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Specific effect of the amount of dietary fat on esterase-1 (ES-1) activity of rat plasma

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Summary: The question addressed is whether the amount of dietary fat rather than that of carbohydrates or protein affects esterase-1 (ES-1) activity in plasma of rats. For this purpose, the effects on plasma ES-1 activity of replacement of dietary fat, by isocaloric amounts of either carbohydrates or protein were studied in male rats. In rats fed high-fat diets, corn oil induced higher plasma ES-1 activities than coconut fat. Plasma ES-1 activity was decreased by a decrease in fat intake. Replacement of fat by carbohydrates produced a similar decrease of plasma ES-1 activity as did replacement of fat by protein. Replacement of carbohydrates by protein did not significantly change plasma ES-1 activity. It is concluded that the amount of fat in the diet specifically influences ES-1 activity in plasma.

Zusammenfassung: Die Frage, die sich stellt, ist: Beeinflußt die Zunahme von Nahrungsfett die Esterase-1-Aktivität (ES-1) im Plasma von Ratten mehr als Kohlenhydrate oder Proteine? Es wurde daher bei männlichen Ratten der Effekt auf die Esterase-1-Aktivität durch das Austauschen des Nahrungsfettes mit isokalorischen Mengen an Kohlenhydraten und Proteinen bestimmt. In hoch fetthaltigem Futter erreichte Maisöl höhere Esterase-1-Aktivität im Plasma von Ratten als Kokosnußöl. Die Esterase-1-Aktivität war abnehmend bei erniedrigter Fettaufnahme. Das Austauschen des Nahrungsfettes durch Kohlenhydrate oder Proteine produziert die gleiche Abnahme der Esterase-1-Aktivität im Plasma. Dagegen tritt keine nennenswerte Veränderung der Esterase-1-Aktivität beim Austausch von Kohlenhydraten mit Proteinen auf. Daraus folgt, daß die Höhe des Fettgehalts in der Nahrung die Esterase-1-Aktivität beeinflußt.

Key words: rat; dietary fat; esterase-1 (ES-1); plasma

Schlüsselwörter: Ratte; diätetisches Fett; Esterase-1 (ES-1); Plasma

Introduction

The physiological function of carboxylesterases (E.C. 3.1.1.1.), which form a complex system of non-specific enzymes present in most living organisms (1), is still obscure, but there is some evidence that at least one of them responds to dietary fats. Replacement of isocaloric amounts of carbohydrates by either corn oil or coconut fat in the diet of rats caused a

pronounced increase of the so-called ES-1 isozyme, an anodal, fast moving esterase zone in the plasma zymogram (2).

It could be argued that both the omission of carbohydrates from the diet as well as the addition of fat to the diet are responsible for the observed effects on ES-1 in plasma. Thus it cannot be concluded whether or not the amount of dietary fat rather than that of carbohydrates has a specific effect on ES-1. This prompted us to perform an experiment with rats in which either dietary coconut fat or corn oil in a high-fat diet was replaced by isocaloric amounts of either carbohydrates or protein. If and when carbohydrates and protein do not affect ES-1 activity, then the replacement of fat by either carbohydrates or protein would reduce ES-1 activity to the same extent. If such an outcome would be obtained, then it can be concluded that the amount of fat specifically influences ES-1 activity, provided that the amounts of carbohydrates and protein do not modulate ES-1 activity in an identical fashion.

Materials and Methods

Animals

Male rats, aged 6 weeks, of an outbred Wistar colony (Cpb:WU), were used. The rats had been fed ad libitum a commercial, pelleted, nonpurified diet (RMH-B®, Hope Farms, Woerden, The Netherlands). The animals were housed individually as described previously (3). On day –2, blood samples of the non-fasted rats were taken by orbital puncture while under light diethyl-ether anesthesia for the determination of plasma cholesterol concentration. On day 0 of the experiment, the rats were divided into 6 dietary groups of 6 animals each. The groups had similar distributions of plasma cholesterol concentration and body weight. The mean values were 2.63 mM and 101 g, respectively.

Diets

Rats were fed purified diets in meal form. The diets contained at least 2.75 % of energy as corn oil, so as to provide sufficient linoleic acid. This implies that the diets to which coconut fat had been added actually contained 2.75 % of energy less in the form of this fat source than indicated. The diets were stored at 4 °C until feeding. The rats had free access to food and tap water.

Diets containing 22.0 % of energy as fat (either coconut fat or corn oil) and 19.6 % of energy from protein were used (Table 1). Of the fat source 16.5 % of energy was replaced by either corn starch plus dextrose in a 1:1 ratio or casein. In this way, 5.5 % fat diets containing either 19.6 or 36.1 energy % of protein and 74.9 or 58.4 energy % of carbohydrates were obtained.

Blood sampling

At the end of the experiment (day 56), between 10.00 and 13.00 h, the rats were anesthetized in the nonfasting state by the intraperitoneal administration of 15 mg of pentobarbital (Nembutal®, Sanofi Sante Animale SA, Paris, France). Blood was taken by aortic puncture and 4.5 ml was mixed with 0.5 ml of distilled water containing 3.8 % (w/w) sodium citrate. Plasma was collected by low speed centrifugation and kept at −20 °C until analysis.

Analyses

Plasma cholesterol was measured enzymatically using the kit (Monotest®) supplied by Boehringer-Mannheim GmbH (Mannheim, F.R.G.). The triglyceride con-

Table 1. Composition of the diets.

	Fat type and macronutrient concentrations (% of energy)					
	Coconut fat			Corn oil		
Fat:	5.5 %	22.0 %	5.5 %	5.5 %	22.0 %	5.5 %
Casein:	19.6 %	19.6 %	36.1 %	19.6 %	19.6 %	36.1 %
Carbohydrates:	74.9 %	58.4 %	58.4 %	74.9 %	58.4 %	58.4 %
Ingredient (g)						
Casein	16	16	29.5	16	16	29.5
Corn starch	25.6	18.8	18.8	25.6	18.8	18.8
Dextrose	25.6	18.8	18.8	25.6	18.8	18.8
Coconut fat	1	7	1	0	0	0
Corn oil	1	1	1	2	8	2
Constant components[1])	30.8	30.8	30.8	30.8	30.8	30.8
Total (g)	100	92.4	99.9	100	92.4	99.9
Chemical analysis (g/100 g diet)						
Crude fat	2.2	9.0	2.1	2.1	8.9	2.3
Crude protein	15.0	16.3	28.0	15.3	16.3	27.3
Fatty acids[2]) (g/100 g fatty acids)						
C 12:0	18.2	36.1	17.0	0	0	0
C 14:0	8.3	16.0	8.0	0.1	0	0.1
C 16:0	11.0	10.9	11.2	10.4	10.2	10.4
C 18:0	2.9	3.3	3.0	2.1	1.9	2.0
C 18:1	21.0	12.7	21.8	29.8	29.3	29.6
C 18:2	32.0	10.9	33.0	55.1	56.1	55.3
Sat. total[3])	46.2	76.0	44.3	13.6	13.1	13.5
Mono. total	21.2	12.8	22.0	30.2	29.7	30.0
Poly. total	32.6	11.1	33.5	56.0	57.0	56.2

[1]) The constant components consisted of (g): molasses, 10; cellulose, 15; dicalcium phosphate, 0.61; calcium carbonate, 0.62; magnesium carbonate, 0.07; magnesium oxide, 0.03; postassium bicarbonate, 1.8; sodium chloride, 0.5; vitamin premix, 1.2; mineral premix, 1.0. The composition of the mineral and vitamin premixes has been described elsewhere (4).
[2]) Selected fatty acids in shorthand notation: the number before and after the colon represents the number of carbon atoms and of double bounds, respectively.
[3]) Sat. = saturated; Mono. = monounsaturated; Poly. = polyunsaturated.

centrations in plasma were measured enzymatically according to Sullivan et al. (5). Crude fat concentrations and fatty acid composition of the diets were determined according to Folch et al. (6) and Metcalfe et al. (7), respectively. Protein contents of the diets were determined as described by Osborne and Voogt (8). Total esterase activity in plasma was measured with p-nitrophenylacetate as substrate as described previously (2). Plasma esterase patterns were determined by vertical 4.5–12.0 % (w/v) polyacrylamide gradient gel electrophoresis at pH 9.0 according to Beynen et al. (9). 10 µl of a mixture containing 67.5 % (v/v) of plasma, 0.285 % (w/w) sodium citrate and 10 % (w/w) glycerol was added to the slots. On each slab gel pooled rat plasma was run as a plasma ES-1 standard. After electrophoresis the gels were stained with Fast Blue BB and α-naphthylbutyrate (2). 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 protein and type of fat. Statistical analyses were carried out using a SPSS^X computer program (10).

Results and Discussion

Final body weight and weight gain were not influenced significantly by the dietary variables (Table 2). Decreasing dietary fat concentrations increased feed intake; there was no effect of fat type. When at a constant fat level the protein or carbohydrate concentration of the diets was increased, feed intake did not change significantly. Thus caloric intake remained essentially similar in rats fed the various experimental diets.

There was no significant effect of the type of fat on plasma cholesterol levels. However, corn oil produced slightly higher group mean plasma cholesterol concentrations than did coconut fat. This corroborates earlier work (2). Changing the macronutrient composition of the diet did not influence plasma cholesterol concentrations.

Decreasing the amount of coconut fat in the diet tended to lower plasma triglyceride concentrations. Decreasing the amount of corn oil in the diet tended to cause an increase in plasma triglycerides, but only if corn oil was replaced by carbohydrates. A triglyceride lowering action of corn oil, when compared with coconut fat, has been shown in earlier investigations (2, 11). When the amount of dietary coconut fat or corn oil was kept constant at 5.5 energy %, an increase in the concentration of protein in the diet, which is associated with a decreased intake of carbohydrates, tended to decrease plasma triglyceride concentrations. This probably relates to a specific effect of dietary carbohydrate concentration (12).

The omission of fat from the diet slightly reduced plasma total esterase activity (Table 2). The magnitude of this effect did not depend on whether fat was replaced by protein or carbohydrates. Neither did it depend on the type of fat. In rats fed the diets containing 22 % energy from fat, significantly higher activities of plasma ES-1 were seen if the fat source was corn oil instead of coconut fat. Substitution of corn starch plus dextrose for isocaloric amounts of fat in the diet produced a decrease in the activity of ES-1 in plasma of male rats. This confirms earlier work (2). However, the present study also demonstrates that replacement of fat by carbohydrates induces a lowering of ES-1 activity that is identical to that seen after replacement of fat by protein. Replacement of carbohydrates by protein did not significantly change plasma ES-1 activity. We therefore conclude that the amount of fat in the diet rather than that of protein or carbohydrates influences plasma ES-1 activity. It could be argued that dietary protein and carbohydrates do affect ES-1 activity but to the same extent. This would also result in an identical change in ES-1 activity after replacement of fat by either carbohydrates or protein. We cannot exclude this possibility but feel that it would be highly coincidental. Since the high fat

Table 2. Effect of decreasing the amount of dietary coconut fat or corn oil on lipid concentrations and esterase activities in plasma of rats.[1])

	Fat type and macronutrient concentrations (% of energy)						
	Coconut fat			Corn oil			
Fat:	5.5 %	22.0 %	5.5 %	5.5 %	22.0 %	5.5 %	
Casein:	19.6 %	19.6 %	36.1 %	19.6 %	19.6 %	36.1 %	
Carbohydrates:	74.9 %	58.4 %	58.4 %	74.9 %	58.4 %	58.4 %	
Parameters							Significance[2])
Body weight (g)							
final, day 56	357 ± 18	366 ± 13	371 ± 13	382 ± 14[a]	350 ± 11[a]	344 ± 11[a]	
gain, day 0–56	256 ± 14	261 ± 11	269 ± 12	281 ± 11[a]	253 ± 6[a]	241 ± 9[a]	
Feed intake (g/day), day 0 to day 56							
	25.5 ± 1.0	23.2 ± 0.4	24.4 ± 0.8	25.9 ± 0.6[a]	22.6 ± 0.7[b]	24.4 ± 0.4[a, b]	F
Plasma lipids (mM), day 56							
cholesterol	2.10 ± 0.11	2.19 ± 0.11	2.10 ± 0.13	2.53 ± 0.16[a]	2.27 ± 0.18[a]	2.20 ± 0.19[a]	
triglycerides	1.64 ± 0.34	1.86 ± 0.22	1.22 ± 0.28	1.71 ± 0.24[a]	1.14 ± 0.12[a]	1.15 ± 0.13[a]	
Plasma esterase activity[3]), day 56							
total	2.2 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.1 ± 0.1[a]	2.4 ± 0.1[b]	2.2 ± 0.1[a, b]	F
ES-1	24 ± 4	66 ± 16	41 ± 11	37 ± 7[a]	132 ± 26[b]	35 ± 2[a]	F, T, F × T

[1]) Results expressed as means ± SEM for 6 animals per group. The diets were fed for 56 d.
[2]) Significance ($p < 0.05$) was calculated by two analyses of variance. In the first analysis, the data were classified by amount of fat and fat type and in the second analysis by type of fat and amount of protein. The amount of protein had no significant effects and there was no interaction with the type of fat. F, effect of amount of fat; T, effect of type of fat; F × T, effect of interaction.
[3]) Total esterase activity expressed as μmol/min/ml plasma. ES-1 activity expressed relative to the plasma ES-1 standard.
None of the groups fed diets containing coconut fat are significantly different ($p < 0.05$, Scheffé's test).
Groups fed diets containing corn oil not sharing a common lower case superscript are significantly different ($p < 0.05$, Scheffe's test).

diets used in this study contained the recommended amount of protein necessary for optimum growth, the concentration of protein in these diets could not be lowered so as to study the effect of substitution of fat for protein.

Wassmer et al. (13) described that an increased activity of lymph ES-2 was seen in mice after infusion of fat into the duodenum. Mouse esterase isozyme ES-1 is assumed to be homologous with rat ES-1 (14, 15). Wassmer et al. (13) have put forward 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 study supports this hypothesis because it suggests that the amount of dietary fat specifically influences plasma ES-1 activities.

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