calculated percentage of energy.

expense of either casein or maize starch plus dextrose. The composition of the constant components was identical to that of Expts 1 and 2, except that casein and molasses were omitted and the amount of cellulose was reduced to 50 g/kg diet.

Preparation of samples

At the end of each experiment (Expt 1 day 62, Expt 2 day 58, Expt 3 day 56), between 10.00 and 13.00 hours, the rats were anaesthetized in the non-fasting state by the intraperitoneal administration of 15 mg pentobarbital (Nembutal®, Sanofi Sante Animale SA, Paris, France). Blood was taken by aortic puncture and 4·5 ml was mixed with 0·5 ml distilled water containing 38 g sodium citrate/kg. Plasma was collected by low-speed centrifugation and kept at −20° until analysis.

After blood sampling, the entire small intestine was removed, trimmed free of fat and mesentery and divided into duodenum, jejunum and ileum. The jejunum was further divided into three parts of equal length. The five small intestinal parts were cut lengthwise and rinsed in ice-cold saline (9 g NaCl/l). The sections of the small intestine were homogenized on ice in 5 vol. buffer (50 mM-Tris–hydrochloric acid, pH 7·5, containing 1 g Triton X-100/kg) with a 60 s burst of an Ultraturrax tissue homogenizer at 20000 rev./min. The homogenates were frozen at −20°, thawed and subsequently centrifuged at 4° for

Table 3. *Expt 3. Composition of the diets*

	Dietary fat and protein concentrations*			
Maize oil...	4·9	39·3	4·9	39·3
Casein...	17·5	17·5	51·9	51·9
Ingredient (g)				
Casein	160	160	475	475
Maize starch	356	198	198	41
Dextrose	356	198	198	41
Maize oil	20	160	20	160
Constant components	108	108	108	108
Total (g)	1000	824	999	825
Chemical analysis (g/kg diet)				
Crude fat	22	183	22	186
Crude protein (nitrogen × 6·25)	143	175	407	506
Fatty acids (g/kg total fatty acids)				
8:0	0	0	0	0
10:0	0	0	0	0
12:0	1	0	2	0
14:0	2	1	5	1
16:0	106	102	111	101
18:0	21	19	24	18
18:1	303	300	301	301
18:2	535	554	521	553
Total Sat	142	130	155	127
Total Mono	309	304	307	305
Total Poly	543	563	529	561

Sat, saturated; Mono, monounsaturated; Poly, polyunsaturated.
* Expressed as calculated percentage of energy.

20 min at 40000 ***g***. The supernatant fractions were stored at −20° until use. Since ES-1 in the proximal part of the jejunum was found to be most sensitive to diet changes in Expt 1, in further experiments only this intestinal section was collected and analysed.

Analyses

Crude fat concentrations and fatty acid compositions of the diets were determined according to Folch *et al.* (1957) and Metcalfe *et al.* (1966) respectively. Protein contents of the diets were determined as described by Osborne & Voogt (1978). Protein contents of the small intestinal extracts were determined by the dye-binding method of Bradford (1976). Total esterase activity in plasma and in the small intestinal extracts were measured with *p*-nitrophenylacetate as substrate as described previously (Van Lith *et al.* 1989). Plasma and small intestinal esterase patterns were determined by vertical 45–120 g polyacrylamide/l gradient gel electrophoresis at pH 9·0 according to Beynen *et al.* (1987). For electrophoresis of intestinal extracts, 10 μl of a mixture containing 150 mg small intestinal protein/l and 100 g glycerol/kg was applied to each slot of the slab gels. Each slab gel contained an ES-1 standard in the form of a pooled, small intestinal extract. For the plasma samples, 10 μl of a mixture containing 675 ml plasma/l, 3 g sodium citrate/kg and 100 g glycerol/kg was added to the slots. On each slab gel, pooled rat plasma was run as a plasma ES-1 standard.

Plasma and small intestinal ES-1 standards were stored in small portions at −20° until use. The same standards were used throughout the experiments. After electrophoresis, the gels were stained for esterase activity as described by Van Lith *et al.* (1989). The intensity of the ES-1 band was measured by densitometric scanning of the stained gels at 530 nm and was expressed relative to the intensity of the ES-1 standard.

Statistics

Data were analysed with Scheffé's test for comparing group means. Analysis of variance was performed to disclose interactions between amount and type of fat or amount of fat and protein. Statistical analyses were carried out using a computer program (SPSS/PC$^+$, 1988).

RESULTS

There were no significant effects of the amount of dietary fat or protein and fat type with regard to final body-weight and body-weight gain in the three experiments (results not shown). Feed intake was significantly reduced with increasing fat concentrations of the diet in each experiment, but there was no effect of fat type or amount of protein (results not shown).

Expt 1

An increase in fat content of the diets resulted in a significant increase of plasma total and ES-1 esterase activity (Table 4). Fat type influenced plasma ES-1 activity, rats fed on the coconut-fat diets showing the lowest activities. In rats fed on diets containing olive oil there was a linear relationship between the activity of plasma ES-1 and amount of olive oil in the diet.

Small intestinal total esterase activities differed between the five sections (one-way analysis of variance: $P < 0{\cdot}05$; Table 5); the lowest activities were found in the ileum. Increasing the amount of dietary fat caused a significant increase in total esterase activity of duodenum and jejunum. In rats fed on the diets containing olive oil this increase was seen only if the diet contained at least 22·0 energy % from fat. The diet containing 44 energy % from maize oil, but not those containing 44·0 energy % from coconut fat or olive oil, caused an increase in total esterase activity of the ileum.

Highest ES-1 activities were detected in the duodenum and proximal jejunum; lowest activities were found in the ileum (one-way analysis of variance; $P < 0{\cdot}05$). Addition of extra fat to the diet enhanced ES-1 activity in the duodenum and jejunum. Increasing the amount of maize oil in the diet caused an increase in ES-1 activity of the ileum; no such effect was seen in rats fed on the diets containing either coconut fat or olive oil. In rats fed on the olive oil diets, maximum ES-1 activity in duodenal and jejunal segments was reached at a dietary fat concentration of 22·0 energy %. A dietary concentration of 44·0 energy % as maize oil produced the highest intestinal ES-1 activities, while there were no clear differences between coconut fat and olive oil; with the diets containing 5·5 energy % as fat, no fat-type effects were seen.

Expt 2

Fat concentrations of 22·0 or 44·0 energy % in the diet slightly but significantly increased plasma total esterase activities (Table 6). This effect was more pronounced with maize oil than with medium-chain triacylglycerols. Plasma ES-1 activity was positively associated with the concentration of fat in the diet. Feeding of medium-chain triacylglycerols resulted in higher plasma ES-1 activities than feeding of maize oil.

Increasing dietary concentrations of maize oil and medium-chain triacylglycerols caused increased proximal jejunal total esterase activities, the increase being somewhat more

Table 4. *Expt 1. Effects of different concentrations of dietary coconut fat, maize oil and olive oil on total esterase and esterase-1 (ES-1) activities in plasma of rats*†

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

Dietary fat concentration (energy %)...		5·5		11·0		22·0		44·0		Statistical significance
	Type of fat	Mean	SE	Mean	SE	Mean	SE	Mean	SE	of effect‡
Plasma esterase activity§ on day 62										
TE	Coconut fat	2·6*	0·1	—	—	—	—	$3·3^{a}$	0·2	—
	Maize oil	2·4*	0·2	—	—	—	—	$3·0^{a}$	0·1	F
	Olive oil	$2·7^{A}$	0·1	$3·3^{A}$	0·1	$3·0^{A}$	0·3	$3·1^{a,A}$	0·2	—
ES-1	Coconut fat	19*	2	—	—	—	—	147^{b}	12	—
	Maize oil	20*	3	—	—	—	—	223^{a}	12	F, T, F × T
	Olive oil	20^{A}	4	44^{A}	6	81^{B}	5	$179^{a,C}$	9	—

F, effect of amount of fat; T, effect of type of fat; F × T, effect of interaction; TE, total esterase.

^{a,b} Mean values from groups fed on diets containing 44·0 energy % from fat with unlike superscript letters were significantly different ($P < 0·05$; Scheffé's test).

^{A,B,C} Mean values from groups fed on diets containing olive oil with unlike superscript letters were significantly different ($P < 0·05$; Scheffé's test).

* Mean values from groups fed on 5·5 energy % from coconut fat or maize oil are significantly different from those of their counterparts fed on 44·0 energy % from fat ($P < 0·05$; two-tailed Student's *t* test).

† The diets (for details of composition, see Table 1) were fed for 62 d; for experimental details, see pp. 380–384.

‡ Statistical significance ($P < 0·05$) was calculated by analysis of variance.

§ Total esterase activity is expressed as μmol/min per ml plasma; ES-1 activity is expressed relative to the plasma ES-1 standard.

pronounced with medium-chain triacylglycerols (Table 7). Increased fat intakes also elevated proximal jejunal ES-1 activities, but there was no differential effect of fat type.

Expt 3

The addition of extra maize oil to the diet caused a significant increase of plasma total esterase and ES-1 activity, irrespective of the amount of protein in the diet (Table 8). When dietary maize oil was kept constant at either 4·9 or 39·3 energy %, an increase in protein concentration of the diet induced significantly elevated activities of ES-1 in plasma.

In rats fed on the diets containing 17·5 or 51·9 energy % as protein, an increase in dietary maize oil produced significantly higher proximal jejunal total and ES-1 esterase activities. Rats fed on the high-protein diets had higher jejunal total and ES-1 esterase activities than their counterparts fed on the diets with 17·5 energy % as protein.

DISCUSSION

It is clear from the present study that substitution of fat for isoenergetic amounts of maize starch plus dextrose in the diet produced an increase in the activity of ES-1 in plasma of male rats. When increasing the amount of fat in the form of either maize oil (Table 6) or olive oil (Table 4) or medium-chain triacylglycerols (Table 6) there was a direct relationship between the percentage of energy from fat in the diet and plasma ES-1 activity. At dietary

Table 5. *Expt 1. Effects of different concentrations of dietary coconut fat, maize oil and olive oil on total esterase and esterase-1 (ES-1) activities in small intestine of rats*†

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

Dietary fat concentration (energy %)...		5·5		11·0		22·0		44·0		Statistical significance
	Type of fat	Mean	SE	Mean	SE	Mean	SE	Mean	SE	effect‡
SIE activity§ on day 62										
D: TE	Coconut fat	2·3*	0·1	—	—	—	—	$3·0^{a}$	0·2	—
	Maize oil	2·4*	0·1	—	—	—	—	$3·8^{a}$	0·4	F
	Olive oil	$2·4^{A}$	0·2	$2·5^{A}$	0·0	$3·1^{A}$	0·2	$3·2^{a,A}$	0·2	—
ES-1	Coconut fat	47*	6	—	—	—	—	74^{a}	6	—
	Maize oil	42*	4	—	—	—	—	101^{a}	16	F, F × T
	Olive oil	51^{A}	5	63^{A}	6	72^{A}	6	$69^{a,A}$	7	—
PJ: TE	Coconut fat	2·1	0·1	—	—	—	—	$2·4^{a}$	0·1	—
	Maize oil	2·2*	0·1	—	—	—	—	$3·1^{a}$	0·1	F, T, F × T
	Olive oil	$2·5^{A}$	0·1	$2·5^{A}$	0·1	$2·7^{A}$	0·2	$3·8^{a,A}$	0·2	—
ES-1	Coconut fat	46	5	—	—	—	—	54^{b}	3	—
	Maize oil	61*	4	—	—	—	—	105^{a}	6	F, T, F × T
	Olive oil	53^{A}	4	$60^{A,B}$	4	70^{B}	5	$70^{a,B}$	2	—
MJ: TE	Coconut fat	1·7*	0·1	—	—	—	—	$2·1^{a}$	0·1	—
	Maize oil	1·8*	0·1	—	—	—	—	$2·7^{a}$	0·1	F, T
	Olive oil	$1·6^{A}$	0·3	$1·9^{A,B}$	0·1	$2·2^{B}$	0·2	$2·4^{a,B}$	0·1	—
ES-1	Coconut fat	42	6	—	—	—	—	50^{b}	3	—
	Maize oil	38*	4	—	—	—	—	71^{a}	3	F, T, F × T
	Olive oil	36^{A}	2	$40^{A,B}$	3	53^{B}	5	$55^{b,B}$	4	—
DJ: TE	Coconut fat	1·4	0·1	—	—	—	—	$1·7^{a}$	0·1	—
	Maize oil	1·4*	0·1	—	—	—	—	$2·1^{a}$	0·1	F
	Olive oil	$1·5^{A}$	0·1	$1·4^{A}$	0·1	$1·9^{A}$	0·2	$1·7^{a,A}$	0·1	—
ES-1	Coconut fat	32	4	—	—	—	—	38^{b}	3	—
	Maize oil	35*	3	—	—	—	—	59^{a}	2	F, T, F × T
	Olive oil	$34^{A,B}$	4	30^{A}	2	46^{B}	4	$46^{b,B}$	3	—
I: TE	Coconut fat	0·8	0·1	—	—	—	—	$0·8^{a}$	0·1	—
	Maize oil	0·8*	0·1	—	—	—	—	$1·2^{a}$	0·1	T, F × T
	Olive oil	$0·8^{A}$	0·0	$0·9^{A}$	0·1	$0·8^{A}$	0·1	$0·7^{a,A}$	0·1	—
ES-1	Coconut fat	25	3	—	—	—	—	$25^{a,b}$	2	—
	Maize oil	23*	2	—	—	—	—	37^{a}	6	T, F × T
	Olive oil	22^{A}	2	20^{A}	3	21^{A}	2	$20^{b,A}$	1	—

D, duodenum; DJ, distal jejunum; I, ileum; MJ, middle jejunum; PJ, proximal jejunum; TE, total esterase; SIE, small intestinal esterase: F, effect of amount of fat; T, effect of type of fat; F × T, effect of interaction.

[a, b] Mean values from groups fed on diets containing 44·0 energy % from fat with unlike superscript letters were significantly different ($P < 0·05$; Scheffé's test).

[A, B] Mean values from groups fed on diets containing olive oil with unlike superscript letters were significantly different ($P < 0·05$; Scheffé's test).

* (Mean values from groups fed on 5·5 energy % from coconut fat or maize oil are significantly different from those of their counterparts fed on 44·0 energy % from fat: ($P < 0·05$; two-tailed Student's t test).

† The diets (for details of composition, see Table 1) were fed for 62 d; for experimental details, see pp. 380–384.

‡ Statistical significance ($P < 0·05$) was calculated by analysis of variance.

§ Total esterase activity is expressed as μmol/min per mg protein; ES-1 activity is expressed relative to the SI ES-1 standard.

Table 6. *Expt 2. Effect of various dietary concentrations of maize oil and medium-chain triacylglycerols (MCT) on total esterase and esterase-1 (ES-1) activities in plasma of rats**

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

Dietary fat concentration (energy %)...		5·5		11·0		22·0		44·0		Statistical significance of effect†
	Type of fat	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Plasma esterase activity‡ on day 58										
TE	Maize oil	$2·3^{a}$	0·1	$2·2^{a}$	0·1	$2·6^{a,b}$	0·1	$2·8^{b}$	0·1	—
	MCT	$2·2^{A}$	0·0	$2·1^{A}$	0·1	$2·3^{B}$	0·1	2·3§·^{B}	0·1	F, T
ES-1	Maize oil	58^{a}	14	75^{a}	5	196^{b}	18	318^{c}	23	—
	MCT	29^{A}	3	60^{A}	6	244^{B}	15	466§·^{C}	29	F, T, F × T

F, effect of amount of fat; T, effect of type of fat: F × T, effect of interaction; TE, total esterase.

^{a,b,c} Mean values from groups fed on diets containing maize oil with unlike superscript letters were significantly different ($P < 0·05$; Scheffé's test).

^{A,B,C} Mean values from groups fed on diets containing MCT with unlike superscript letters were significantly different ($P < 0·05$; Scheffé's test).

* The diets (for details of composition, see Table 2) were fed for 58 d. The group fed on the diet containing 11·0 energy % from medium-chain triacylglycerols consisted of five animals (on day 36, one animal in this group died); for details of experimental procedures, see pp. 380–384.

† Statistical significance ($P < 0·05$) was calculated by analysis of variance.

‡ Total esterase activity is expressed as μmol/min per ml plasma; ES-1 is expressed relative to the plasma ES-1 standard.

§ Means with their standard errors for five animals; in one animal aortic puncture failed.

concentrations of 22·0 or 44·0 energy % there were differential effects of fat type on plasma ES-1. Increasing ES-1 activities were induced by coconut fat, olive oil, maize oil and medium-chain triacylglycerols, in this order.

Replacement of dietary carbohydrates by fat also induced increased activities of ES-1 in the intestine. ES-1 in the duodenum and jejunum, rather than in the ileum, responded to the amount of dietary fat (Table 5). There was no proportional increase in jejunal ES-1 activity with increasing fat concentration in the diet. The findings in Tables 5 and 7 suggest that the minimum fat concentration at which intestinal ES-1 will be increased is between 11·0 and 22·0 energy %. There is some evidence that the type of fat influences jejunal ES-1 activity. At the concentration of 44·0 energy % maize oil induced higher activities than coconut fat or olive oil (Table 5) but not medium-chain triacylglycerols (Table 7).

In Expts 1 and 2 extra fat was added to the diet at the expense of isoenergetic amounts of carbohydrates. Expt 3 was carried out in an attempt to see whether replacement of carbohydrates by protein would affect ES-1. The incorporation of extra casein into the diet increased plasma ES-1 activity at both dietary fat levels (Table 8). In this experiment maize oil was added to the diet instead of either carbohydrates or protein. In both cases the ingestion of extra fat caused an increase in plasma ES-1 activity. This indicates that fat is a more powerful determinant of plasma ES-1 than protein.

Replacement of carbohydrates by fat resulted in increased activities of jejunal ES-1, but replacement of protein by fat did not affect jejunal ES-1 activity. This agrees with the observation that substitution of protein for carbohydrates induced an increase in jejunal

Table 7. *Expt 2. Effect of various dietary concentrations of maize oil and medium-chain triacylglycerols (MCT) on total esterase and esterase-1 (ES-1) activities in proximal jejunum of rats**

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

Dietary fat concentration (energy %)...		5·5		11·0		22·0		44·0		Statistical significance of effect†
	Type of fat	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Proximal jejunal esterase activity‡ on day 58										
TE	Maize oil	$1·9^{a}$	0·1	$2·0^{a,b}$	0·1	$2·3^{b}$	0·1	$2·8^{c}$	0·1	—
	MCT	$1·9^{A}$	0·1	$2·0^{A,B}$	0·1	$2·8^{B}$	0·1	$3·3^{C}$	0·2	F, T, F × T
ES-1	Maize oil	53^{a}	3	55^{a}	3	74^{a}	11	79^{a}	5	—
	MCT	51^{A}	2	$57^{A,B}$	5	$75^{B,C}$	5	88^{C}	5	F

F, effect of amount of fat; T, effect of type of fat; F × T, effect of interaction; TE, total esterase.

^{a,b,c} Mean values from groups fed on diets containing maize oil with unlike superscript letters were significantly different ($P < 0·05$; Scheffé's test).

^{A,B,C} Mean values from groups fed on diets containing MCT with unlike superscript letters were significantly different ($P < 0·05$; Scheffé's test).

* The diets (for details of composition, see Table 2) were fed for 58 d. The group fed on the diet containing 11·0 energy % from medium-chain triacylglycerols consisted of five animals (on day 36, one animal in this group died); for details of experimental procedures, see pp. 380–384.

† Statistical significance ($P < 0·05$) was calculated by analysis of variance.

‡ Total esterase activity is expressed as μmol/min per mg protein; ES-1 is expressed relative to the small intestinal ES-1 standard.

ES-1 activity. Thus the amount of both dietary fat and protein influences plasma ES-1 activity, the effect of fat being more pronounced. With regard to jejunal ES-1 activity, the effects of isoenergetic amounts of protein and fat may be similar.

Lewis & Hunter (1966) observed in rats an increase in the activity of esterases of high electrophoretic mobility in lymph after administration of fats by stomach tube. Wassmer *et al.* (1988) described that an increased activity of lymph ES-2 was seen in mice after infusion of fat into the duodenum. Mouse esterase isozyme ES-2 is assumed to be homologous with rat ES-1 (Augustinsson & Henricson, 1966; Van Zutphen & Den Bieman, 1988). Wassmer *et al.* (1988) have suggested that mouse ES-2 becomes associated with lipid droplets during fat resorption by the enterocyte and remains associated during the formation of primary chylomicrons and their extrusion into the lymph. Thus it would follow that ES-1 might be involved in fat absorption and that it is released from the intestine during this process.

The present findings do not provide evidence for a role of ES-1 in the formation of chylomicrons. Dietary medium-chain triacylglycerols caused an increase in the activity of ES-1 in plasma and the proximal jejunum, the effect being similar to that of isoenergetic amounts of maize oil (Tables 6 and 7). Medium-chain triacylglycerols are rapidly hydrolysed and absorbed from the gut, and transported as medium-chain fatty acids by the portal vein directly to the liver. Thus, in contrast to long-chain fatty acids in maize oil, medium-chain fatty acids do not require chylomicron formation for absorption. Since

Table 8. *Expt 3. Effect of increasing the amounts of dietary maize oil at the expense of either carbohydrates or protein on total esterase and esterase-1 (ES-1) activities in plasma and proximal jejunum of rats**

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

	Dietary fat and protein concentrations (energy %)								
Maize oil...	4·9		39·3		4·9		39·3		
Casein...	17·5		17·5		51·9		51·9		Statistical significance of effect†
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Plasma esterase activity‡ on day 56:									
Total esterase	2·3	0·1	3·0	0·0	2·3	0·1	3·2	0·2	F
ES-1	37	4	333	13	77	5	448	13	F, P, F × P
Proximal jejunal esterase activity§ on day 56:									
Total esterase	1·8	0·1	2·1	0·1	2·4	0·1	2·7	0·1	F, P
ES-1	37	3	53	8	53	3	76	7	F, P

F, effect of amount of fat; P, effect of amount of protein: F × P, effect of interaction.

* The diets (for details of composition see Table 3) were fed for 56 d; for experimental details, see pp. 380–384.

† Statistical significance ($P < 0·05$) was calculated by analysis of variance.

‡ Total esterase activity is expressed as μmol/min per ml plasma: ES-1 activity is expressed relative to the plasma ES-1 standard.

§ Total esterase activity is expressed as μmol/min per mg protein; ES-1 activity is expressed relative to the small intestinal ES-1 standard.

dietary medium-chain triacylglycerols induced increased ES-1 activities, it seems unlikely that ES-1 is involved in the formation of chylomicrons.

The previously stated reasoning would not exclude the possibility that ES-1 plays a role in the absorption of fat at the intestinal lumen level. However, other observations in the present study may do so. Substitution of dietary protein for carbohydrates at a constant concentration of fat in the diet was found to enhance ES-1 activity in plasma and in the jejunum (Table 8). As fat absorption may not be influenced under these conditions, it seems unlikely that ES-1 is uniquely involved in this process.

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