TRANSFER OF VERY LOW DENSITY LIPOPROTEIN FROM HEN PLASMA INTO EGG YOLK

G. HOLDSWORTH, R.H. MICHELL and J.B. FINEAN

Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, England

Received 19 December 1973

1. Introduction

When a hen starts to produce eggs, enhanced synthesis by the liver brings about large increases in the plasma concentrations of very low density lipoprotein (VLDL) and of a heterogeneous high density 'lipophosphoprotein' component [1–3]. These lipoproteins closely resemble the low density (LDF) and the high density lipoprotein fractions of egg yolk. There are strong similarities with respect to the lipid [4,5], carbohydrate [5,6], and protein [7,8] components, and identical antigens occur in equivalent lipoproteins from the two sources. Such marked similarities of composition, together with the lack of substantial lipid-synthesising systems in the hen ovary [9], indicate that the ovary probably takes these lipoproteins directly from the blood and incorporates them relatively unchanged into the egg yolk.

This paper describes a demonstration of the direct transfer of L-[4,5-^{3}H] leucine-labelled VLDL from hen plasma into the LDF of egg yolk.

2. Materials and methods

All studies were carried out on White Leghorn or Rhode Island Red (Warren strain) hens which were laying regularly.

In the first set of experiments, a 200 μCi sample of L[4,5-^{3}H] leucine in Eagle's minimal essential medium was injected into a wing vein of each of four laying hens. At intervals up to 72 hr, blood was withdrawn from wing veins into heparin ($>$ 1 unit per ml of blood), and the cells removed by centrifugation. VLDL was purified from each sample of plasma by repeated flotation on a medium of density 1.006. [10]. Samples of VLDL were dispersed in a 1:1 (vol/vol) mixture of Triton X-100 and scintillation fluid, and the radioactivity counted in a Philips liquid scintillation analyser. The protein content was assayed by the method of Lowry et al. [11].

The second set of experiments involved injection of L[^{3}H] leucine into hens as before, followed by the sacrifice of the animals by decapitation at the time of maximum labelling of the plasma VLDL (see Results). Up to 50 ml of blood were collected and the VLDL isolated as previously described. The lipoprotein was suspended in 0.9% NaCl solution 'for injection', sterilised by filtration through an 0.8 μm Millipore filter and injected intravenously into fresh hens. The eggs laid subsequently by these animals were collected, and their LDF and high density lipoprotein fractions isolated [12,13]. Samples of these lipoproteins were counted and their radioactivities compared with those of the lipoprotein samples isolated from eggs laid by hens injected with labelled leucine.

Electrophoresis in sodium dodecyl sulphate of the apoproteins [14] of egg LDF and plasma VLDL was carried out in 7.5% acrylamide according to Weber and Osborn [15].

3. Results

Maximal labelling of the protein moiety of plasma VLDL was reached about 7 hr after injection of labelled leucine (fig. 1). In 24 hr the activity had decreased again to a relatively constant level.

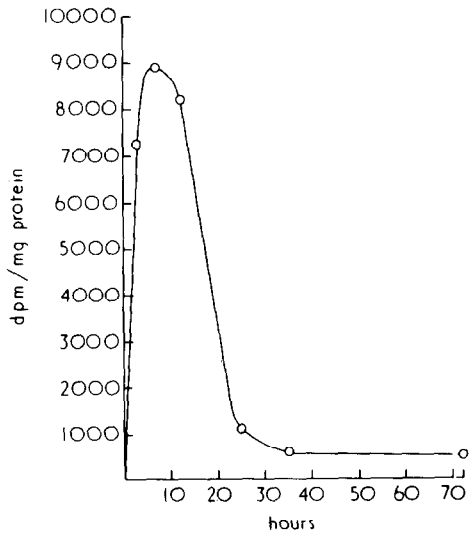

Fig. 1. The incorporation of L[^{3}H] leucine into hen plasma VLDL. The values shown are collated from four experiments.

The pattern of incorporation of label into egg lipoproteins following injection of ^{3}H-labelled leucine is illustrated in fig. 2. There was parallel incorporation of the label into the LDF and high density lipoproteins, both of which showed maximum radioactivity in the fourth egg after injection. Following injection of the labelled VLDL, the label was found only in the LDF fractions of the eggs. Again maximum activity was observed at about the fourth egg, but no activity was present in the high density lipoproteins (fig. 3).

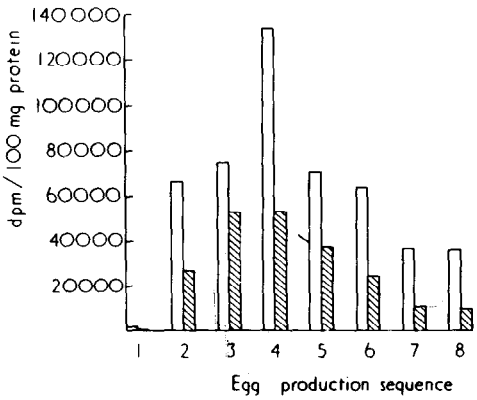

Fig. 2. The incorporation of radioactivity into the lipoprotein fraction of eggs laid by hens which had been injected with L[^{3}H] leucine. □, LDF; ▨ high density lipoproteins.

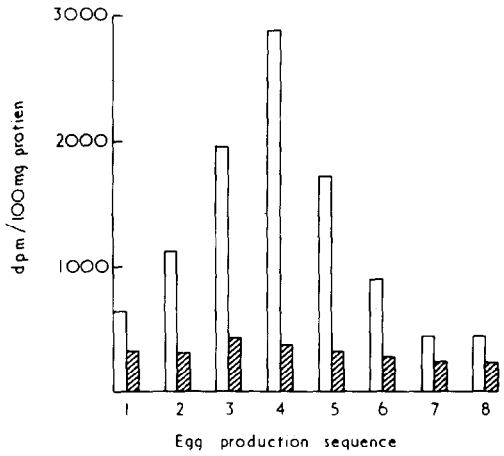

Fig. 3. The incorporation of radioactivity into the lipoprotein fractions of eggs laid by hens which had been injected with labelled VLDL. □; LDF; ▨ high density lipoproteins.

Experiments involving 3 'donor' hens and 4 recipients gave closely similar results.

The polypeptide profiles of egg LDF (gel a) and of plasma VLDL (gel b), as revealed by SDS polyacrylamide gel electrophoresis, are shown in fig. 4. There are close similarities in the mobilities and staining intensities (Coomassie brilliant blue®) of the major bands.

4. Discussion

The appearance after injection of labelled VLDL into laying hens of radioactivity in the egg yolk LDF, but not in the high density fraction, indicates that there is direct transfer of protein from plasma VLDL into egg LDF. Such a conclusion is consistent with the detailed similarities in amino acid compositions [7] of plasma VLDL and egg LDF apoproteins, and the close correspondence of their principal polypeptide components in SDS acrylamide gels. The absence of radioactivity in the egg yolk high density lipoprotein fraction suggests that breakdown and reutilisation of the VLDL protein moiety during transfer are probably negligible.

Extensive structural investigations [16–19] have indicated that these low-density lipoproteins probably consist of a neutral lipid core surrounded by a

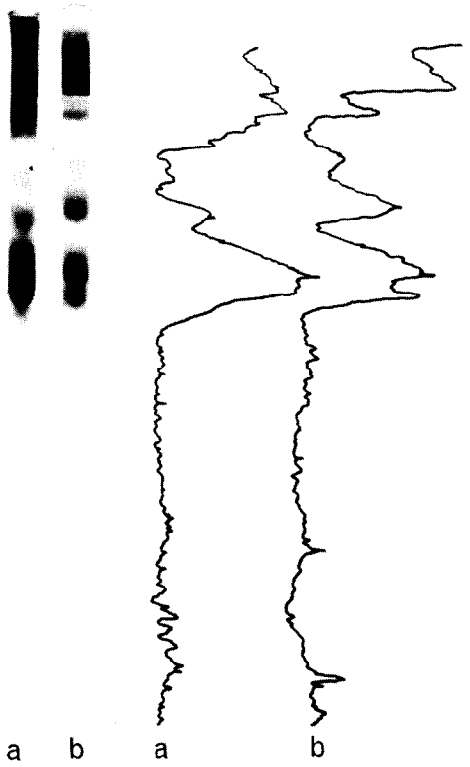

Fig. 4. SDS-polyacrylamide gel electrophoresis. (a) The apoproteins of plasma VLDL; (b) the apoproteins of egg LDF. 7.5% Acrylamide.

surface layer of phospholipid and protein. If this structural picture is correct, the evidence of direct transfer of protein might suggest that the whole lipoprotein particle is transferred from plasma to yolk relatively intact. Their similar lipid compositions provide further support for this view, as does the absence of substantial lipid-synthesising systems in the hen ovary.

An equivalent transfer of specific high density components from plasma into egg HDF is suggested by similarities in composition [1,5,8]. A direct assessment of this idea would be feasible using a technique similar to that employed in the present study.

Acknowledgements

G. Holdsworth was supported by Rank Hovis McDougall (Research), Ltd.

References

[1] Schjeide, O.A and Urist, M.R. (1960) Nature 188, 291–293.
[2] Hillyard, L.A., Entennam, C. and Chaikoff, I.L. (1956) J. Biol. Chem. 223, 359–368.
[3] Bos, E.S., Bergink, E.W., Kloosterboer, H.J. and Vonk, R.J. (1972) Abstr. Commun. Meet. Fed. Eur. Biochem. Soc. 8, 526.
[4] Gornall, D.A. and Kuksis, A. (1973) J. Lipid Res. 14, 197–205.
[5] Schjeide, O.A. (1963) Prog. Chem. Fats and other Lipids. 6, 253–289.
[6] Abraham, S., Hillyard, L.A. and Chaikoff, L.A. (1960) Arch. Biochem. Biophys. 89, 74–78.
[7] Hillyard, L.A., White, H.M. and Pangburn, S.A. (1972) Biochemistry 11, 511–518.
[8] Heald, P.J. and McLachlan, P.M. (1963) Biochem. J. 87 571–576.
[9] Gornall, D.A., Kuksis, A. and Morely, N. (1972) Biochim. Biophys. Acta 280, 225–230.
[10] Hatch, F.T. and Lees, R.S. (1968) Adv. Lipid Res. 6, 1–68
[11] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[12] Martin, W.G., Augustyniak, J. and Cook, W.H. (1964) Biochim. Biophys. Acta. 84, 716–720.
[13] Burley, W.R. and Cook, W.H. (1961) Can. J. Biochem. Physiol. 39, 1295–1307.
[14] Scanu, A. (1966) J. Lipid Res. 7, 295–306.
[15] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[16] Martin, W.G. and Cook, W.H. (1962) Can. J. Biochem. Physiol. 40, 1273–1285.
[17] Kamat, V.B. and Lawrence, G.A. (1972) Chem. Phys. Lipids 9, 1–25.
[18] Holdsworth, G. and Finean, J.B. (1972) Chem. Phys. Lipids 9, 217–229.
[19] Schneider, H., Morrod, R.S., Colvin, J.R. and Tattrie, N.H. (1973) Chem. Phys. Lipids 10, 328–353.