RELATIVE INCORPORATION OF PALMITIC AND OLEIC ACIDS INTO TRIACYLGLYCEROL OF SUBFRACTIONS SEPARATED BY GEL CHROMATOGRAPHY OF THE VERY LOW DENSITY LIPOPROTEIN SECRETED BY THE LIVER

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1. Introduction

The VLDL synthesized and secreted by the liver can vary in particle diameter and volume. The size of the VLDL particles secreted by the isolated perfused rat liver can be modulated by, among other factors, the quantity [1–3] and structure [4,5] of the fatty acid provided the liver. Furthermore, the fatty acid composition of the VLDL triacylglycerol secreted by the liver depends on the free fatty acids available to the liver [2–5]. It had not been clear, however, whether classes of triacylglycerol with different fatty acid composition are distributed randomly or whether distribution of the fatty acid is ordered during biosynthesis and secretion among newly synthesized VLDL particles of various sizes.

An answer to this problem was sought in the present investigation by perfusing livers from normal rats in vitro with equimolar quantities of [^{3}H]palmitic acid and [^{14}C]oleic acid; radioactivity incorporated into triacylglycerol was measured in different subfractions of the VLDL isolated from the perfusate by gel chromatography.

2. Materials and methods

2.1. *Chemicals*

Palmitic and oleic acids (99% purity) were obtained from Nu-Chek Prep., Elysian MN. [9,10-^{3}H] Palmitic (spec. act. 350 mCi/mmol), [9,10-^{3}H]oleic acid (spec. act. 9.28 Ci/mmol) and [1-^{14}C]oleic acid (spec. act. 55 mCi/mmol) were purchased from New England Nuclear Corp., Boston MA. Bovine serum albumin (Fraction V powder), obtained from Pentex Inc., Kankakee IL, was purified by a modification [5] of the Goodman method [6]. Agarose (A-150m) was purchased from Bio-Rad Laboratories, Richmond CA. Other chemcials used were reagent grade and were obtained from standard sources. Silica gel G plates, 250 μm thick, were purchased from Analtech, Inc., Newark DE.

2.2. *Animals*

Male Sprague-Dawley rats (Charles River Laboratory) weighing between 200 g and 300 g were used; the animals had free access to water and Purina Chow at all times.

2.3. *Perfusion of the livers*

Livers from normal fed male rats were perfused using the procedures and apparatus described for a medium devoid of erythrocytes [7]. The livers were perfused initially for an equilibration period of 20 min with 40 ml Krebs-Ringer bicarbonate buffer, pH 7.4 [8] containing 100 mg glucose and 3 g bovine serum albumin/dl. The experiment was initiated (T_0) by addition of the fatty acid complex (vide infra), and was continued for another 60 min, at which time (T_1) the perfusate was replaced competely with 40 ml fresh medium. The experiment was then continued for an additional 120 min. At the termination of the experiment (T_3), perfusate was collected for isolation of the VLDL. A pulse dose (5 ml) of the fatty acid

Abbreviations: VLDL, very low density lipoprotein

complex [9] was added to the medium at T_0 and at T_1 and the same complex was infused at a constant rate (11.6 ml/h) during each of the experimental periods. The complex contained equimolar quantities of [^{3}H]palmitic and [^{14}C]oleic acid (10 μmol each acid, 0.2 μCi of oleate and 0.2 μCi palmitate and 60 mg albumin/ml complex) such that the pulse dose was 100 μmol, and 232 μmol were infused/h.

2.4. *Separation and fractionation of VLDL*

The VLDL was isolated from about 50 ml perfusate by ultracentrifugation at native density for 18 h at 153 000 X *g* [10]. The VLDL, which had been concentrated to about 5 ml by centrifugation, was subjected to column chromatography on agarose gel to allow separation based on particle size [11,12]. The VLDL was applied directly to a 1% A-150m agarose column (2.6 X 95 cm) and eluted in ascending manner at 16°C with 0.9% NaCl containing 0.02% NaN_3 and 0.01% EDTA.

2.5. *Lipid analysis and measurement of the radioactivity*

Lipids from the VLDL fractions and from samples of perfusate were extracted with $CHCl_3/CH_3OH$ (2:1, v/v) [13], and free fatty acids and triacylglycerol were isolated by thin layer chromatography on silica gel [2]. The bands of lipids on the plates were visualized with iodine vapors and were extracted with chloroform or scraped directly into counting vials [2]. Free fatty acids [14] and triacylglycerol [15] were analyzed by colorimetric methods.

The incorporation of ^{3}H and ^{14}C into subfractions of the VLDL triacylglycerol was estimated by liquid scintillation counting (Beckman LS 3155T Counter) after isolation by thin-layer chromatography [2]. Correction for double-isotope counting was carried out with internal standards. A more constant spill-over of ^{14}C counts into the ^{3}H counting window was obtained by use of the automatic quench compensation accessory.

3. Results and discussion

The elution pattern on agarose A-150m of the VLDL secreted by the perfused rat liver, and the incorporation of [^{3}H]palmitic acid and [^{14}C]oleic acid into consecutive fractions of the VLDL triacylglycerol are depicted in fig.1 (panels A and B). Although the liver was provided with equimolar amounts of palmitic and oleic acids, radioactivity from oleic acid was incorporated preferentially into VLDL triacylglycerol. However, the relative content of both ^{3}H and ^{14}C were similar for the different lipoprotein fractions separated by gel chromatography (fig.1C). Such random distribution of [^{3}H] palmitate and [^{14}C]oleate among newly synthesized particles of various sizes may result from uniform incorporation of both isotopic fatty acids into VLDL triacylglycerol, or, alternatively, could result from exchange of lipid and subsequent equilibration among different sized VLDL particles during the process of separation.

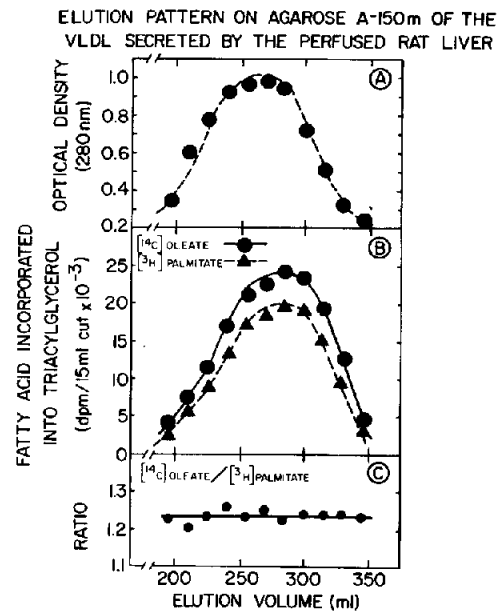

Fig.1. Elution pattern on agarose A-150m of the VLDL secreted by the liver. The liver was perfused with equimolar amounts of [1-^{3}H]palmitic acid and [1-^{14}C]oleic acid, and incorporation of radioactivity into triacylglycerol of the VLDL was measured in consecutive fractions isolated by column chromatography. Refer to the text for additional details. In this experiment, uptake of FFA was 22.7 μmol/g/liver/h, while output of triacylglycerol was 1.2 μmol/g/liver/h. The experiment was repeated 3 times, although the data from only one representative experiment are shown. In the two other experiments, the relative distribution of ^{3}H and ^{14}C was also uniform among the VLDL subfractions; average ratios of ^{14}C/^{3}H were 1.28 and 1.41.

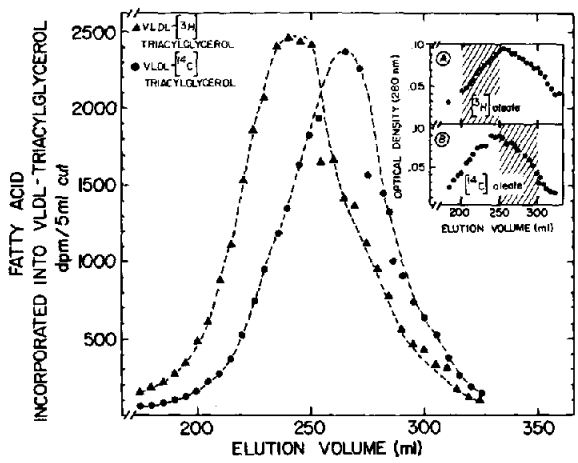

Fig.2. Distribution of ^{3}H and ^{14}C radioactivity among consecutive chromatographic subfractions of a mixture of ^{3}H-labeled 'large' and ^{14}C-labeled 'small' VLDL particles secreted by the isolated livers perfused with [^{3}H]oleate and [^{14}C]oleate, respectively. Livers were perfused with either [9,10-^{3}H]oleic acid or [1-^{14}C]oleic acid. The conditions of perfusion were identical to those described in the section on Materials and methods, except that separate perfusions were required for the [^{3}H]oleate and [^{14}C]oleate, respectively. The VLDL synthesized by livers perfused with [^{3}H]oleate and [^{14}C]oleate were subfractionated by column chromatography on A-150m agarose gel. Elution patterns are presented in inset A ([^{3}H]oleate) and B ([^{14}C]oleate). Fractions indicated by shaded areas in inset A and B were pooled and centrifuged, at native density, for 18 h at 153 000 × g. The concentrated ^{3}H-labeled VLDL and ^{14}C-labeled VLDL were mixed in the proportions of 1:1 (dpm/dpm). An aliquot was applied on agarose column and eluted. Fractions of 5 ml were collected, lipids were extracted and the radioactivity was measured in triacylglycerol separated by thin-layer chromatography. Refer to the text for additional details.

This latter explanation seems unlikely since, using identical procedures, separation of two populations of VLDL was obtained when a mixture of 'large' ^{3}H-labeled and 'small' ^{14}C-labeled VLDL particles secreted by the isolated liver perfused with [^{3}H]oleic acid and [^{14}C]oleic acid, respectively, was applied to the agarose column (fig.2). These data suggest that when the liver is exposed to mixtures of saturated and unsaturated fatty acids, the fatty acid composition of the newly synthesized triacylglycerol released as VLDL is not the primary regulator of the spectrum of sizes among the VLDL particles secreted by the livers.

Since the quantity [1–3] and the structure [3–5] of the fatty acids provided the perfused liver change the relative proportions of different sized VLDL particles secreted by the liver, as measured by mobility in the zonal ultracentrifuge, and yet preferential incorporation of the two different fatty acids among the VLDL subfractions was not observed in the present study, it is conceivable that the quantity of the triacylglycerol to be transported is a fundamental determinant of the spectrum of sizes among the newly synthesized VLDL particles. It is of interest in this regard that the differences in size of VLDL secreted by livers from male and female rats, respectively, provided with equimolar amounts of oleate, were abolished when the VLDL were compared at equal output of triglyceride but unequal uptake of free fatty acid by livers from both sexes [2].

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