CARBOHYDRATE COMPOSITION OF LIPOPROTEIN APOPROTEINS ISOLATED FROM RAT PLASMA AND FROM THE LIVERS OF RATS FED OROTIC ACID

L. A. Pottenger, L. E. Frazier, L. H. DuBien,
G. S. Getz and R. W. Wissler

Departments of Pathology and Biochemistry
University of Chicago
Chicago, Illinois 60637

Received July 31, 1973

Summary: Dietary orotic acid has been shown to inhibit secretion of plasma lipoproteins from the liver and to cause the accumulation of lipoprotein apoproteins within the liver. Comparison of the carbohydrate composition of plasma apoproteins and corresponding apoproteins isolated from orotic acid induced fatty livers indicates that the latter are deficient in N-acetyl glucosamine, galactose, and N-acetyl neuraminic acid. These differences can be explained by a lack of exposure of liver apoproteins to glycosylating enzymes present in the Golgi apparatus.

Introduction:

Rats fed an adenine-free diet containing one percent orotic acid develop fatty livers (1) and are unable to secrete plasma very low density (VLDL) and low density (LDL) lipoproteins from their livers into their blood (2). We have demonstrated that, during the time of inhibition, all but one of the apoproteins of plasma VLDL accumulate within fatty particles (liposomes)[1] in the liver (3). The apoprotein missing from the liposome is one of a pair of smaller apoproteins of VLDL which have been shown to be antigenically identical but to differ in their content of sialic acid (4). The other member of this pair is present in the liposome in high concentrations. These observations suggest that the inhibition in secretion of serum lipoproteins might be related to glycosylation of the apoproteins within the liver. In this report we present carbohydrate composition of some of the apoproteins isolated from plasma VLDL and orotic acid induced fatty livers.

Materials and Methods:

Isolation and purification of apoproteins from the liver: Male Sprague-Dawley rats (175-250g) were fed a semisynthetic diet containing 1 per cent orotic

[1]The term liposome, as used in this paper, refers to a lipid-laden vesicle bounded by membrane probably derived from rough endoplasmic reticulum.

acid (3) for 7 days. Liposomes were isolated by ultracentrifugation and delipidated in alcohol and ether as previously described (3). Apoproteins were resuspended in 0.01 M borate buffer at pH 11.5 containing 6 M urea. Sephadex gel filtration of apoproteins in 6 M urea was performed according to the method of Koga et al. (5). Effluent fractions were pooled and concentrated by pressure dialysis. Preparative polyacrylamide electrophoresis was then performed using a Canalco Prep-disc apparatus according to the method described in the Canalco Prep-disc instruction manual, except that all solutions contained 6 M urea. The length of the separating gel and the time of electrophoresis depended upon the particular apoprotein to be isolated. Protein determinations were performed by the method of Lowry et al. (6) using bovine serum albumin as a standard.

Isolation and Purification of Plasma VLDL apoproteins:

Male Sprague-Dawley rats were fed a hyperlipoproteinemia-inducing diet (7) for ten days prior to sacrifice and isolation of plasma VLDL by ultracentrifugation at density 1.006 g/ml (3). VLDL was purified by 2 additional centrifugations at the same density. Delipidation, gel filtration and preparative electrophoretic procedures used to purify plasma VLDL apoproteins were identical to those used for liver apoproteins.

Figure 1 shows analytical electrophoregrams of purified liposomal and VLDL apoproteins after preparative electrophoresis. The apoproteins were operationally designated 22, 31 and 32; an L or V prefix indicates whether the fraction was derived from liposomes or VLDL, respectively. The apoprotein that VLDL holds in common with low density lipoprotein does not migrate in the polyacrylamide and was not purified because it could not be satisfactorily resuspended in aqueous media after delipidation. Its presence within the liver liposomes, as determined by immunological methods, has already been reported (3). With these methods of purification, 100 ml of hyperlipemic plasma yielded approximately 6 mg of V22, 0.3 mg of V32 and 0.5 mg of V31, while 100 g of orotic acid induced fatty liver yielded approximately 0.3 mg of L22 and 3 mg of L31.

Carbohydrate Analysis:

Carbohydrate analyses were performed by gas-liquid chromatography using the

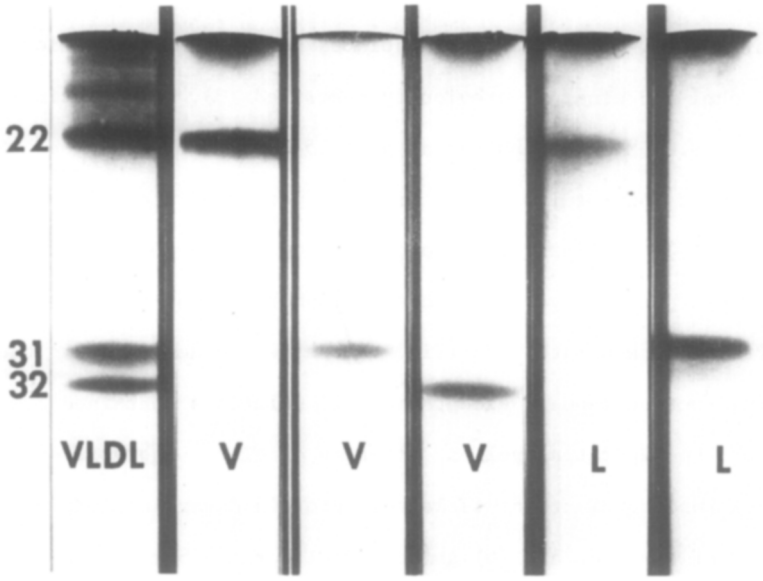

Fig. 1.
Polyacrylamide electrophoresis of delipidated VLDL and purified VLDL and liposomal apoproteins in 6 M urea. Acrylamide concentration was 7.5%. The apoproteins were operationally designated 22, 31 and 32 with a prefix of L for apoproteins isolated from liposomes and V for apoproteins isolated from plasma VLDL.

method of Dawson et al. (8). To eliminate non-covalently bound sugars prior to analysis, each sample was dialyzed extensively against distilled water, then filtered through 2 layers of glass fiber filter followed by precipitation with 5% trichloroacetic acid, centrifugation and resuspension of the precipitate in 0.01 N NaOH.

Results:

Of the apoproteins that can be solubilized, comparable apoproteins in liposomes and VLDL have been found to be identical immunologically, electrophoretically and by peptide mapping, and the 2 small VLDL apoproteins (V31 and V32) have been found to be identical to each other by immunologic and peptide mapping criteria. The peptide mapping and further comparative characterization of VLDL and liposomal apoproteins will be reported upon separately.

Figure 2 gives the results of carbohydrate analyses of L31, V31 and V32. Four separate isolations from different groups of rats were made for each apoprotein. L31 and V31 were found to be almost devoid of carbohydrate while

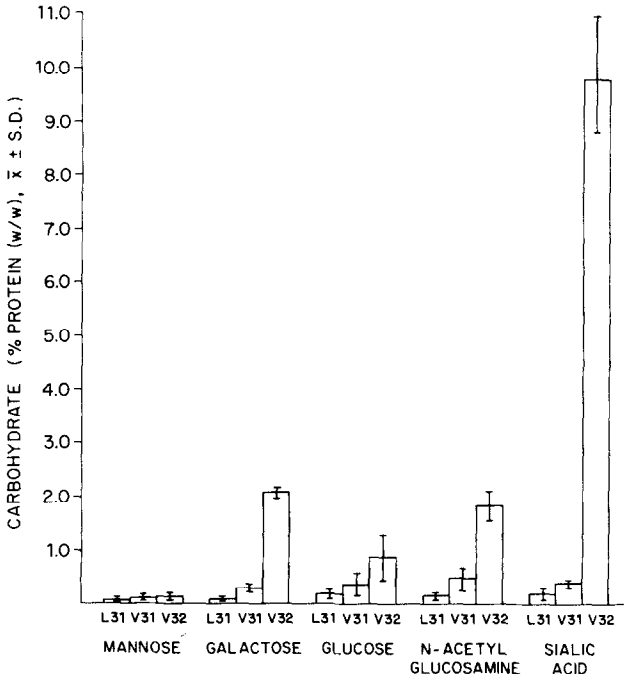

Fig. 2.

Carbohydrate analysis of L31, V31 and V32. Four separate isolations from different groups of rats were made for each apoprotein: the height of the bars represents the average of 4 determinations while the error bars represent plus and minus the standard deviation.

V32 contained approximately 2% galactose and N-acetyl glucosamine and 9% sialic acid. The data are consistent with one residue each of N-acetyl glucosamine and galactose and 3 residues of sialic acid per 10,000 daltons of protein. All three apoproteins are completely lacking in mannose. This clearly demonstrates that the polymorphism of the small VLDL apoproteins is due to the presence or absence of an entire carbohydrate chain.

Figure 3 shows the results of a similar study of L22 and V22 (note the scale of % protein on the ordinate is different from figure 2). These apoproteins were found to contain both glucosamine and galactosamine which could not be quantitively separated on our chromatograms. The amount of glucose in all of the apoproteins examined was extremely variable; we consider it most likely that this variability is due to contamination, probably from the Sephadex chromatography. L22 contains more mannose and about half as much hexosamine, galactose and sialic acid as V22.

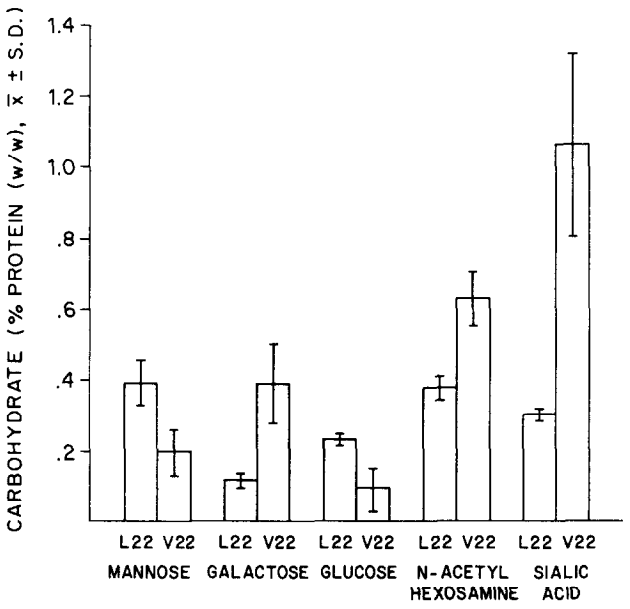

Fig. 3.

Carbohydrate analysis of V22 and L22. The data are the result of determinations of 4 separate isolations of each apoprotein. The height of the bar represents the average of four determinations while the error bars represent plus and minus the standard deviation.

Discussion:

Feeding orotic acid to rats produces a fatty liver in which fat accumulates along with apoproteins as liposomes in endoplasmic reticulum which is studded with ribosomes. The data presented here suggest that liposomal apoproteins are relatively deficient in N-acetyl glucosamine, galactose and sialic acid.

Redman and Cherian (9) have examined the incorporation of labeled sugar precursors into rat liver glycoproteins in different parts of the endoplasmic reticulum. They find that most of the mannose and about half of the glucosamine is attached to protein in the rough endoplasmic reticulum; the remaining glucosamine and most of the galactose and sialic acid is incorporated in smooth endoplasmic reticulum and Golgi apparatus. Perhaps the glucosamine attached to protein in the rough endoplasmic reticulum is differently linked to its protein than that attached in the smooth endoplasmic reticulum and Golgi apparatus. In other words, there might be two classes of enzymes responsible for the addition of glucosamine to

protein, one of which is localized to the rough endoplasmic reticulum while the other is confined to the smooth endoplasmic reticulum and Golgi apparatus. This could account for the apparent acceptor specificity for glycosylation revealed by the presence of all sugar components, albeit in lower concentration, in L22 while the accumulated L31 contains virtually no carbohydrate. Since liposomal apoproteins are found in rough endoplasmic reticulum, their relative lack of carbohydrate might be the result of not having been exposed to the glycosylating enzymes of the Golgi apparatus, where the missing carbohydrate would presumably be attached. This suggests that the missing carbohydrate is a result of the inhibition of lipoprotein secretion rather than its cause. The accumulated apoproteins may be regarded as secretory precursors of the VLDL apoproteins.

The normal dimorphism seen in the small VLDL apoproteins (V31 and V32) might be due to the relative position of the apoproteins in the lipoprotein particle at the time of glycosylation i.e. proteins on the surface of the lipoprotein particle might be more accessible to glycosylation as the particle traverses the smooth endoplasmic reticulum and Golgi. An alternate possibility is that the dimorphism is due to a relatively low efficiency of enzymes which specifically attach carbohydrate moieties to these particular proteins. That this dimorphism exists at the time of secretion of VLDL is suggested by electrophoresis of VLDL apoproteins isolated from rat Golgi apparatus (10) which show the presence of both apoproteins.

This work was supported by Life Insurance Medical Research Fund and USPHS HL 12332, HL 15062 and GM 0093.

References:

1. Standerfer, S. B. and Handler, P. (1955) Proc. Soc. Exp. Biol. Med. 90, 270-271.
2. Windmueller, H. G. and Levy, R. I. (1968) J. Biol. Chem. 243, 4878-4884.
3. Pottenger, L. A. and Getz, G. S., (1971) J. Lipid Res. 12, 450-459.
4. Bersot, T. P., Brown, W. V., Levy, R. I., Windmeuller, H. G., Frederickson, D. S. and LeQuire, V. S. (1970) Biochemistry, 9, 3427-3433.
5. Koga, S., Bolis, L. and Scanu, A. M. (1971) Biochim. Biophys. Acta 236, 416-430.
6. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.

7. Thomas, W. A. and Hartroft, W. S. (1959) Circulation 19, 65-72.
8. Dawson, C. and Clamp, J. R. (1970) Methods in Medical Research. 12, 131-136
9. Redman, C. M. and Cherian, M. G. (1972) J. Cell Biol. 52, 231-245.
10. Mahley, R. W., Bersot, T. P., LeQuire, V. S., Levy, R. I., Windmueller, H. G. and Brown, W. V. (1970) Science 168, 380-382.