Changes in serum cholinesterase (*EC* 3 · 1 · 1 · 8) activity in rats consuming a high-fat diet*

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Adult male rats were fed on a control diet containing (g/kg) carbohydrate 600, lipid 35 and protein 190, or on a high-fat diet containing carbohydrate 360, lipid 420 and protein 120. After 30 d, the high-fat diet provoked a decrease in serum cholinesterase (*EC* 3 · 1 · 1 · 8) activity which was reversed by feeding rats on the control diet. The observed decrease after 90 d on the high-fat diet was not seen if a simultaneous daily intraperitoneal injection of a lipotrophic agent containing (mg/kg) S-adenosyl-L-methionine 3, coenzyme A 0·1, UDP-glucose 30 and CDP-choline 1·5 was given to rats on the high-fat diet. The findings are discussed in relation to the apparent susceptibility of serum cholinesterase to dietary components and its possible role in lipid metabolism.

Cholinesterase activity: Dietary fat: Rat

The biological function of serum cholinesterase (*EC* 3 · 1 · 1 · 8, ChE) has not been clearly established. Several reports in the literature present evidence suggesting that cholinesterase may be involved in lipoprotein metabolism, as it has been demonstrated in patients with hyperlipidaemia, hyperlipoproteinaemia (Kutty & Jacob, 1972; Chu *et al.* 1978; Jain *et al.* 1983; Magarian & Dietz, 1987), obesity and diabetes (Antopol *et al.* 1973).

A direct relation was observed between enzyme activity and low-density lipoprotein (LDL) concentrations. Inhibition of the enzyme was associated with a decrease in serum total cholesterol and LDL, and increases in high-density lipoprotein (HDL) concentrations (Kutty *et al.* 1973, 1975; Ryhanen *et al.* 1982, 1984).

In mice with a high carbohydrate intake, an increase in serum cholinesterase was seen (Kutty *et al.* 1981). A similar result was also reported by Deshmukh (1986) in experimental diabetic rats.

The aim of the present work was to determine the influence of a high-fat diet on rat serum cholinesterase activity. As cholinesterase activity appeared to be reduced by the diet, a lipotrophic agent was administered to restore the diminished cholinesterase activity. These results are the basis for the present report.

MATERIALS AND METHODS

Materials

All reagents for enzymic analysis were supplied by Sigma Chemical Co., St Louis, MO. The rat diet was obtained from Sander S.A., Barcelona. The lipotrophic agent was obtained

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from Alter Pharmaceutical Co., Madrid. The fat supplement was provided by Agra S.A., Madrid.

Animal treatments

Inbred male Wistar rats (200–250 g, aged 2 months) were kept under different experimental conditions.

Group 1. Control animals were fed on a standard commercial diet, the control diet, which was composed of (g/kg): carbohydrate 600, lipid 35 and protein 190; gross energy 18·3 kJ/g.

Group 2. Rats were fed on a high-fat diet (HFD) composed of (g/kg) carbohydrate 360, lipid 420 and protein 120; gross energy 21·5 kJ/g. The experimental high-fat diet (/kg) was prepared by mixing 600 g control diet with 400 g margarine. The fatty acid composition of the margarine was (g/100 g fatty acids): 12:0, 3·2; 14:0, 5·6; 16:0, 21; 16:1, 8·8; 18:0, 8·1; 18:1, 27·2; 18:2, 12·3; 18:3, 4·8; 18:4, 3·8; 20:0, 1·4; 20:3, 0·2; 20:4, 1. The margarine also contained 80 mg cholesterol/100 g.

Both groups of rats were fed *ad lib.* After 30 and 90 d, blood samples were obtained and serum cholinesterase activity determined.

Group 3. Rats were fed *ad lib.* for 30 d on the high-fat diet and then received the control diet for 15 d. After this time-period, blood samples were collected for serum cholinesterase assay.

Group 4. Rats received the control diet with a daily intraperitoneal (i.p.) injection of a lipotrophic agent containing 3 mg S-adenosyl-L-methionine/kg, 0·1 mg coenzyme A/kg, 30 mg UDP-glucose/kg and 1·5 mg CDP-choline/kg.

Group 5. Rats received the high-fat diet with a daily i.p. injection of the lipotrophic agent.

The samples from rats in groups 4 and 5 were obtained after 90 d of treatment.

Rats were kept on a fasting regimen with free access to water for 18 h; they were then anaesthetized with pentobarbital and blood was drawn from the abdominal aorta. Blood collected was allowed to clot and centrifuged at 3000 ***g*** for 10 min. Sera were obtained and immediately frozen until further analyses were performed.

Enzymic assay of cholinesterase

Serum cholinesterase activity was assayed according to the modified method of Knedel & Böttger (1967). The final reaction mixture (total volume 2·05 ml) contained (/l): 41·87 mmol sodium phosphate buffer (pH 7·7), 0·1635 mmol 5-5′-dithio-bis(2-nitro-benzoic acid), 5·2 mmol butyrylthiocholine iodide, to which 50 μl serum (volume fraction of sample 1:41) were added. Reagents were pre-incubated for 5 min at 37° and the reaction initiated by addition of the serum sample. The enzyme assays were monitored at 37° in a Varian Cary 210 Spectrometer equipped with a plotter. Absorbance changes at 415 nm during the initial 3 min were linear and were used to calculate reaction rates. For calculations an extinction coefficient of $13{\cdot}6 \times 10^6$/mol per cm^2 (Moss *et al.* 1986) was used. One unit of enzyme activity was defined as the hydrolysis of 1 μmol butyrylthiocholine/min in 41·87 mmol sodium phosphate/l buffer (pH 7·7) at 37°.

Statistical analysis

Results are presented as means and standard deviations. The Shapiro–Wilk test was applied to establish the behaviour of distributions. Whenever the Shapiro–Wilk test rejected the hypothesis of normal distribution or the F ratio of variances was significantly different, the significance between two mean values was calculated using the Mann–Whitney U test. Differences were considered not significant when $P > 0{\cdot}05$ (Sokal & Rohlf, 1969).

RESULTS

The mean energy intakes as well as the initial and final body-weights for animals on the different dietary regimens are presented in Table 1. Rats kept on the high-fat diet gained less weight than rats on the control diet as shown in Table 1.

Animals fed for 30 d on the high-fat diet which then received the control diet for 15 d (group 3) had a similar final weight to that of the control rats.

Rats which received the lipotrophic agent simultaneously with the high-fat diet (group 5) did not show differences in their final body-weight when compared with the control group.

The enzyme analysis was conducted with a 6·3 % imprecision expressed as a within-run coefficient of variation of around 136 IU/l at 37°; the detection limit was 10 IU/l.

In Table 2, it can be observed that the high-fat diet provoked a significant decrease in the serum cholinesterase activity assayed after 30 and 90 d on this diet. The observed decrease after 1 month of treatment was reversed when rats were fed for 15 d on the control diet.

Administration of the lipotrophic agent alone did not cause significant differences in enzyme activity in comparison with the control group. When this agent was administered simultaneously with the high-fat diet no variation in the serum cholinesterase activity was observed when compared with the control group, and the lipotrophic agent restored the decreased activity observed in rats fed on the high-fat diet.

DISCUSSION

The present study was undertaken to determine whether a high-fat diet would change the cholinesterase activity of rat serum, particularly since a high-carbohydrate diet provokes an increase in the activity of this enzyme (Kutty *et al.* 1981). However, the high-fat diet produced the opposite result to that found with carbohydrate. The decreased activity cannot be considered an analytical artifact nor an effect due to differences in carbohydrate and protein intake between rats fed on the standard commercial control diet and rats fed on the high-fat diet, because rats treated concomitantly with the high-fat diet and lipotrophic agent for 90 d did not show any significant change in enzyme activity. Furthermore, injection of the lipotrophic agent had no effect on serum cholinesterase activity in rats fed on the control diet when compared with the enzyme activity in serum from rats identically fed and not treated with the lipotrophic agent. Because of differences in lipid composition of the control and high-fat diets, it is not possible to know whether some specific components of the high-fat diet are responsible for, or at least contribute to, the observed fall in cholinesterase activity.

The mechanism underlying the observed decrease in cholinesterase activity is unknown and, at present, we do not know whether it is a decrease in activity or a decrease in the mass of enzyme. Evaluation of hepatic function and histology did not reveal any significant damage or dysfunction (Aylagas, 1988). Serum cholinesterase is known to be synthesized in the liver (Moss *et al.* 1986). The maintenance of hepatic function suggests that there was not a diminution in the mass of enzyme, and the observed results could be ascribed to a modulation of serum cholinesterase activity. How this inhibition occurs is a challenging question worthy of further research. Kutty *et al.* (1981) referred to a decrease in cholinesterase in adipose tissue of obese mice, and our work shows a special susceptibility of this enzyme to lipid intake. An approach such as the one reported here may provide a basis for further study of the biological role of serum cholinesterase.

Table 1. *The effect of a high-fat diet (HFD) and an intraperitoneal (i.p.) injection of lipotrophic agent (LT) on energy intake and weight of rats*

(Food intakes were measured daily and the results are means and standard deviations)

Group no.	Experimental conditions	Period of experiment (d)	No. of animals	Energy intake (kJ/d per rat)*		Initial body-wt (g)		Final body-wt (g)	
				Mean	SD	Mean	SD	Mean	SD
1	CD	30	20	470	75	202	6	335	26
2	HFD	30	17	365[a]	80	207	19	277[a]	46
3	HFD+CD	30+15	6	439[b]	63	209	4	337[b]	22
1	CD	90	20	450	50	201	13	337	44
2	HFD	90	17	370[a]	55	207	20	286[a]	30
4	CD+LT	90	8	393	58	200	6	402	30
5	HFD+LT	90	5	320[a]	64	201	7	331[c]	21

Group 1, rats fed on the control diet (CD) for 30 or 90 d; group 2, rats fed on the HFD for 30 or 90 d; group 3, rats fed for 30 d on HFD and then fed on CD for 15 d; group 4, as for group 1 plus a daily i.p. injection of LT for 90 d; group 5, as for group 2 plus a daily i.p. injection of LT for 90 d.

* Commercial standard diet (CD) contained 18270 kJ/kg, HFD contained 21496 kJ/kg.

[a] HFD and HFD+LT *v.* CD ($P < 0.01$).

[b] HFD+CD *v.* HFD ($P < 0.01$).

[c] LT+HFD *v.* HFD ($P < 0.01$).

Table 2. *The effect of a high-fat diet (HFD) and an intraperitoneal (i.p.) injection of lipotrophic agent (LT) on serum cholinesterase (EC 3 · 1 · 1 · 8) activity in rats*

(Results are means with their standard deviations)

Group no.	Experimental conditions	Period of experiment (d)	No. of animals	Cholinesterase activity (IU/l)	
				Mean	SD
1	CD	30	20	62	19
2	HFD	30	17	48[a]	5
3	HFD+CD	30+15	6	67[b]	18
1	CD	90	20	64	23
2	HFD	90	17	47[a]	9
4	LT+CD	90	8	63	12
5	LT+HFD	90	5	67[c]	15

Group 1, rats fed on the control diet (CD) for 30 or 90 d; group 2, rats fed on the HFD for 30 or 90 d; group 3, rats fed for 30 d on HFD and then fed on CD for 15 d; group 4, as for group 1 plus a daily i.p. injection of LT for 90 d; group 5, as for group 2 plus a daily i.p. injection of LT for 90 d.

[a] HFD *v.* CD ($P < 0{\cdot}01$).

[b] HFD+CD *v.* HFD ($P < 0{\cdot}02$).

[c] LT+HFD *v.* HFD ($P < 0{\cdot}02$).

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