EFFECTS OF A MIXTURE OF A SATURATED WITH AN UNSATURATED FATTY ACID ON SECRETION OF THE VERY LOW DENSITY LIPOPROTEIN BY THE LIVER[1]

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SUMMARY:

Palmitic acid (16:0) and palmitoleic acid (16:1), as the complex with bovine serum albumin, were infused at rates of 62 and 124 μmoles/hr into an albumin-buffer medium perfusing livers isolated from normal fed male rats. In other experiments, equimolar mixtures (124 μmoles/hr, total) of 16:0 + 16:1, or myristate (14:0) + 16:1 were infused. The output of triglyceride when 16:1 was infused was greater than when equivalent amounts of 14:0 or 16:0 were infused; output with equimolar mixtures of 14:0 and 16:1, or 16:0 and 16:1 was intermediate between that of saturated and unsaturated fatty acids alone. Rate-zonal mobility of the VLDL in the ultracentrifuge was more rapid as the quantity of 16:1 available to the liver increased, but did not change with increasing amounts of 16:0. The rate-zonal mobility of the mixtures of 14:0 and 16:1, or of 16:0 and 16:1, was not different than that of 16:1 alone. The ratios of phospholipid and cholesterol relative to triglyceride in the VLDL decreased with increasing output of triglyceride and with unsaturation of the fatty acid. Ratios resulting from mixtures of the fatty acids appeared to be in an intermediate position. The composition and properties of the secreted VLDL clearly are dependent on the structure and quantity of FFA available to the liver; with mixtures of saturated and unsaturated fatty acids, the unsaturated fatty acid seems to exert a dominant effect.

INTRODUCTION:

The very low density lipoprotein (VLDL)[4] synthesized and secreted by the isolated perfused rat liver when infused with an unsaturated free fatty acid had a faster flotation rate in a density gradient in the zonal ultracentrifuge

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[4]Abbreviations used: VLDL, very low density lipoprotein; TG, triglyceride; PL, phospholipid; C, free cholesterol; CE, cholesteryl esters; FFA, free fatty acids.

and contained less phospholipid and cholesterol per mole triglyceride than did the VLDL secreted when an equimolar amount of a saturated FFA was provided (1,2). It was concluded from these data that the VLDL secreted in response to the infusion of an unsaturated FFA consisted of particles which had a larger mean volume and which contained relatively more core lipids (TG + CE) than surface lipids (PL + C) than did those secreted in response to a saturated FFA. It was inferred from these observations that mixtures of various FFA might give rise to VLDL particles possessing intermediate properties. To study this problem in a simplified manner, equimolar mixtures of pairs of saturated and unsaturated FFA were infused, and the properties of the VLDL secreted by the isolated perfused rat liver were investigated. The properties of the VLDL were evaluated by flotation in the zonal ultracentrifuge and by determination of lipid composition. It was observed that the lipid composition and rate-zonal mobility of the VLDL were, in part, dependent on the rate of output of TG by the liver, and, in part, on the structure of the fatty acid substrate, when the individual fatty acids were infused. When equimolar mixtures of a saturated and unsaturated FFA were infused, the rate-zonal characteristics of the VLDL were observed to be similar to those of the particle produced from the unsaturated fatty acid alone, while the lipid class composition was intermediate between that of VLDL secreted when either a saturated or unsaturated FFA was infused.

EXPERIMENTAL PROCEDURES:

Livers for perfusion, obtained from normal fed male rats (The Holtzman Co., Madison, Wisconsin) weighing 225-250 g, were maintained on Purina Laboratory Chow and water ad libitum. The livers were perfused in vitro using the apparatus (3) and procedures described previously for the bloodless system (2,4). The livers were perfused for a 20 minute period of equilibration with 70 ml Krebs-Henseleit bicarbonate buffer, pH 7.4 (5) containing 100 mg glucose and 3 g purified bovine serum albumin (2) per 100 ml medium. The medium did not contain any erythrocytes or serum. After the period of equilibration, a complex (6) containing either 265 or 530 μmoles FFA/3 g bovine serum albumin/50 ml was infused at a rate of either 62 or 124 μmoles/hr into the recycling perfusate for the duration of the 4-hour experiment. Complexes containing either palmitate (16:0) or palmitoleate (16:1) alone were infused in these concentrations. Additionally, equimolar mixtures of palmitate and palmitoleate or of myristate (14:0) and palmitoleate were infused at the combined rate of 124 μmoles/hr. Albumin alone was infused as the control. The VLDL

Table 1

Output of TG and Characteristics of VLDL Secreted by Liver in Response to Various Free Fatty Acids Infused Individually or in Mixtures.

FFA Infused	Rate of FFA Infusion, μmoles/hr	FFA Fractional Uptake Rate[a]	TG Secretion Rate, μmoles/g Liver/4 hr	Relative Position of VLDL in Density Gradient[b]	Molar Ratio of Lipids in VLDL[c]			Percent Composition of VLDL-TG Fatty Acids	
					PL	C	CE	16:0	16:1
A. None (4)[d]	-	-	3.1±0.3	12-13	31.0±1.6	25.2±2.0	5.7±0.9	27.1±1.5	4.4 ± 0.4
B. 16:0 (3)	62	8.3±0.5	3.3±0.4	12-13	37.9±2.2	29.3±1.5	7.3±1.0	49.0±0.6	6.1 ± 1.4
C. 14:0 (5)[d]	124	-	4.6±0.3	12-13	30.1±1.5	18.5±1.9	2.8±0.3	e	g
D. 16:0 (6)[d]	124	-	5.6±0.1	13	27.7±1.4	17.4±1.2	3.7±0.2	58.7±1.2	6.6 ± 0.6
E. 14:0/16:1 (2)	62/62	9.1,9.1/ 8.9,9.0	6.0,7.2	10	26.4,28.0	12.9,14.6	2.2,4.2	f	28.7,29.0
F. 16:0/16:1 (5)	62/62	8.7±0.4/ 9.4±0.4/	6.3±0.4	8-9	24.2±0.3	16.8±1.5	3.6±0.3	35.3±0.6	28.7±0.2
G. 16:1 (4)	62	11.1±0.7	6.4±0.7	10	27.8±1.7	15.8±1.4	5.0±0.2	17.8±0.2	33.3±1.4
H. 16:1 (4)[d]	124	-	8.9±0.7	8	22.7±1.4	15.0±0.7	3.0±0.9	16.2±0.2	46.8±1.2

All data are given as Mean ± S.E. Figures in parentheses indicate number of observations.

a. Fractional uptake = $\frac{(\mu\text{moles FFA removed/g liver/hr})}{(\mu\text{moles FFA infused/hr})}$ (100)

b. Lower numbers mean more rapidly migrating VLDL particles. See text and reference 2.

c. Moles of lipid relative to TG, set = 100.

d. Data are reproduced by permission of the Editors of Biochimica Biophysica Acta (2).

e. Per cent of 14:0 = 41.9±2.1. When no fatty acid was infused, 0.7±0.4% 14:0 was observed.

f. Per cent of 14:0 = 15.4 and 16.0.

g. None detected.

was isolated from the perfusate at the termination of the experiment and characterized by rate-zonal ultracentrifugation in the Ti-14 zonal rotor as described previously (2,7). Lipids were extracted from samples of perfusate and VLDL with chloroform-methanol (2:1, v/v), and aliquots of the lipid extract were separated by thin-layer chromatography (2). The individual lipid bands were eluted from the plate and analyzed colorimetrically for TG (8,9), PL (10), and C and CE (11). Gas-liquid chromatographic analysis was carried out as reported previously (2).

All solvents were reagent grade and were redistilled from glass prior to use. Chromatographically pure free fatty acids were obtained from Supelco or Nu-Check Prep. Precoated (silica gel G, schedule A) thin-layer plates for chromatography were obtained from Analtech, Inc. Bovine serum albumin (Fraction V) was purchased from Pentex, Inc., and was purified before use (2).

RESULTS AND DISCUSSION:

The total output of TG, the position of the VLDL in the density gradient in the zonal ultracentrifuge, the molar ratios of the VLDL lipids, and the per cent of 16:0 or 16:1 in the VLDL triglyceride are summarized in Table 1, arranged in order of increasing output of TG.

As observed previously, in experiments with perfusate containing diluted defibrinated rat blood, the output by the liver of TG and the VLDL under the experimental conditions reported here was stimulated by the addition of FFA to the medium (12). When fatty acids were infused, the output of TG was largest with the higher level of 16:1 and least with the lower level of 16:0. Clearly, output of TG appears to be a function of the structure, as well as the quantity of the free fatty acid substrate. Output of TG by the liver was increased as more FFA, either 16:0 or 16:1, was provided. When equimolar mixtures of 16:0 and 16:1 were infused, the output of TG was 6.3 μmoles/g liver/4 hr, intermediate between that amount of TG secreted when 124 μmoles of either fatty acid were infused separately. When equimolar mixtures of 14:0 and 16:1 were infused, the output of TG was similar to that when 16:0 plus 16:1 were infused. The output of TG when the smaller amount of 16:1 was infused was not different from that when fatty acid was not infused.

The fractional uptakes of 16:0 and 16:1 were similar when infused together. Fractional uptakes of 14:0 and 16:1 infused together were also similar. When infused separately, the fractional uptake of 16:1 was slightly more rapid than that of 16:0 ($p<0.05$).

The position of the peak of the VLDL in the density gradient in the zonal ultracentrifuge is presented as the number of the fraction collected from the rotor. Fractions of 25 ml volume were collected; therefore, the separation between fraction 8 and 13 is 125 ml (total rotor volume = 650 ml). The smaller the number, the more rapid the rate-zonal mobility of the VLDL. Since overall density of the total VLDL lipids was relatively constant, even though lipid composition varied, a rapid rate-zonal mobility was interpreted to mean a larger volume of the VLDL particle (2). The slowest mobility was characteristic of the VLDL secreted by livers perfused without exogenous fatty acid, or with infusion of saturated fatty acids regardless of quantity. A more rapid rate-zonal mobility was observed when either the unsaturated fatty acids alone or mixtures were infused. The impression is conveyed that, as the quantity of 16:1 infused increased from 0 to 62 to 124 μmoles/hr, the peak position of the VLDL in the gradient changed from 12-13 to 10 to 8, respectively. Related observations have been made recently in experiments in which oleic acid (18:1) was the substrate, and the perfusate was one containing diluted defibrinated rat blood and buffer (13). When the saturated fatty acid was infused, however, the position of the peak remained in fraction 13 even at the infusion rate of 124 μmoles 16:0/hr. When 16:1 was infused, either singly or in combination with an equimolar quantity of 14:0 or 16:0, the rate-zonal mobility of the VLDL was essentially that of the VLDL produced by the 16:1 alone.

In agreement with earlier observations (1,2), the VLDL produced when 16:0 was infused alone (62 μmoles/hr) contained a larger proportion of PL and C relative to TG than when 16:1 was infused alone (62 μmoles/hr). The same relationship appeared to hold when 124 μmoles/hr of either fatty acid were infused although the magnitude of the difference was diminished. The ratio of PL and C relative to TG in the VLDL, when mixtures of either 16:0 or 14:0 with 16:1 were infused, appear to occupy an intermediate position; the ratios were similar for each of the two combinations of fatty acids. Furthermore, when the larger quantity (124 μmoles/hr) of either 16:0 or 16:1 was infused,

the ratio of PL and C to TG in the VLDL was lower than when the smaller amounts of each FFA were infused, respectively. Clearly, as the output of VLDL increased, the proportion of surface lipids (PL and C) relative to the major core lipid (TG) decreased.

The data suggest that the ratio of CE relative to TG in the VLDL was larger when 16:0 rather than 16:1 was infused. When 62 μmoles/hr of 16:0 or 16:1, or no FFA was infused, the proportion of CE in the VLDL relative to TG exceeded that when 124 μmoles/hr FFA were infused, with either 16:0 or 16:1 as substrates. It may be that CE, transported as a core lipid in the VLDL, is displaced by TG when increased quantities of TG are secreted. Two factors appear to determine the amount of CE in the VLDL. These are the structure of the fatty acid (saturation vs. unsaturation) and the rate of secretion of TG, which itself is dependent upon the structure and quantity of the FFA available to the liver. Similarly, the amount of total cholesterol (C + CE) synthesized and secreted by the liver is also dependent upon these two factors (14)[5].

The percentage of 16:0 and 16:1 in the VLDL triglyceride fatty acids indicates the enrichment of TG by the infused individual fatty acid. This enrichment increased where the larger amount of fatty acid, either 16:0 or 16:1, was infused. When the mixture of 16:0 and 16:1 was infused, lesser percentages of the individual fatty acids were found than when the individual fatty acid was infused alone. There appears to be greater enrichment (i.e., increase above composition when exogenous fatty acid was not infused) of TG with 16:1 than with 16:0 when either the mixtures or the individual fatty acids are infused, suggesting that 16:1 is preferable to 16:0 as a substrate for formation of VLDL triglyceride. The data also suggest that 16:1 is a better substrate than is 14:0.

Unsaturated fatty acids may give rise to larger VLDL particles, in part, through stimulation of TG output. The increase in the volume of the VLDL may also be related to the greater volume occupied by the TG synthesized from

[5]E. H. Goh and M. Heimberg, unpublished observations.

unsaturated fatty acids due to the bend in the hydrocarbon chain at the position of the double bond(s) (15). A larger volume of core lipid (primarily TG) synthesized from unsaturated fatty acids, but a constant molar ratio of PL + C on the surface of the particle (2), results in an apparent decrease in the molar proportions of PL and C relative to TG, assuming the physical limits of the particular volume are set by certain biological constraints. It is of interest that the stimulation by 16:0 of output of TG did not increase the rate-zonal mobility of the VLDL even though the proportions of PL and C relative to TG decreased. An explanation for this observation might be secretion of larger numbers of particles whose volume did not change enough to alter the rate-zonal mobility under our conditions of analysis. The infusion of 62 μmoles/hr of 16:0 did not alter the basal output of TG by the liver, yet the VLDL secreted under these conditions contained more PL and C relative to TG than did the VLDL produced from endogenous fatty acids only. Furthermore, the TG was enriched substantially with 16:0 (22% enrichment). Because of the greater hydrophobic nature of saturated fatty acids, perhaps greater proportions of PL and C are incorporated for stability of the VLDL particles in the aqueous medium of the blood.

In contrast to 16:0, the stimulation of output of TG by 16:1 increased the rate-zonal mobility (increased particle volume) and decreased the ratio of PL and C relative to TG in the VLDL. Presumably, even with 16:1 as the substrate, a point would be reached where the maximum size of the particles is achieved and the liver is required to secrete more identical particles or store the TG in the liver. These data suggest that differences in particle size (rate zonal mobility) and lipid class composition of the VLDL are influenced by the rate of TG secreted by the liver, particularly when unsaturated FFA is the substrate. In previous studies (2) similar effects on lipid composition of the VLDL were observed after the infusion of 18:1 or linoleic acid (18:2). We might, therefore, expect that the lipid class composition of a VLDL containing TG with high proportions of any unsaturated fatty acid is similar. Our results

lead us to suggest that if the plasma FFA pool *in vivo* is enriched with unsaturated FFA, as would result, for example, when the content of the diet is enriched in unsaturated fat, the liver will produce larger VLDL particles, which have lower proprotions of PL and C relative to TG (16).

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