THE EFFECT OF SEX ON CERTAIN PROPERTIES OF THE VERY LOW DENSITY LIPOPROTEIN SECRETED BY THE LIVER[1]

Henry G. Wilcox[2], William F. Woodside[3], Kerry J. Breen[4], Howard R. Knapp, Jr., and Murray Heimberg[5]

Departments of Pharmacology and Medicine
Vanderbilt University, School of Medicine
Nashville, Tennessee 37232

Received April 25,1974

SUMMARY: Livers from normal fed male and female rats were perfused with a diluted blood-buffer medium containing various amounts of oleic acid as the complex with bovine serum albumin. Livers from female rats secreted greater quantities of triglyceride into the perfusate, over a wide range of concentration of free fatty acid, than did livers from male animals. The very low density lipoprotein (VLDL) carrier for triglyceride, which was secreted by livers from female animals, was a larger particle and contained fewer moles phospholipid and sterol per mole triglyceride than the VLDL secreted by livers from male rats. Since plasma concentrations of triglyceride in female rats are similar to or less than those in male animals, but yet hepatic output of VLDL triglyceride is greater in the female, it can be postulated that extrahepatic uptake and metabolism of VLDL triglyceride is also more rapid in the female than in the male; the more rapid rate of utilization of VLDL triglyceride may also apply to phospholipid and cholesterol, and, in part, result from the differences in properties and composition of the VLDL secreted by livers from male and female animals, respectively.

INTRODUCTION

It had been reported earlier from this laboratory that the output of triglyceride by isolated perfused livers from normal female rats exceeded that of livers from male animals, and that the output by livers from ovariectomized rats was less than that of livers from unoperated female rats (1). The output of triglyceride by livers from ovariectomized rats could be returned toward normal

---

1. This research was supported by Grants AM-01677 and AM 15498 from the National Institutes of Health, United States Public Health Service. Part of this work was presented by M. H. at the Benzon Symposium on Hepatic Metabolism, Royal Danish Academy of Sciences, Copenhagen, Denmark, 1973.
2. Career Development Awardee of the U.S. Public Health Service, No. 1-K4-HL 70329.
3. Present Address: Department of Pharmacology, University of New Mexico, School of Medicine, Albuquerque, New Mexico 87106.
4. Present Address: Department of Medicine, St. Vincent's Hospital, University of Melbourne, Melbourne, Australia.
5. To whom requests for reprints should be addressed.

by treatment with estrogens. We wish to report here that the output of triglyceride by livers from female rats not only exceeds that of livers from male rats over a wide range of concentration of perfusate free fatty acid, but also that the rate-zonal mobility of the VLDL* in the ultracentrifuge, the apparent size of the secreted particle, and the lipid composition of the VLDL differ between the sexes.

EXPERIMENTAL METHODS

Male and female rats (obtained from Holtzman) weighing 230-270 g, maintained on Purina Laboratory Chow and water ad libitum, were used in the fed state as liver donors. Procedures and apparatus for perfusion of the isolated liver were described previously (2). The initial perfusate (hematocrit approximately 30) contained 47 ml defribrinated rat blood and 23 ml of Krebs-Ringers bicarbonate buffer, pH 7.4 (2). A complex of oleic acid (purchased from Nu Chek or Supelco) (18:1) and purified (3, 4) bovine serum albumin (Pentex, fraction V), which contained 0-1773 μmoles oleate and 5 g albumin per 50 ml of complex (2), was infused at a constant rate (11.7 ml/hr) during the experiment. The livers were perfused with the recycling medium for a 20 minute period when an aliquot of perfusate was removed for analysis, and infusion of the fatty acid complex started. The experiment was terminated four hours later. Aliquots of perfusate were taken for analysis at hourly intervals. In those experiments in which the VLDL was isolated, the final volume of perfusate was collected and EDTA was added to give a concentration of 1.0 mg/ml. The perfusate plasma was separated from the erythrocytes by centrifugation, and an aliquot (60 ml) was adjusted to d 1.4 with NaBr (40 g/60 ml plasma) giving a final volume of about 70 ml. The VLDL in the perfusate plasma was isolated and characterized partially by zonal ultracentrifugation in a density gradient (5).

The fractions containing the VLDL were reduced in volume by ultrafiltration (5). Lipids were extracted from the concentrated VLDL with $CHCl_3$-CH_3OH (2:1 v/v),

* Abbreviations used: VLDL, very low density lipoprotein; TG, triglyceride; PL, phospholipid; C, cholesterol; CE, cholesteryl esters; FFA, free fatty acids; d, density; EDTA, ethylenediamine tetraacetic acid.

aliquots were applied to 250μ silica gel G plates (Anatech); individual lipid bands were eluted and analyzed (5).

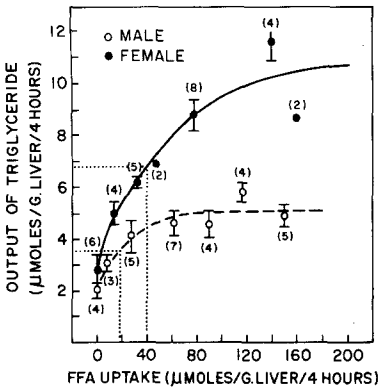

Figure 1: Dependence of hepatic output of triglyceride on uptake of FFA. The output of triglyceride is shown as a function of hepatic uptake of oleic acid. The average body weight in the female group was 241±3.5 (S.E.M.) g and 233±2.2 g in the male group. The average liver weight was 7.65±0.13 g for the females and 8.95±0.15 g for the males. The difference in the rates of secretion of triglyceride between livers from male and female rats would be diminished were the output calculated per 100 g body weight or per total liver. The Michaelis-Menten plot (reciprocal of output of triglyceride, 1/v, vs. reciprocal of uptake of fatty acid, 1/s) was constructed from the data in figure 1, except that the output of triglyceride when oleic acid was omitted was subtracted (2.09 μmoles/g/4 hours for males and 2.77 for females). Values for V_{max} and K_S were calculated. The values for K_S (39.8 μmoles FFA/g/4 hours for females and 19.1 for males) on the abscissa are related to the half-maximal velocity on the ordinate. The calculated V_{max} was 3.4 μmoles TG/g/4 hours for males and 8.4 for females, above the rate at 0 uptake of FFA.

RESULTS AND DISCUSSION

The uptake of FFA from the medium by livers from normal fed male and female rats was proportional to the concentration of FFA in the medium and was similar for livers from both sexes when expressed per total liver, or per 100 g body weight (6). Nevertheless, because of differences in liver mass between males and females, it might be considered that the rate of uptake (per g liver) was slightly more rapid in the female. The output of triglyceride by livers from male or female rats was proportional to the uptake of FFA by the liver (Figure 1). The output of triglyceride by the livers from female animals exceeded that of livers from male animals regardless of the quantity of oleic acid infused. Both the apparent Michaelis-Menten constant, K_S (for oleate), and the maximal

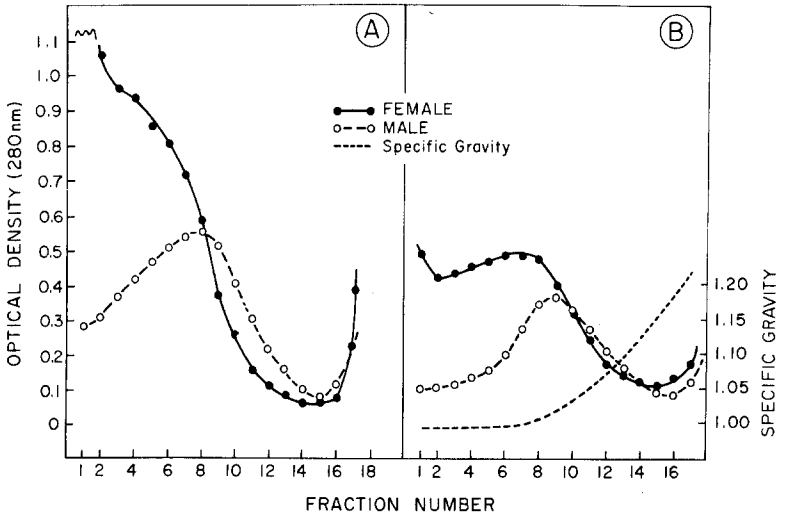

Figure 2: Pattern of the VLDL following rate-zonal centrifugation of perfusate plasma in the Ti-14 rotor.

The procedure for zonal ultracentrifugation was modified from that reported previously (5). Gradient (d 1.0-1.4, 380 ml) was introduced behind 200 ml overlay of distilled water into the Ti-14 zonal rotor (Spinco) revolving at 3000 rpm. The sample and additional NaBr solution (d 1.4) to fill the rotor was introduced, the rotor was accelerated to 30,000 rpm, allowed to run at speed for 10 minutes, decelerated to 3000 rpm, and the contents of the rotor collected. Each fraction contained 25 ml. The direction of migration of the VLDL is from left to right.

A. 290.5 μmoles of oleic acid were infused per hour into the medium perfusing livers from male and female rats.

B. 66.5 μmoles of oleic acid were infused per hour into the medium perfusing livers from male and female rats.

---

velocity, V_{max} (for output of triglyceride), were larger for the female than for the male (Figure 1). The V_{max} for the female liver was attained when uptake of oleic acid was approximately 90-100 μmoles/g/4 hours, corresponding to a steady state concentration of about 1.0-1.1 mM fatty acid in the perfusate plasma; V_{max} for the male liver was attained when the uptake of fatty acid was approximately 50-60 μmoles/g/4 hours, corresponding to a steady state concentration of about 0.7-0.75 mM FFA. It is of interest, although it may be only coincidence, that the K_s for both the male and the female was observed at a concentration of about 0.3-0.4 mM oleate in the perfusate plasma, a concentration similar to that found in plasma of intact fed animals at rest.

The VLDL secreted by the female liver appeared to possess a faster rate-zonal mobility in the zonal ultracentrifuge than the VLDL secreted by the male liver (Figure 3). This behavior of the VLDL in the zonal ultracentrifuge

TABLE 1

Lipid Composition, Relative to Triglyceride, of the VLDL Secreted by the Perfused Liver[1]

Group	TG	PL	C	CE	C+CE
Male (3)	100	21.7±0.5**	13.1±1.6	3.0±0.1**	16.1±1.6 *
Female (3)	100	14.0±0.3	10.0±0.2	1.7±0.3	11.7±0.4

1. Values are moles of lipid relative to TG, set = 100. 290.5 μmoles oleic acid were infused per hour.
* Indicates significance of differences between groups to be <0.05
** Indicates significance of differences between groups to be <0.01
Figures in parentheses indicate number of observations.

suggests to us that the VLDL secreted by the female was a larger, less dense particle than that produced by the male.

The lipid class composition of the secreted VLDL is presented in Table 1. It is of interest that the VLDL produced by the female liver contained fewer moles of PL relative to TG than did the VLDL secreted by the male. Although the amount of C relative to TG tended to be less in the VLDL secreted by the female, the differences between groups with this small number of observations were not statistically significant. These differences are now being studied further.

The sizes of the VLDL particles secreted in response to the infusion of 290.5 μmoles/hr of 18:1 were estimated in one experiment from electron-micrographs. The average diameter of the VLDL secreted by the male was 570±10 Å (SEM) whereas in the case of the female, it was significantly larger, 780±10 Å ($p<.006$).

Differences in the properties of the VLDL secreted by livers from male or female rats, respectively, did not appear to result from any difference in the fatty acid composition of the triglyceride component (Table 2).

The implications of these data are that the VLDL secreted by the female may be a larger and/or less dense particle, and that more triglyceride is transported per molecule phospholipid or sterol, than is the case with the VLDL se-

TABLE 2

Fatty Acid Composition of the VLDL Triglyceride Secreted by the Liver Perfused with Oleic Acid[1]

Group	Fatty Acid Analyzed					
	16:0	16:1	18:0	18:1	18:2	20:4
Male (3)	10.5±0.6	3.7±0.3	1.0±0.3	76.5±1.1	6.8±0.9	0.1±0.1
Female (4)	11.4±0.3	3.8±0.4	1.3±0.1	76.3±1.2	5.9±0.6	0.7±0.2

1. Values are percent fatty acid and are means±standard error. Figures in parentheses indicate number of observations. 290.5 μmoles oleic acid were infused per hour.

creted by the male. The inherently greater capacity of livers from female rats to secrete triglyceride is probably the result of certain hormonally-mediated control mechanisms; the hormones which may be responsible most likely include gonadal steroids and pituitary gonadotrophins. The observation was made recently in our laboratory that livers from normal fed female rats perfused with oleate-1-^{14}C esterified a greater proportion of FFA to triglyceride and oxidized less to CO_2 and ketone bodies than did livers from male animals.* This greater potential of the female to esterify FFA to triglyceride undoubtedly is a stimulant to the increased output of the VLDL, which is the primary transport form for triglyceride from the liver. The greater output of triglyceride by livers from female rats might have been expected to be transported in a VLDL similar in all ways to the lipoprotein by the male. However, rather than synthesize and secrete more VLDL particles of similar composition, livers from female animals apparently produce larger VLDL particles than the male; these larger particles contain more triglyceride relative to the other lipid components, and have a

* C. Soler-Argilaga and M. Heimberg, unpublished information

more rapid rate-zonal mobility in the ultracentrifuge. It is important to learn whether these differences in the properties of the VLDL result from inherent sex-dependent differences in the livers to assemble a VLDL, or whether they result secondarily from differences in the capacity of the livers to synthesize triglyceride. The structure and properties of the VLDL particles were compared in response to a given load of infused fatty acid, but not under conditions of equal output of triglyceride, since, clearly, the female secreted more TG per mole FFA taken up than did the male. It is conceivable that, were these comparisons made under conditions of equal output of TG (but unequal availability of FFA), that the size, rate-zonal mobility, and lipid composition of the VLDL might be identical. Another possibility which must be considered to explain the differences in properties of the VLDL is that the female may possess, in addition to an increased capacity for biosynthesis of triglyceride in comparison to the male, a relatively decreased capacity for provision or biosynthesis of phospholipid, cholesterol, or protein, all of which are necessary constituents of the VLDL. It is of interest, therefore, that livers from female rats esterified more oleate-1-^{14}C to triglyceride but less to phospholipid than did livers from male animals.*

It is of particular interest to relate the data reported here to the concentration of plasma lipids observed in the intact animal. The concentration of triglyceride in the serum of the normal fed rat was observed to be similar in both the male and female (1). In the fasting rat (16 hr), however, the concentration of the VLDL triglyceride was observed to be 0.16±0.02 (S.E.M.) and 0.43±0.05 μmoles/ml plasma, for the female and male, respectively. Plasma triglyceride concentrations have also been reported to be lower in fasting women than in men (7). If output of triglyceride by livers from female animals exceeds that of the male *in vivo*, it is necessary to assume that the rate of utilization of the VLDL triglyceride is more rapid in the female, as has been reported for humans (8). Conceivably, differences in rates of utilization of VLDL triglyc-

* C. Soler-Argilaga and M. Heimberg, unpublished information

eride between the male and the female *in vivo* may be dependent on the lipid composition and physical properties of the VLDL secreted by the liver.

REFERENCES

1. Watkins, M. L., Fizette, N. and Heimberg, M. (1972) Biochim. Biophys. Acta 280, 82-85.

2. Breen, K., Schenker, S. and Heimberg, M. (1972) Biochim. Biophys. Acta 270, 74-80.

3. Goodman, DeW. S. (1957) Science 125, 1296-1297.

4. Goh, E. H. and Heimberg, M. (1973) Biochem. Biophys. Res. Com. 55, 382-388.

5. Heimberg, M. and Wilcox, H. G. (1972) J. Biol. Chem. 247, 875-880.

6. Heimberg, M., Wilcox, H. G., Dunn, G. D., Woodside, W. T., Breen, K. J. and Soler-Argilaga, C. (1974) Proceedings of the Benzon Symposium on Hepatic Metabolism. Royal Danish Academy of Sciences, Copenhagen, Denmark (Munksgaard Press) In press.

7. Furman, R. H., Alaupovic, P. and Howard, R. P. ed. by D. Kritchevsky, R. Paoletti and D. Steinberg (1967) Progress in Biochemical Pharmacology Vol. 2, S. Karger, Basel (Switzerland) p. 215-249.

8. Nikkila, E. A. and Kekki, M. (1970) Ann. Med. exp. Fenn. 48, 246-248.