SHORT COMMUNICATION

THE METABOLISM OF LOW-DENSITY LIPOPROTEIN IN A PATIENT WITH FAMILIAL HYPERBETALIPOPROTEINAEMIA

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SUMMARY

1. Plasma low-density lipoprotein (LDL) from a patient with homozygous familial hyperbetalipoproteinaemia was labelled with $^{125}$I and LDL from a matched normal subject was labelled with $^{131}$I. The two samples of LDL were injected simultaneously into the patient and into a second normal subject.

2. In each subject the fractional rate of turnover of the two LDL samples was the same, but the fractional rate in the patient was less than half that in the normal subject.

3. It is concluded that the hyperbetalipoproteinaemia in the patient was not due to an abnormality in her LDL protein recognizable by her own body or by that of the normal recipient.

Key words: familial hyperbetalipoproteinaemia, turnover of low-density lipoprotein.

Familial hyperbetalipoproteinaemia (FH) is characterized by an abnormally high plasma concentration of low-density lipoprotein (LDL) and is inherited as a partially dominant trait, homozygotes being more severely affected than heterozygotes. Attempts to demonstrate abnormal metabolism of LDL in FH have led to contradictory results. Scott & Hurley (1969) found that the half-life of the apoprotein of LDL (apo-LDL) in four patients with primary hypercholesterolaemia was normal, but Langer, Strober & Levy (1972) found abnormally low fractional rates of turnover in patients with FH in the heterozygous form. This discrepancy may be due partly to differences in the selection of patients.

In this communication we describe the behaviour of LDL from a patient in whom the diagnosis of FH in the homozygous form was established beyond reasonable doubt. Fractional rates of turnover of the patient's LDL and of LDL from a normal subject of the same age and sex (normal donor) were compared in the body of the patient and in the body of a second normal subject (normal recipient). In order to ensure that the metabolism of LDL from the

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patient and normal donor was measured under identical conditions within each recipient, LDL from the patient and normal donor were labelled with different radioisotopes, the patient and normal recipient then receiving a single injection containing the two labelled samples.

**METHODS**

**Clinical**

The clinical and biochemical findings in the patient (a female aged 21 years) and her first-degree relatives have been described elsewhere (patient 2; Moutafis & Myant, 1969). During the present investigation, and throughout the previous year, the patient was given cholestyramine (20 g/day), nicotinic acid (2.5–3.5 g/day) and a corn-oil diet. Despite this treatment her plasma cholesterol concentration had remained between 16.9 and 19.5 mmol/l (650–750 mg/100 ml) for the previous 6 months. The two normal subjects were in good health and ate a normal diet. All three subjects were given potassium iodide [1.2 mmol (200 mg)/day] during the study and for the following 4 weeks. Antibodies to Australia antigen were not detectable in the plasma of the patient or the normal donor.

**Labelled lipoproteins**

For the preparation of labelled lipoprotein, the density of the serum was adjusted to 1.019 with a solution of NaCl containing EDTA (1 mmol/l) and centrifuged for 16 h at 105 000 g. The bottom layer (the layer of fluid below the clear, colourless intermediate zone) was removed and centrifuged again under the same conditions. The bottom layer obtained from this centrifugation was adjusted to a density of 1.063 and centrifuged for 18 h at 105 000 g. The top layer (the layer of fluid above the intermediate zone) was removed and centrifuged again at the same density. The top layer obtained from this centrifugation, containing the lipoproteins of density 1.019–1.063, was removed and dialysed for 24 h in 0.05 mol/l potassium phosphate buffer (pH 8.2) containing EDTA (1 mmol/l) and NaCl (0.15 mol/l). The dialysed solution was iodinated with $^{125}$I (patient's LDL) or $^{131}$I (normal donor's LDL) by the method of Hunter (1970). Unbound radioiodine was removed from the iodinated sample by passage through a column of Sephadex G-10 suspended in 0.15 mol/l NaCl, followed by dialysis for 24 h against 0.15 mol/l NaCl containing EDTA (1 mmol/l). The dialysed LDL solution was washed once by centrifugation at density 1.063 for 18 h at 105 000 g to remove denatured protein. All density adjustments were carried out by the method of Havel, Eder & Bragdon (1955) and all operations were carried out at 5°C. The degree of iodination was equivalent to about 0.5 g-atom of iodine/mol of LDL (mol. wt. of LDL protein assumed to be 100 000) in each of the two samples. Less than 5% of the radioiodine in the labelled LDL samples was extractable with chloroform–methanol (2:1, v/v), and more than 97% was precipitated when the LDL protein was precipitated with trichloroacetic acid (10%). The two labelled samples were mixed and then sterilized by passage through a Millipore filter. The patient and normal recipient (a male aged 56 years) each received an intravenous injection containing measured amounts of $^{125}$I- and $^{131}$I-labelled LDL (total protein 4–10 mg; total radioactivity 40–50 μCi). The normal donor received 40–6 μCi of $^{131}$I-labelled LDL (3 mg of protein). The three subjects gave informed consent to the injection of labelled lipoproteins.

**Collection and analysis of samples**

Blood samples were obtained at 12 min, 1, 3, 8 and 18 h, and then at the later intervals
Turnover of LDL

shown in Fig. 1. Total urine was collected in 24 h periods. Blood samples sufficient for measurement of serum LDL protein concentration (Lowry, Rosebrough, Farr & Randall, 1951) were taken from each subject at the beginning and end of the study. Radioactivity as $^{125}$I and $^{131}$I was assayed in samples (2 ml) of whole plasma and in portions (10 ml) of each urine collection in a Wallac Instruments double-channel gamma-ray spectrometer with a sodium iodide crystal detector. All counting rates were corrected to the time of the injection. In a preliminary investigation of two other human subjects, it was found that more than 95% of the total radioactivity of whole serum obtained after intravenous injection of $^{125}$I-labelled LDL was present as $^{125}$I-labelled LDL.

RESULTS AND DISCUSSION

Fig. 1 shows the plasma radioactivity curves for the two labelled samples of LDL in the normal recipient and the patient. In each recipient the curves for the two samples of LDL were similar, but the slopes of the log-linear portions of the curves were steeper in the normal recipient.
than in the patient. Each curve was analysed in terms of two exponentials (Fig. 1). The fractional rate of turnover of the intravascular pool of labelled LDL, calculated by the method of Matthews (1957), was similar for the two samples in each recipient, but the rate in the normal recipient (0.385/day for $^{125}$I and 0.384/day for $^{131}$I) was almost twice that in the patient (0.210/day for $^{125}$I and 0.225/day for $^{131}$I). The fractional rate of turnover of the normal donor’s labelled LDL in her own circulation was 0.426/day. Fractional rates of turnover, calculated from the renal clearance rate of radioiodine and the plasma volume (4.5% of body weight), were in good agreement with rates calculated from the plasma radioactivity curves. The absolute rate of turnover of apo-LDL in the patient (43.2 mg day$^{-1}$ kg$^{-1}$) was higher than that in the normal recipient (13.5 mg day$^{-1}$ kg$^{-1}$) and the normal donor (15.9 mg day$^{-1}$ kg$^{-1}$).

We conclude that the hyperbetalipoproteinaemia of the patient was not due to an abnormality in her LDL recognizable by her own body or by that of the normal recipient. In this respect, our results confirm and extend those of Langer et al. (1972), who found similar fractional rates of turnover of LDL from normal subjects and patients with familial hyperbetalipoproteinaemia when both types of LDL were injected successively into the same patient. It is not possible to say, from the present results alone, whether the low fractional rate of turnover of LDL in the patient, compared with the values obtained in the normal recipient and the normal donor, was due to a defect in the mechanisms for catabolizing LDL or to an abnormally high rate of synthesis leading to an increase in plasma LDL concentration. The higher absolute rate of turnover in the patient is unlikely to have been due to her treatment, since the absolute rate of turnover of apo-LDL is unaffected by cholestyramine (Langer, Levy & Fredrickson, 1969) and is decreased by nicotinic acid (Langer & Levy, 1971). This raises the possibility that the rate of synthesis of apo-LDL is increased in FH.

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REFERENCES


