The effect of sex on the quantity and properties of the very low density lipoprotein secreted by the liver in vitro

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Abstract Livers from normally fed male and female rats were perfused in vitro with different amounts of oleate, and the production and properties of the very low density lipoprotein (VLDL) were studied. The mobility of the VLDL in the zonal ultracentrifuge was dependent on the uptake of free fatty acid and on the sex of the animal from which the liver was obtained. A higher proportion of the VLDL secreted by livers from females displayed a more rapid mobility in the zonal ultracentrifuge and, in addition, contained less phospholipid and cholesterol per mole triglyceride than the VLDL from the male, suggestive of larger size of the VLDL secreted by livers from the female rats. Such differences were diminished when the VLDL was compared at equal output of triglyceride but unequal uptake of free fatty acid. These data suggest that the properties of the VLDL are only secondarily modulated by sex, and primarily result from differences in the capacities of livers from either male or female rats to synthesize triglyceride for transport as VLDL. The quantity of triglyceride secreted, regardless of sex, may be an important determinant of both size and number of the VLDL particles. The incorporation of endogenous hepatic fatty acid into VLDL triglyceride was diminished in livers from both sexes by increased uptake of oleate. The greater output of VLDL triglyceride by livers from female animals was dependent on both exogenous and endogenous fatty acids when relatively small quantities of exogenous oleate were available for uptake by the liver. The proportion of palmitate and oleate in the phospholipid of the VLDL secreted by livers from male rats decreased and the content of arachidonate increased with increasing uptake of oleate; no differences were observed in the composition of the phospholipid fatty acids among the various experimental female groups, although these contained more stearate and less oleate and linoleate compared to the male groups. The change of fatty acid composition of the VLDL phospholipid may reflect inclusion of specific types of phospholipid in the VLDL structure for transport of triglyceride from the liver under particular conditions.

Supplementary key words free fatty acids • triglycerides • cholesterol • phospholipids • zonal ultracentrifugation • perfused rat liver • oleate

It has been concluded from the results of studies with the isolated perfused rat liver that the output of triglyceride as a moiety of the VLDL is proportional to the uptake of exogenous FFA and that, for the same hepatic uptake of FFA, livers from female rats secrete more triglyceride than do livers from male animals (1–3). The rate-zonal mobility and the relative lipid composition of VLDL particles secreted by the liver under these conditions were also reported; it was observed that the VLDL secreted by livers from female animals had a more rapid mobility in the zonal ultracentrifuge and contained fewer moles of PL and C per mole of TG than did the VLDL secreted by livers from male rats (2, 3). The present report extends these observations and describes further how sex may modulate the influence of increasing quantities of exogenous oleic acid on the rate-zonal mobility of the VLDL in the ultracentrifuge, and on the lipid class and fatty acid composition. A preliminary report of this work has appeared (2).

EXPERIMENTAL PROCEDURES

Chemicals

All chemicals used were reagent grade and all organic solvents were redistilled from glass before use. Oleic acid (99% purity) was obtained from Applied Science Inc., State College, Pa. and Nu-Chek Prep., Elysian, Minn. Bovine serum albumin (fraction V powder), obtained from Pentex, Inc., Kankakee, Ill., was purified prior to use as described previously (4). BF_3 was obtained from Supelco, Inc., Bellefonte, Penn. [1-^{14}C]oleic acid (sp act 55 mCi/mmol) was purchased from Amersham/Searle, Arlington Heights, Ill. Silica gel G plates, 250 μm thick were purchased from Analtec, Inc., Newark, Del.

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Abbreviations: C, cholesterol; d, density; EDTA, ethylenediaminetetraacetic acid; FFA, free fatty acid; PL, phospholipid; TG, triglyceride; VLDL, very low density lipoprotein.

Perfusion of livers

Livers isolated from normal Sprague-Dawley male and female rats (250–300 g body wt) obtained from the Holtzman Company, Madison, Wisconsin, were perfused in a recycling system (5). The rats were maintained on Purina Laboratory Chow and water ad libitum for at least two weeks prior to removal of the livers for perfusion. The perfusate consisted of 96 ml of defibrinated rat blood and 48 ml of Krebs-Henseleit bicarbonate buffer, pH 7.4 (6). After 20 min of equilibration, a complex (5 ml) of bovine serum albumin and [1-^{14}C]oleic acid containing 12.5, 50 or 100 μmoles of oleic acid was added to the medium at 0 hr; 29, 116, or 232 μmoles of oleate–albumin complex were infused/hr (11.6 ml/hr for 4 hr) for groups I, II, or III, respectively. The average concentrations of FFA maintained in the erythrocyte-free perfusate (measured at T_1 and T_4) were 0.35 ± 0.01, 0.55 ± 0.04 and 0.89 ± 0.08 μmoles/ml for groups I, II, and III, respectively, in experiments with livers from male rats. Corresponding concentrations in experiments with livers from female animals were 0.27 ± 0.01, 0.43 ± 0.03 and 0.80 ± 0.07 for groups I, II, and III respectively. The oleic acid–albumin complex was prepared as reported earlier (5). One hour after the fatty acid was added (T_1), an aliquot of perfusate was removed for analysis; the experiment was continued for another 3 hr, at which time the remaining perfusate was removed (T_4).

Isolation of the VLDL

The VLDL was isolated from the perfusate by zonal ultracentrifugation (7). Aliquots of cell-free perfusate (45–60 ml) containing EDTA (final concentration 1 mM) were centrifuged in the Spinco Ti-14 zonal rotor (Beckman Instruments, Spinco Div., Palo Alto, Cal.). Gradient (d = 1.0–1.4 NaBr, 300 ml) was introduced behind a 200 ml overlay of distilled water into the zonal rotor revolving at 3000 rpm. The sample and sufficient additional NaBr solution (d = 1.4) to fill the rotor was injected at the periphery of the rotor. The rotor was then accelerated to 30,000 rpm, allowed to run at speed for 20 min, decelerated to 3000 rpm and the contents of the rotor collected in 25-ml fractions. The contents of tubes 1–15 from the zonal rotor were combined and lyophilized.

Lipid analysis

Lipids were extracted from the cell-free perfusate and oleic acid–albumin complex (8), and FFA were analyzed (9). The lyophilized VLDL was extracted three times with 25 ml of $CHCl_3$–CH_3OH 2:1 (v/v). The extracts were washed with 0.5 volume of aqueous 0.02% $MgCl_2$, dried in vacuo, and dissolved in 100 ml of $CHCl_3$. Fractionation of the neutral lipids was accomplished by thin-layer chromatography on silicic acid in a solvent system containing petroleum ether–ethyl ether–acetic acid 85:15:1 (v/v). The bands of lipid were visualized with ultraviolet light after spraying the plates with 0.1% rhodamine 6G in methanol. The bands were scraped from the plates, collected in stoppered tubes, and the lipids were extracted. The band of triglyceride was extracted twice with 10 ml of chloroform, and aliquots were analyzed for TG (10, 11). Lipid-soluble phosphorus was measured in the total lipid extract of the VLDL (12). Methyl esters of fatty acids of various lipid classes were prepared (13) and analyzed by gas–liquid chromatography (14).

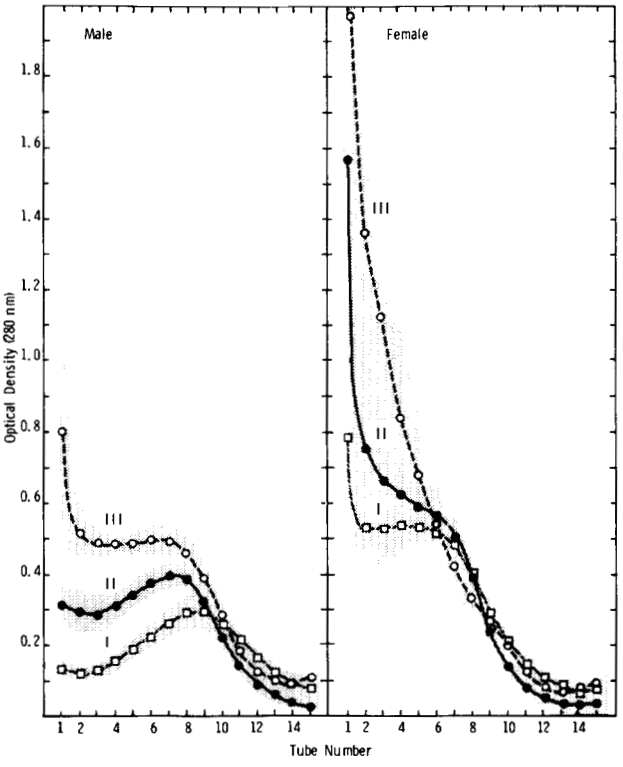

Fig. 1. Pattern of the VLDL after ultracentrifugation in the Ti-14 zonal rotor. The effect of uptake of FFA (Groups I, II, and III) on the rate-zonal mobility of the VLDL secreted by perfused livers from normal male (Panel A) and female (Panel B) rats is shown. Details of the conditions of perfusion and separation of the VLDL are given in the text. Shaded areas indicate ± one standard error (n = 4).

Measurement of the radioactivity

The radioactivity incorporated into the various lipids was measured by liquid scintillation counting in a Beckman (CPM-100) counter with diluted Permaflour. Radioactivity was estimated directly in the bands scraped from the thin-layer plates (15).

Calculations

Uptake of FFA (μmoles or dpm/g liver/hr) was calculated as follows:

$$(1)\quad \text{Net uptake} = \frac{[(FFA)_{T1} + (FFA)_{infused}] - (FFA)_{T4}}{(3)\ (\text{liver weight, g})},$$

where (FFA) = concentration (μmoles or dpm/ml) × volume (ml).

Output of TG, PL, C and fatty acid of various lipids (μmoles or dpm/g liver hr) was calculated by difference between quantities present at T_4 and T_1:

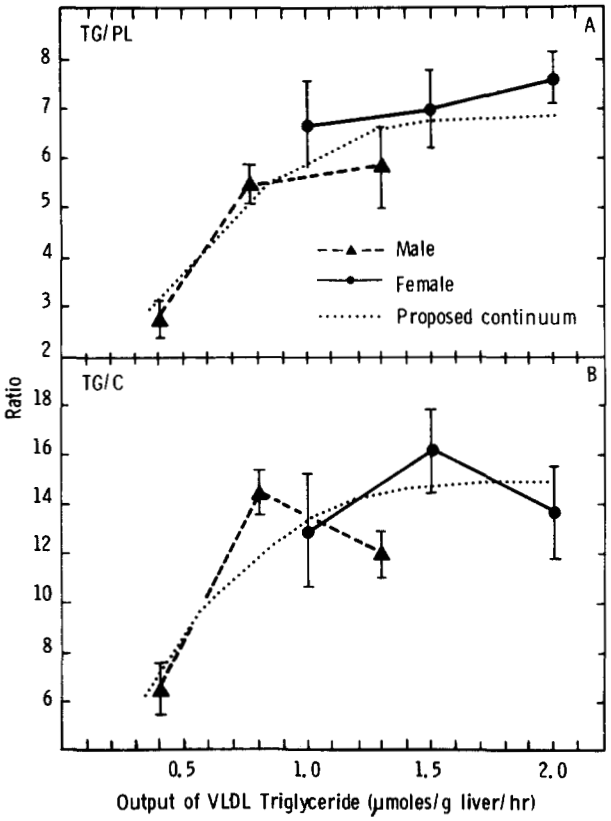

Fig. 2. Molar ratio of the VLDL lipids. Livers from normally fed male and female rats were perfused with increasing amounts of oleic acid and the relative proportion of triglyceride to phospholipid and cholesterol was studied as a function of the total output of VLDL triglyceride. A complete description of the groups, conditions of the perfusion, and calculations is presented in the text. Each point is the mean ± SEM (n = 4).

$$\text{(2)} \qquad \text{Net output} = \frac{(\text{lipid})_{T4} - (\text{lipid})_{T1}}{(3)\ (\text{liver weight, g})}$$

where (lipid) = concentration (μmoles or dpm/ml) × volume (ml).

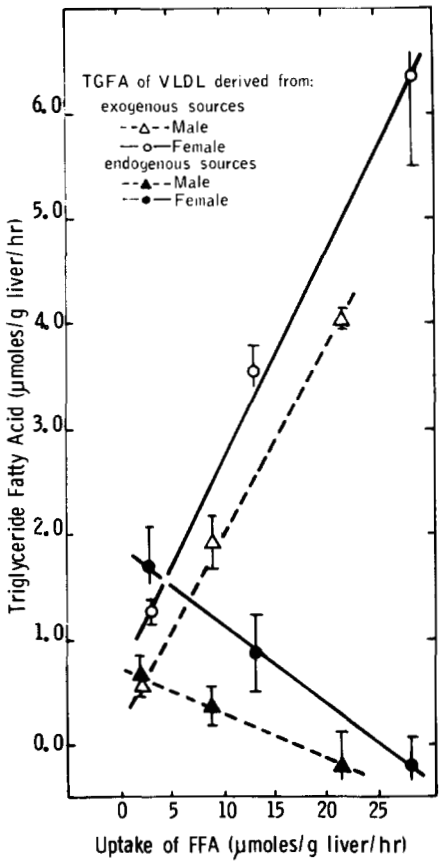

Fig. 3. Hepatic output and source of triglyceride fatty acids (TGFA) of VLDL. A description of the conditions of perfusion is presented in the text. The amounts of VLDL-TGFA derived from exogenous sources were calculated by dividing the radioactivity recovered in TG (equation 2) by the specific activity of FFA taken up by the liver. The specific activity (dpm/μmole) of the FFA taken up by the liver was calculated from radioactivity (dpm/g liver/hr) and mass (μmoles/g liver/hr) of FFA disappearing from the medium using equation (1). The amounts of TGFA derived from endogenous sources were calculated by differences: TGFA derived from endogenous sources = total TGFA − TGFA derived from exogenous sources ([1-^{14}C]oleic acid). Each point is the mean ± SEM (n = 4).

RESULTS

Zonal ultracentrifugation pattern of VLDL secreted by the liver

The pattern of the VLDL after ultracentrifugation of the perfusate in the Ti-14 zonal rotor shows differences in rate-zonal mobility of the VLDL dependent on the amount of FFA infused and on the sex of the animal (**Fig. 1**). It is of particular interest that, within each sex, the proportion of the VLDL which exhibited a more rapid rate-zonal mobility increased as the FFA available to the liver increased. For the same amount of FFA infused, the VLDL secreted by livers from female rats tended to have a more rapid rate-zonal mobility in the ultracentrifuge. This more rapid mobility is indicative of a larger average particle size and/or lower density than that of the VLDL produced by the male, particulary apparent at lower concentrations of FFA.

Lipid composition of the VLDL secreted by liver

The differences in physical behavior of the VLDL in the zonal ultracentrifuge are related to the lipid composition and particle size of the lipoprotein. The composition varied with rate of uptake of FFA by the liver. The VLDL particles secreted by livers from male rats infused with small amounts of oleate (group I) contained the highest proportion of PL relative to TG, about twice that of the other male groups. The amount of PL relative to TG in the VLDL secreted by livers from females of group I was less than in the VLDL secreted by the males of the same group; no differences due to sex were observed in groups II and III. It is possible, therefore, that the ratio TG/PL depends primarily on the

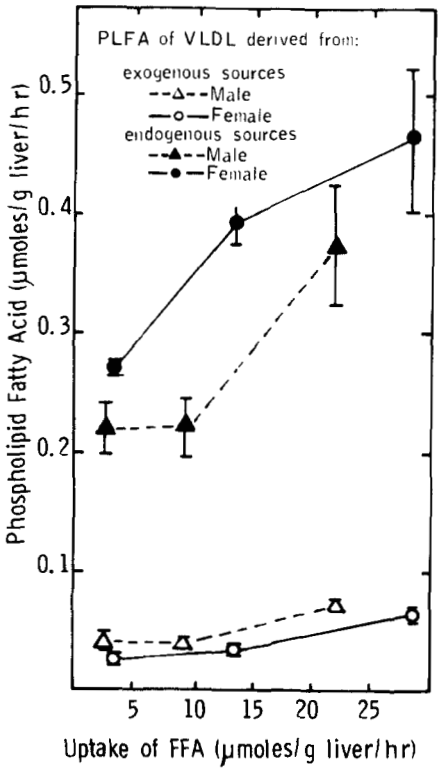

Fig. 4. Hepatic output and source of phospholipid fatty acid (PLFA) of VLDL. A description of the conditions of perfusion is presented in the text. For calculations, see the legend for Fig. 3. Each point is the mean ± SEM (n = 4).

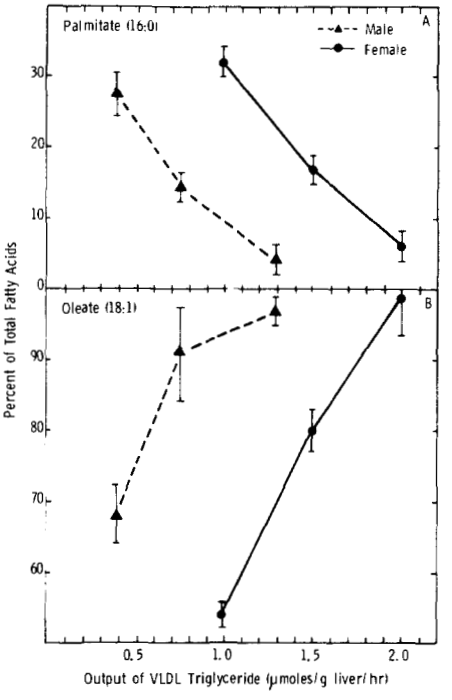

Fig. 5. Percent of palmitate and oleate in VLDL triglyceride fatty acids secreted by the liver. Livers from normally fed male and female rats were perfused with increasing amounts of oleic acid as described in the text. Output of total triglyceride fatty acids was calculated using equation 2. Output of 16:0 and 18:1 was calculated similarly, following determination of the percent 16:0 and 18:1 in the total triglyceride fatty acids by gas–liquid chromatography. These values were related to the output of total triglyceride fatty acids. The percent 16:0 or 18:1 in TGFA depicted in Fig. 5 was calculated as the ratio: 100 × (output of 16:0 or 18:1 in TGFA)/(total TGFA output). Each point is the mean ± SEM (n = 4).

total output of VLDL triglyceride (**Fig. 2A**). The molar ratio of TG/PL for males was much higher in group II than in group I, but did not increase further in group III. The ratio TG/PL for all female groups was similar and did not differ from males of groups II and III. These data suggest that the ratio TG/PL is a continuous function, varying with output of VLDL, and reaching a maximum. The molar content of VLDL cholesterol relative to TG, about half that of PL, was also higher in group I than in II or III (males). The molar ratio of TG/C was higher in the males of groups II and III than in those of group I. No significant differences were observed in the ratio TG/C among the several groups of females (Fig. 2B).

Origin of VLDL triglyceride fatty acids

The total output of VLDL triglyceride and the fraction derived from exogenous FFA[3] were proportional to the uptake of FFA by the liver and were greater with livers from female rats than with livers from male animals at equal uptake of oleate (**Fig. 3**). VLDL triglyceride fatty acids derived from endogenous fatty acids were negligible in group III, but accounted for about 50% of the TG fatty acids secreted by livers of group I of either sex (Fig. 3). Clearly, the higher rate of production of VLDL triglyceride by livers from female animals depended on both exogenous and endogenous fatty acids when the uptake of FFA by liver was small. The rate of incorporation of endogenous hepatic fatty acid into VLDL triglyceride was diminished in both sexes by the increased uptake of FFA.

Origin of VLDL phospholipid fatty acids

The net output of VLDL phospholipid by livers from male rats was similar in groups I and II despite differences in output of TG in these groups; output of PL was higher in group III than in groups I and II. The origin of the fatty acids of the VLDL phospholipids is shown in **Fig. 4.** Exogenous fatty acids (oleate under our experimental conditions) accounted for 13.9 ± 4.1, 11.2 ± 2.3, and 16.9 ± 2.0% of the total VLDL phospholipid fatty acids secreted by livers from male rats of the groups I, II, and III, respectively. The corresponding values were 8.0 ± 2.6, 6.9 ± 0.8 and 12.8 ± 0.5 for groups I, II, and III when livers from female rats were perfused. The respective differences between male and female in groups II and III were statistically significant ($P <$

[3] Exogenous fatty acids denotes the fatty acids taken up from the perfusate. Endogenous fatty acids denotes fatty acid derived from in vivo synthesis in the liver and from intrahepatic lipolysis of fatty acid esters.

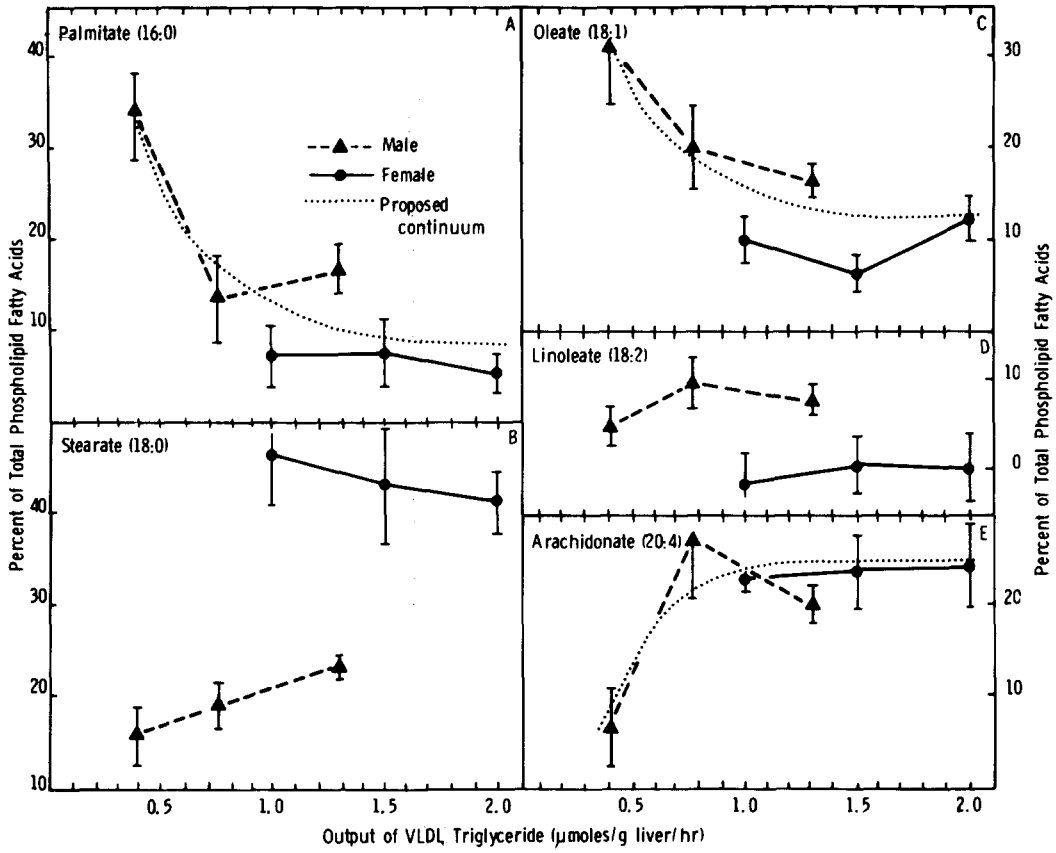

Fig. 6. Percent fatty acids in VLDL phospholipid fatty acids secreted by the liver. Livers from normally fed male and female rats were perfused with increasing amounts of oleic acid as described in the text. Calculations of total and individual phospholipid fatty acids are similar to those described in the legend for Fig. 5. Each point is the mean ± SEM (n = 4).

0.05). It is of interest that endogenous fatty acids represented the more important source of VLDL phospholipid fatty acids even when the liver was provided with large amounts of oleate and oleate was the predominant fatty acid transported in the VLDL triglyceride.

Fatty acid composition of lipids of the VLDL

The content of palmitate (16:0) and oleate (18:1) VLDL triglyceride is shown in **Fig. 5.** As expected, the percent 18:1 in TG increased while the proportion of palmitate fell with increasing availability of exogenous oleate. For the same amount of VLDL triglyceride secreted, the TG secreted by livers from male rats contained relatively more oleate and less palmitate than the VLDL triglyceride secreted by the female.

The fatty acid composition of the VLDL phospholipid secreted by livers infused with different amounts of oleate can be seen in **Fig. 6.** The fatty acid composition of the PL secreted by livers from male rats was relatively similar in groups II and III; the proportion of palmitate and oleate in groups II and III (male) was lower and, reciprocally, the content of arachidonate was higher compared to that of group I. No significant differences were observed in the relative composition of VLDL phospholipid fatty acids in the different groups of female rats. In all groups the VLDL phospholipid fatty acids secreted by livers from female animals contained more stearate and less oleate and linoleate than the VLDL secreted by livers from male rats.

DISCUSSION

The data presented here confirm earlier reports from this laboratory that the output of TG by the liver is stimulated by FFA (16, 17), and that livers from female rats secrete more TG at equal quantities of infused oleate than do livers from male animals (2, 3); the greater output of VLDL triglyceride by the female may result from increased rates of synthesis of TG from FFA in comparison to livers from male rats (2). The secretion of PL and C as moieties of the VLDL is probably proportional to the output of TG (18), at least in some circumstances. The output of TG, in turn, is dependent on the availability of FFA to the liver. This proportionality of output of lipid moieties of the VLDL was observed originally with livers from normally fed male rats when relatively small quantities of palmitate were provided (19). Presumably the proportionality resulted from the stimulation by the

FFA substrate of the output of more VLDL particles of similar composition.

It is not necessary, however, that such a mechanism be invoked for all conditions or with all fatty acids. It is possible that the composition of the VLDL particle may be altered, as appears to be the case here, by increasing concentrations of the substrate oleate. Output of TG may be increased without a proportionate increase in output of PL or C as moieties of the VLDL, and would explain the change in lipid composition, particle size, and rate-zonal mobility of the secreted VLDL reported here. This lack of proportionate increase may be related to the physical properties of the FFA substrate (e.g., oleate) and may be manifested primarily as a change of volume of the VLDL particle (e.g., disproportionate increase in TG) rather than of the number of particles. On the basis of the data reported in this paper, it is probable that the VLDL particles increase in size (volume), resulting in a more rapid rate-zonal mobility in response to an increase in content of TG relative to PL and C with increasing output of lipoprotein. As additional FFA becomes available to the liver, both the size and the number of VLDL particles secreted by the liver increase. When the rate-zonal mobility and lipid composition of the VLDL secreted by livers from male and female rats were compared under conditions of equal output of TG (but unequal uptake of FFA), differences were not observed. These data lead us to suggest that the properties of the VLDL are only secondarily sex dependent, and result from major differences between livers from male or female rats in their capacity to synthesize TG for transport as VLDL (2).

It is probable from the data reported here that the relative content of oleate and palmitate in the VLDL triglyceride fatty acid is modulated by sex; inherent genetic and metabolic differences between the sexes may affect the fatty acid composition of the VLDL triglyceride. In addition, the uptake and structure of specific FFA by the liver also affects the fatty acid composition of the VLDL triglyceride. The proportion of exogenous FFA (those taken up from the medium) resecreted in the VLDL triglyceride fatty acids increases with greater availability of the FFA to the liver. With increasing uptake of exogenous FFA, incorporation of the fatty acid into the triglyceride and output of the TG is stimulated, but incorporation of the endogenous fatty acid into the secreted VLDL triglyceride is suppressed. This latter effect may result from inhibition of hepatic lipogenesis by exogenous FFA (20–22), and, possibly, from decreased lipolysis of storage pool(s) of hepatic lipids and reduced reentry of the fatty acid into a metabolic pool for subsequent retransport as VLDL triglyceride.

The change in phospholipid fatty acids of VLDL secreted by livers from male animals with increased uptake of oleate suggests that the output of TG may be, under certain circumstances, a determinant of phospholipid fatty acid composition. It is conceivable that certain microsomal lecithin species (23) may preferentially be utilized for synthesis of VLDL, depending on conditions. Furthermore, the existence of two hepatic metabolic pathways which may synthesize lecithins with different degrees of unsaturation (23, 24) may be a means of controlling the formation of specific lecithins for transport of TG from the liver. The sex differences in the composition of the VLDL phospholipid fatty acids may be related, in part, to the sex differences in hepatic phospholipid metabolism reported previously (25, 26).

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