Comparison of metabolism of free fatty acid by isolated perfused livers from male and female rats[1]

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Abstract Livers from normal, fed male and female rats were perfused with different amounts of [1-^{14}C]oleate under steady state conditions, and the rates of uptake and utilization of free fatty acid (FFA) were measured. The uptake of FFA by livers from either male or female rats was proportional to the concentration of FFA in the medium. The rate of uptake of FFA, per g of liver, by livers from female rats exceeded that of the males for the same amount of FFA infused. The incorporation by the liver of exogenous oleic acid into triglyceride, phospholipid, and oxidation products was proportional to the uptake of FFA. Livers from female rats incorporated more oleate into triglyceride (TG) and less into phospholipid (PL) and oxidation products than did livers from male animals. Livers from female rats secreted more TG than did livers from male animals when infused with equal quantities of oleate. The incorporation of endogenous fatty acid into TG of the perfusate was inhibited by exogenous oleate. At low concentrations of perfusate FFA, however, endogenous fatty acids contributed substantially to the increased output of TG by livers from female animals. Production of $^{14}CO_2$ and radioactive ketone bodies increased with increasing uptake of FFA. The partition of oleate between oxidative pathways (CO_2 production and ketogenesis) was modified by the availability of the fatty acid substrate with livers from either sex. The percent incorporation of radioactivity into CO_2 reached a maximum, whereas incorporation into ketone bodies continued to increase. The output of ketone bodies was dependent on the uptake of FFA, and output by livers from female animals was less than by livers from male rats. The increase in rate of ketogenesis was dependent on the influx of exogenous FFA, while ketogenesis from endogenous sources remained relatively stable. The output of glucose by the liver increased with the uptake of FFA, but no difference due to sex was observed. The output of urea by livers from male rats was unaffected by oleate, while the output of urea by livers from females decreased as the uptake of FFA increased. A major conclusion to be derived from this work is that oleate is not metabolized identically by livers from the two sexes, but rather, per gram of liver, livers from female rats take up and esterify more fatty acid to TG and oxidize less than do livers from male animals; livers from female animals synthesize and secrete more triglyceride than do livers from male animals when provided with equal quantities of free fatty acid.

Supplementary key words oleic acid · triglycerides · phospholipids · CO_2 production · ketogenesis · sex

A number of differences in the hepatic metabolism of lipids have been observed between male and female rats. For example, the concentration of triglyceride in livers from normal, fed female rats exceeds that of the males (2). The increased susceptibility of the female to induction of fatty liver after treatment with hepatotoxins (2–5) may be related to an increased rate of synthesis of TG in the female. In agreement with these data are the observations that the output of TG by livers from female rats exceeds that of livers from male animals in vitro (6, 7) and in vivo (8–10). The biosynthesis of phosphatidylcholine by methylation of phosphatidylethanolamine is accelerated in the female rat in comparison to the male (11, 12). A related observation is that phosphatidylcholine in livers from female rats contains a higher proportion of stearic and arachidonic acids than in the males (13, 14). Sex also influences certain parameters related to the hepatic metabolism of cholesterol (15).

The present study was undertaken to elucidate the influence of sex on the disposition of FFA by the isolated perfused rat liver. Therefore, livers from normal, fed male and female rats were perfused with increasing amount of [1-^{14}C]oleate; uptake and incorporation of fatty acid into products of esterification and oxidation, as well as net output of TG

Abbreviations: TG, triglyceride; DG, diglyceride; PL, phospholipid; C, cholesterol; CE, cholesteryl esters; FFA, free fatty acids; VLDL, very low density lipoproteins.

[1] Some of this work was presented at the 58th annual meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N. J., April, 1974 (1).

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and ketone bodies were measured. We observed that the uptake of FFA, the output of TG, the relative partition of FFA between esterification and oxidation products, the partition between different esters (TG and PL), and partition of fatty acid carbon between CO_2 and ketone bodies, and also the rate of ketogenesis were sex dependent. These data confirm and extend earlier reports from this laboratory (1, 6, 7) and are an approach to the study of the mechanism(s) by which livers from female animals secrete more TG (and VLDL) than do livers from male animals.

EXPERIMENTAL PROCEDURES

Animals

Male and female rats (250–300 g body wt), obtained from the Holtzman Company, Madison, Wisconsin, were maintained on a balanced laboratory ration and water ad libitum for at least two weeks before removal of the livers for perfusion.

Chemicals

All chemicals used were reagent grade and all solvents were redistilled before use. Oleic acid (99% purity) was obtained from Applied Science Laboratories, State College, Pa., and Nu-Chek Prep., Elysian, Minn. Bovine serum albumin (Fraction V powder), obtained from Pentex, Inc., Kankakee, Ill., was purified by a modification (16) of the Goodman method (17). [1-^{14}C]Oleic acid (sp act 55 mCi/mmole), ethyl [3-^{14}C]acetoacetate (sp act, 11.3 mCi/mmole) and sodium [3-^{14}C]DL-β-hydroxybutyrate (sp act, 11.8 mCi/mmole) were purchased from Amersham/Searle, Arlington Heights, Ill. or New England Nuclear Corp., Boston, Mass. Silica gel G plates, 250 μm thick, were purchased from Analtech, Inc., Newark, Del.

Perfusion of livers

Livers, isolated from normal, fed male and female rats, were perfused in vitro using the apparatus described previously (18). The livers were removed surgically from the animals (19), placed in the perfusion apparatus and perfused with a medium consisting of 96 ml of defibrinated rat blood and 48 ml of Krebs-Henseleit bicarbonate buffer, pH 7.4 (20). The medium was gassed continuously with 95% O_2–5% CO_2. After a 20 min period of equilibration, 5 ml of a complex of bovine serum albumin and [1-^{14}C]oleic acid (containing 14.5 ± 1.6, 52.1 ± 1.0, or 112.0 ± 4.6 μmoles of oleic acid for the males of groups I, II, and III, respectively, or 15.0 ± 1.3, 54.5 ± 0.7, or 111.3 ± 3.7 μmoles for the females of groups I, II, and III, respectively) were added to the medium as a pulse dose; immediately thereafter 11.6 ml/hr of the same complex (33.7 ± 3.7, 120.8 ± 2.2, or 259.9 ± 10.6 μmoles/hr for the males or 34.8 ± 3.0, 126.2 ± 1.6 or 258.1 ± 8.7 μmoles/hr for the females of groups I, II, or III, respectively) were infused at a constant rate during a 4 hr experimental period. The oleic acid–albumin complex was prepared as reported earlier (19). One hour later (T_1), about 80 ml of perfusate was removed for analysis; the experiment was continued for another 3 hr, at which time the remaining perfusate was removed (T_4). At the termination of the experiment, the livers were perfused with 30 ml of ice-cold 0.9% NaCl to remove residual perfusate; they were then cleansed of adherent nonhepatic tissue, blotted, weighed, homogenized, and extracted for analysis of lipid content. Radioactive CO_2 generated during the experiment was trapped by passing the gas through three traps in series, each containing 125 ml of 10% KOH (w/v). The KOH in each trap was replaced hourly to insure complete collection of the radioactive CO_2.

Analytical methods

Lipids were extracted from the cell-free perfusate (21) and from the liver (22). The extracts were washed, fractionated, and analyzed as reported elsewhere (23).

Samples of perfusate were hemolyzed and deproteinized with $Ba(OH)_2$–$ZnSO_4$ (24). Aliquots of the protein-free supernatant were analyzed for ketone bodies (24–26), glucose (27), and urea (28).

The radioactivity incorporated into the various lipid classes was measured by liquid scintillation counting in diluted Permafluor using a Beckman CPM-100 counter (Beckman Instruments, Fullerton, Cal.). Radioactivity was estimated directly in the bands scraped from the thin-layer plates (29). Measurement of ^{14}C in ketone bodies was carried out as described by Bieberdorf, Chernick, and Scow (30). Radioactive CO_2 was measured in aliquots of KOH from each trap. CO_2 was distilled into 1 M hyamine in CH_3OH from 1 ml of KOH solution, the hyamine solution was then transferred to counting vials with Permafluor, and the radioactivity was measured. Quenching was corrected by use of internal standards.

Calculations

The uptake of FFA and output of metabolites between T_1 and T_4 was calculated as follows.

FFA uptake (μmoles or dpm/g liver per hr)

$$= \frac{(C_1V_1 + C_0V_0) - C_4V_4}{(LW)\,(3)} \quad \text{Eq. 1}$$

TABLE 1. Experimental parameters: weights of animals and perfused livers, perfusate flow rate and hepatic output of bile

Experimental Parameters	Male			Female		
	I	II	III	I	II	III
Animal wt. (g)	272.7 ± 34.6	272.7 ± 11.5	263.5 ± 16.8	293.7 ± 11.5	287.0 ± 19.7	272.7 ± 6.3
Liver wt. (wet) (g)	11.3 ± 0.9	11.2 ± 0.5	11.2 ± 1.2	8.4 ± 0.3	8.9 ± 0.4	8.5 ± 0.6
$\frac{\text{Liver wt.}}{\text{Body wt.}} \times 100$	4.2 ± 0.3	4.1 ± 0.2	4.2 ± 0.3	2.9 ± 0.3	3.1 ± 0.2	3.1 ± 0.2
Perfusate flow rate (ml/g liver/min)	2.4 ± 0.2	2.4 ± 0.1	2.7 ± 0.2	3.3 ± 0.3	3.0 ± 0.2	3.8 ± 0.2
Hepatic bile output (μl/g liver/hr)	38.4 ± 6	39.9 ± 5	37.6 ± 2	40.7 ± 4	36.8 ± 8	34.2 ± 2

A complete description of each group (I, II, III) is presented in the section on Experimental Procedure. Data are means ± SEM for four experiments in each group. Values for the liver weight relative to body weight, and for the perfusate flow rate of male groups differ significantly ($P < 0.05$) from those of female groups.

where:

C_1 = μmoles or dpm (FFA)/ml cell-free perfusate at T_1
V_1 = volume (ml) of cell-free perfusate at T_1
C_0 = μmoles or dpm (FFA)/ml oleic acid–albumin complex infused between T_1 and T_4
V_0 = volume (ml) of oleic acid–albumin complex infused between T_1 and T_4
C_4 = μmoles or dpm (FFA)/ml cell-free perfusate at T_4
V_4 = volume (ml) of cell-free perfusate at T_4
LW = liver weight, g (wet)

Triglyceride output (μmoles or dpm/g liver per hr)

$$= \frac{C_4V_4 - C_1V_1}{(LW)\ (3)}, \quad \text{Eq. 2}$$

where:

C_1 = μmoles or dpm (TG)/ml cell-free perfusate at T_1
V_1 = volume (ml) of cell-free perfusate at T_1
C_4 = μmoles or dpm (TG)/ml cell-free perfusate at T_4
V_4 = volume (ml) of cell-free perfusate at T_4
LW = wet weight of liver, g.

Output of glucose, ketone bodies, and urea was calculated similarly, except that volume of whole perfusate was used.

Statistics

Statistical differences between regression lines were determined as described by Hewett and Tsutakawa.[3] Their procedure consisted essentially of fitting one line to the pooled data from both groups. One would expect about the same number of points above the line from each group, if the groups were the same. The test statistic used was the number of points in each group falling above the line.

[3] Manuscript in preparation.

RESULTS

The body weights of the animals in all groups were similar (**Table 1**). Weights of the livers, however, were less in the female than in the male groups. The liver weights were about 4% and 3% of the body weights of male and female rats, respectively. The rate of flow

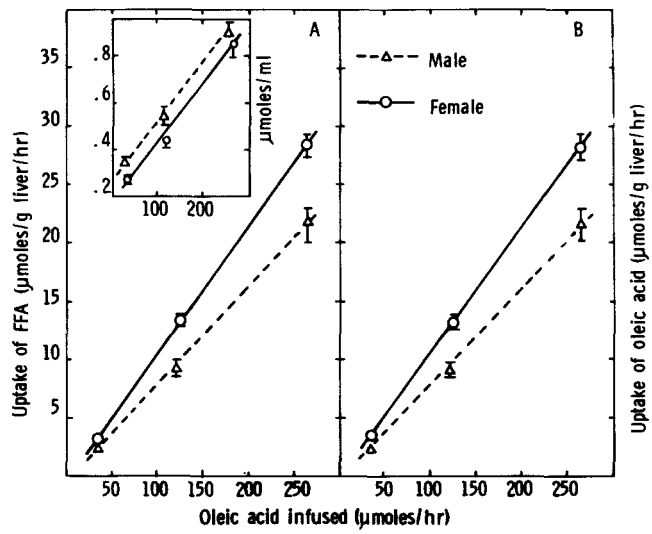

Fig. 1. Uptake of FFA by perfused rat livers from normal, fed male and female rats. This figure demonstrates the effects of rate of oleic acid infusion on the rate of uptake of FFA (panel A) or oleic acid (panel B) by the isolated perfused liver. Uptake of total FFA was calculated as described in the text. Oleic acid uptake was calculated similarly following determination of the percent 18:1 in the total FFA by gas–liquid chromatography. Values given are means ± SEM (n = 4). Regression equations and significance of differences are as follows: Panel A, $y = -0.31 + 0.08x$ (male), and $y = -0.44 + 0.11x$ (female), $P < 0.02$; Panel B, $y = -0.14 + 0.08x$ (male) and $y = -0.15 + 0.11x$ (female), $P < 0.001$. The inset in panel A shows concentration of FFA in the cell-free perfusate (ordinate = μmoles/ml) as a function of rate of infusion of the oleic acid–albumin complex (abscissa = μmoles/hr). The mean concentrations of FFA in the cell-free perfusate were calculated from those values obtained at T_1 and T_4 with all loads of oleic acid. Values are given as means ± SEM (n = 4). Regression equations and significance of differences: $y = 0.26 + 0.003x$ (male), $y = 0.15 + 0.003x$ (female), $P < 0.001$.

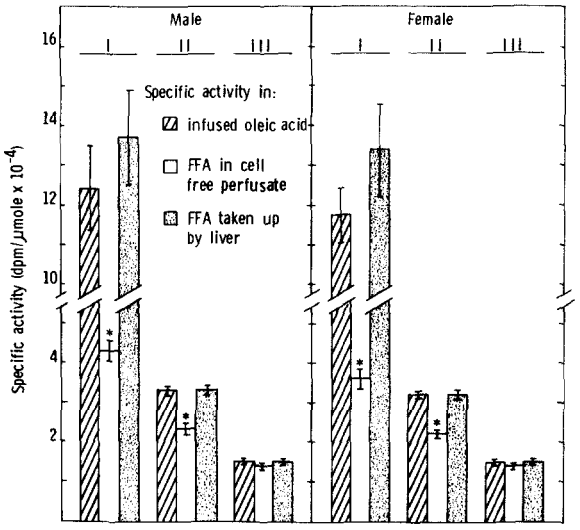

Fig. 2. Specific activity of precursor FFA. In the preparation of the oleic acid–albumin complex (58 ml), 10 μCi of [1-^{14}C]oleic acid was added to 50, 200, and 400 mg of nonisotopic oleic acid for groups I, II, and III, respectively. The specific activity of the infused oleate and the total FFA in the cell-free perfusate were calculated from the radioactivity (dpm/ml) and concentration (μmoles/ml of FFA), at T_1 and T_4 after separation of FFA by thin-layer chromatography from lipid extracts of aliquots of the oleate–albumin complex and from the cell-free perfusate. The specific activity of the FFA taken up by the liver was calculated from the radioactivity (dpm/g liver per hr) and mass (μmoles/g liver per hr) of FFA as indicated in the text under Experimental Procedures. Values given are means ± SEM (n = 4). Those means that are statistically different ($P < 0.05$) from the specific activity of the infused oleic acid are indicated by an asterisk.

of perfusate through the liver in the female groups, expressed per g of liver, exceeded that of the males. Bile flow was maintained at relatively constant rates throughout the duration of the experiment, indicative of good liver function for that period of time. Output of bile was similar in all groups.

Uptake of FFA by liver

With livers from both sexes, the rate of FFA uptake was proportional to the amount of oleate infused. When expressed per g of liver, the rate of FFA uptake was more rapid with livers from female than from male rats as the quantity of infused oleate increased (**Fig. 1A**). It should be noted that the concentration of FFA in the cell-free perfusate was slightly lower when livers from female animals were perfused, despite the larger weights of livers from male animals, although the rate of infusion of FFA was identical in comparable groups (Inset Fig. 1A). The uptake of oleic acid by the liver was similar to the uptake of total FFA in all groups, and was so even in group I, where the concentration of oleic acid accounted for only 61.3 ± 1.3% (male) or 58.0 ± 1.3 (female) of the total chemically measurable FFA in the cell-free perfusate (Figure 1B).

It is necessary to know the specific activity of the precursor ^{14}C-labeled fatty acid in order to evaluate conversion to various metabolic products. We therefore compared the specific activity of the infused oleic acid–albumin complex with that of the FFA of the cell-free perfusate and of the FFA taken up by the liver (**Fig. 2**). These specific activities were similar in group III for both sexes (infusion of larger quantities of oleate). However, in group II, and especially in group I, the specific activity of the FFA in the cell-free perfusate was significantly less than either the specific activity of the infused fatty acid, or the fatty acid taken up by the liver. We have interpreted these data to mean that oleic acid was the only fatty acid taken up by the livers in all groups (compare panels A and B in Fig. 1), and that the FFA, exclusive of added oleate present in the perfusate after the first hour of

TABLE 2. Fate of infused [1-^{14}C]oleate in perfused lives from normal fed male and female rats

Metabolites		Male I	Male II	Male III	Female I	Female II	Female III
A. Products of esterification							
1. In liver	TG	35.3 ± 4.7	36.3 ± 5.7	35.1 ± 2.1	39.1 ± 1.4	41.7 ± 3.6	45.3 ± 3.6
	DG	2.2 ± 0.3	3.3 ± 1.0	1.4 ± 0.2	1.8 ± 0.3	2.7 ± 0.4	1.1 ± 0.1
	PL	12.5 ± 1.2	9.6 ± 0.6	7.5 ± 0.8	8.2 ± 0.6	6.2 ± 0.4	5.6 ± 0.3
	CE	0.9 ± 0.1	0.9 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.4 ± 0.03
2. In perfusate	TG	38.9 ± 5.2	25.8 ± 2.1	22.5 ± 2.5	40.5 ± 0.7	34.0 ± 3.2	28.1 ± 3.9
	DG	0.6 ± 0.1	0.4 ± 0.1	0.9 ± 0.2	0.7 ± 0.1	0.5 ± 0.1	0.8 ± 0.2
	PL	1.2 ± 0.03	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
	CE	0.5 ± 0.05	0.3 ± 0.04	0.3 ± 0.04	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.02
B. Products of oxidation							
1. Carbon dioxide		6.7 ± 1.7	15.7 ± 2.6	12.5 ± 2.4	3.7 ± 1.0	10.8 ± 1.5	7.6 ± 1.5
2. Ketone bodies		1.3 ± 0.1	3.1 ± 0.8	6.6 ± 0.5	0.4 ± 0.1	0.9 ± 0.2	4.5 ± 0.6
C. Total recovery		100.3 ± 5.0	96.4 ± 5.5	89.4 ± 2.4	96.6 ± 3.0	101.3 ± 3.0	94.5 ± 5.8

Details of the conditions of perfusion and description of different groups (I, II and III) are given in the text. Values, expressed as percent of FFA taken up between T_1 and T_4 by the liver, represent means ± SEM for four experiments in each group.

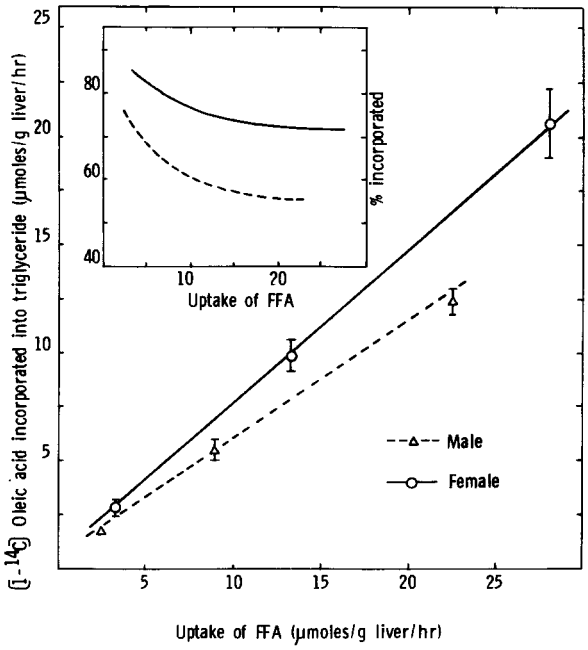

Fig. 3. Incorporation of [1-^{14}C]oleic acid into total triglyceride of liver plus perfusate. Livers from normal fed male and female rats were perfused with increasing amounts of oleic acid, and incorporation of fatty acid into TG was measured as a function of FFA uptake. Details of the conditions of perfusion are given in the text. μMoles of oleic acid incorporated into TG of liver plus perfusate (equation 2) equal total radioactivity recovered in TG divided by specific activity of FFA take up by the liver. Each point is the mean ± SEM (n=4). Regression equations and significance of differences are: $y = 0.72 + 0.53x$ (male), and $y = 0.60 + 70x$ (female), $P < 0.02$. The inset shows the relative incorporation of oleic acid (% of ^{14}C taken up incorporated into TG) as a function of FFA uptake (μmoles/g liver per hr). The curves were calculated from the data generated by the regression equations.

perfusion, represents an albumin-bound FFA pool that has a very slow turnover rate and that is not in rapid equilibrium with the total FFA pool (31). When the molar ratio FFA/albumin is decreased, the relative proportion of the slow turnover-rate pool to that of the fast turnover-rate pool becomes larger.

In all experimental groups, the specific activity of perfusate FFA remained unchanged during the course of the perfusion. It is, therefore, unlikely that the reported differences in specific activities resulted from influx of unlabeled FFA from the liver or from lipolysis of glycerides present in the perfusate. Such dilution of specific activity of FFA taken up by the liver had been observed when heparin was added to the medium, and when the animal from which the liver was removed was treated with heparin (32). Under such conditions significant lipolytic activity was measured in the perfusate (33). For these reasons, the specific activity of the fatty acid taken up by the liver was used to calculate the metabolic disposition of the infused [1-^{14}C]oleate.

Utilization of oleate by the liver

The fate of the [1-^{14}C]oleic acid taken up by the liver is summarized in **Table 2**. The majority of FFA was esterified to TG in all groups. Oxidation products accounted for less than 20% and 13% of FFA taken up for male and female animals, respectively, with the highest concentration of infused oleate. The incorporation of [1-^{14}C]oleic acid into DG and CE represents, at most, 3–4% of total radioactivity.

The rate of net incorporation of [1-^{14}C]oleate into TG, PL, and oxidation products, the major pathways of metabolism of exogenous FFA, was proportional to the amount of FFA taken up by the liver (Figs. 3, 4, and 5). The percentage of ^{14}C incorporated into TG (**Fig. 3,** inset) and PL (**Fig. 4,** inset) decreased, whereas the percentage incorporated into oxidation products (**Fig. 5,** inset) increased with increasing uptake of FFA by the liver. The incorporation of exogenous FFA into TG by livers from female rats exceeded that of livers from male animals, the difference being magnified as the uptake of FFA increased. In contrast, the incorporation of [^{14}C]oleate into PL and oxidation

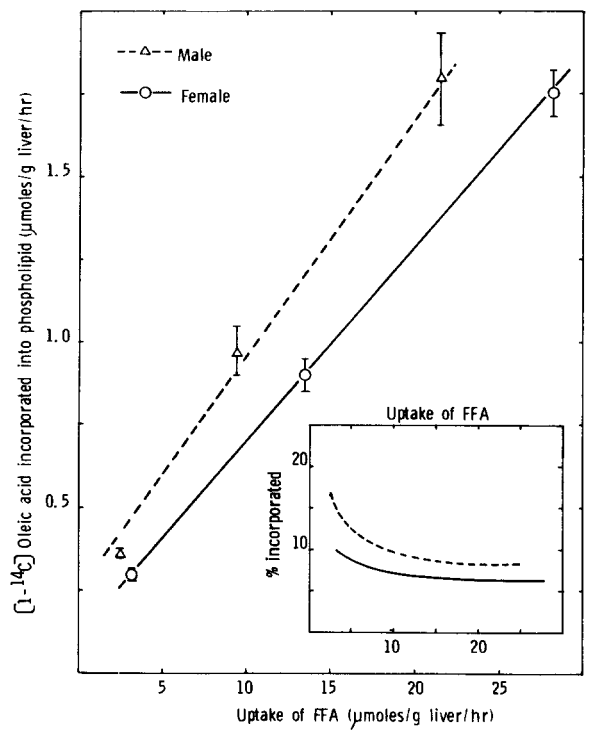

Fig. 4. Incorporation of [1-^{14}C]oleic acid into total phospholipid of liver plus perfusate. Calculations of rates of incorporation are similar to those described in the legend for Fig. 3. Each point is the mean ± SEM (n=4). Regression equations and significance of differences are: $y = 0.24 + 0.07x$ (male), and $y = 0.12 + 0.06$ (female), $P < 0.01$. The inset shows the relative incorporation of oleic acid (% of ^{14}C taken up incorporated into PL) as a function of FFA uptake (μmoles/g liver per hr). The curves were calculated from the data generated by the regression equations.

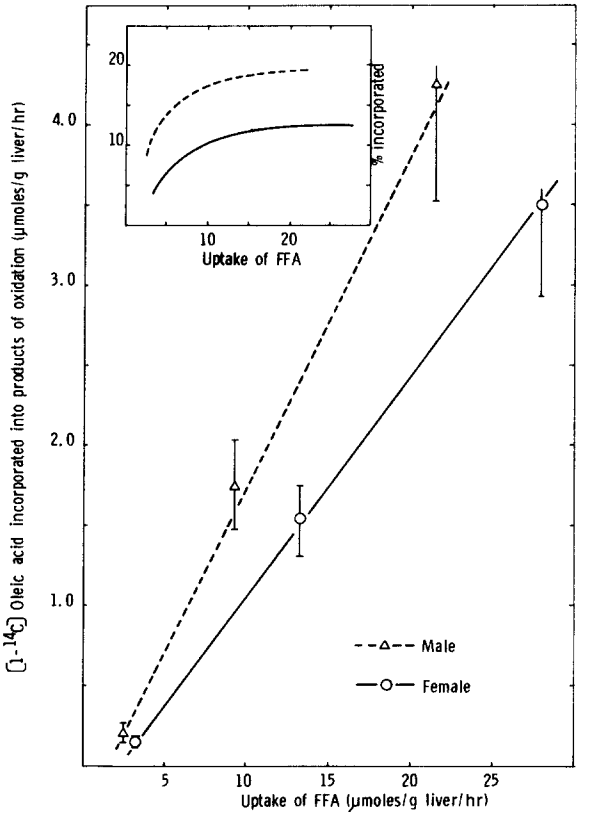

Fig. 5. Incorporation of [1-^{14}C]oleic acid into total products of oxidation (CO_2 plus ketone bodies). Calculations of rates of incorporation are similar to those described in the legend for Fig. 3. Each point is the mean ± SEM (n=4). Regression equations and significance of differences are: $y = -0.36 + 0.21x$ (male), and $y = -0.37 + 0.14x$ (female), $P < 0.02$. The inset shows the relative incorporation of oleic acid (% of ^{14}C taken up incorporated into products of oxidation as a function of FFA uptake (μmoles/g liver per hr)). The curves were calculated from the data generated by the regression equations.

products by livers from male rats exceeded that of livers from female animals. Apparently, esterification of oleate to triglyceride is favored in livers from female rats.

A fraction of the exogenous ^{14}C taken up by the liver is retained in the liver as TG, while another portion is resecreted into the perfusate as the TG component of the very low density lipoprotein (**Figs. 6A** and **B**). The percentage of [1-^{14}C]oleate incorporated into hepatic TG was about equal to that secreted into the perfusate in group I of both sexes (i.e., at the low uptake of FFA). The percent of ^{14}C incorporated into hepatic TG decreased slightly as the quantity of oleate perfusing livers from male animals was increased. In contrast, the percentage of ^{14}C retained in TG of livers from female rats increased moderately with increase in availability of oleate (Fig. 6A, inset). With livers from both sexes, the percentage of TG secreted into the perfusate decreased with increasing uptake of FFA (Fig. 6B, inset). No differences were observed between the sexes in the relative amount of ^{14}C incorporated into the TG of liver in comparison to that of the perfusate; rather the ratios, increasing with the availability of substrate, were relatively similar at all levels of oleate infused. Ratios of the number of moles of [1-^{14}C]oleic acid incorporated into triglyceride of liver to that in perfusate were 1.0 ± 0.3, 1.4 ± 0.2, and 1.6 ± 0.2 for the males of groups I, II, and III, respectively. The corresponding values for groups of female rats were 1.0 ± 0.1, 1.2 ± 0.1, and 1.7 ± 0.1, respectively.

Triglyceride accumulated in the livers of rats of both sexes as a function of FFA taken up, but to a slightly greater extent in the female, as suggested by the differences between the slopes of the lines indicated by the regression equations given in the footnote to **Table 3**.

The total output of TG fatty acids was proportional to the uptake of FFA by the liver, and was higher for livers from female rats than livers from male animals in all groups (**Fig. 7A**). It is of interest that the rate of utilization of exogenous fatty acids, proportional to the uptake of FFA by the liver, inhibited the utilization of endogenous fatty acids for formation of TG in proportion to the uptake of FFA by the liver (Fig. 7B). Exogenous fatty acids are defined as fatty acids taken up by the liver from the perfusate, whereas endogenous fatty acids denote fatty acids derived from de novo hepatic biosynthesis or from lipolysis of hepatic lipids. Exogenous [1-^{14}C]oleic acid ac-

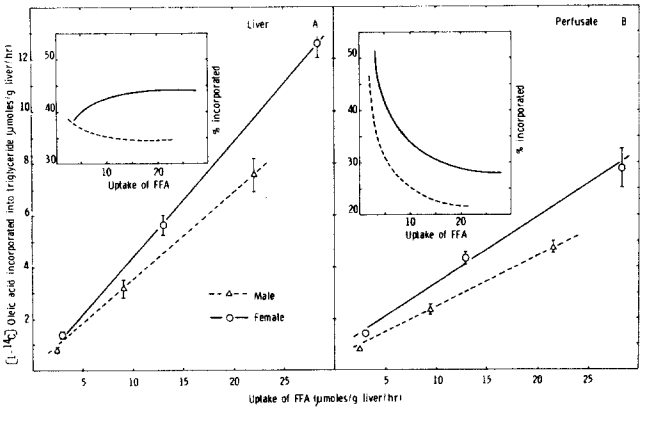

Fig. 6. Incorporation of [1-^{14}C]oleic acid into triglyceride retained in the liver (Panel A) and secreted into the perfusate (Panel B). Calculations of rates of incorporation are similar to those described in the legend for Fig. 3. Each point is the mean ± SEM (n=4). Regression equations and significance of the differences: Panel A, $y = 0.13 + 0.34x$ (male), $y = -0.23 + 0.45x$ (female), $P < 0.02$; Panel B, $y = 0.58 + 0.19x$ (male), $y = 0.81 + 0.25x$ (female). The insets show the relative incorporation of oleic acid (% of ^{14}C taken up incorporated into TG) as a function of FFA uptake (μmoles/g liver per hr). The curves were calculated from the data generated by the regression equations.

counted for 45.0 ± 5.8, 81.2 ± 7.7, and 104.5 ± 4.1% (male) and 45.1 ± 3.1, 85.7 ± 5.6, and 107.5 ± 4.0% (female), for groups I, II, and III, respectively, of the total triglyceride fatty acid secreted.

The incorporation of [1-^{14}C]oleate into oxidation products is shown in **Fig. 8**. Production of both $^{14}CO_2$ (Fig. 8A) and radioactive ketone bodies (Fig. 8B) increased with increasing uptake of FFA by the liver, this response being more marked in the male than in the female. The percentage of [1-^{14}C]oleate incorporated into $^{14}CO_2$ was higher with livers from male than from female animals. The percent incorporation into CO_2 rose between groups I and II and then remained relatively constant with both sexes (Fig. 8A, inset); the percent incorporation of ^{14}C into ketone bodies increased as a function of uptake of FFA by the liver and was higher for the male than for the female (Fig. 8B, inset). The mass output of ketone bodies by the liver was similarly dependent on the uptake of FFA and was lower in livers from female than from male rats (**Fig. 9A**). The increase in rate of ketogenesis was dependent primarily on the influx of larger amounts of exogenous FFA, while ketogenesis from endogenous fatty acid remained relatively stable (Fig. 9B).

Output of glucose and urea by liver

The output of glucose by the liver increased with the uptake of FFA. No consistent differences due to sex were observed (**Table 4**). The output of urea by livers from female animals decreased as the uptake of FFA increased; output by livers from male rats remained essentially constant in all groups. The higher output of urea by livers from female animals (Group I) disappeared so that output by males and females was similar in Group III (Table 4).

TABLE 3. Terminal concentration of triglyceride in perfused livers from normal, fed male and female rats

Group	Concentration of Triglyceride	
	Male	Female
	μmoles/g liver	
I	7.2 ± 0.4	9.1 ± 1.0
II	10.2 ± 0.9	17.1 ± 1.4
III	13.9 ± 0.5	23.7 ± 0.8

Triglycerides were measured in the liver at the termination of the experiments. A complete description of the groups and the conditions of perfusion is presented in the text. Each value is the mean ± SEM (n = 4). Regression equations and significance of the differences are: $y = 6.66 + 0.33x$ (male), $y = 8.17 + 0.57x$ (female), where y is terminal concentration of triglyceride in the liver (μmole/g liver) and x is hepatic uptake of FFA (μmoles/g liver per hr), ($P < 0.02$.). The y intercepts (6.66 and 8.17) are theoretical values for concentration of liver TG when no fatty acid was infused; these values agree with previously published results (2).

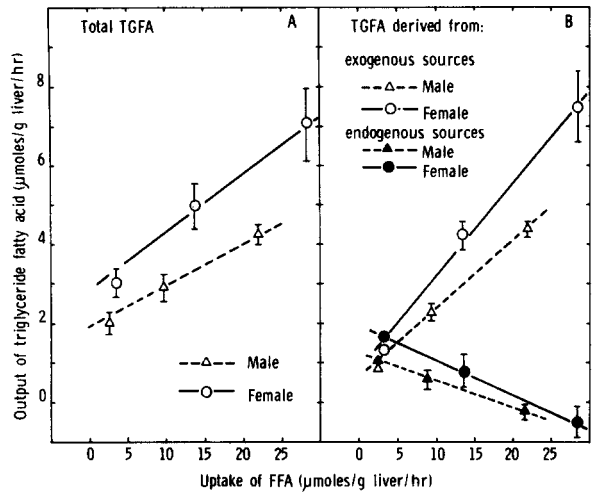

Fig. 7. Hepatic output and source of triglyceride fatty acids (TGFA). Calculations of rates of incorporation of exogenous oleic acid into TG are similar to those described in the legend for Fig. 3. The amounts of TGFA derived from endogenous sources were calculated by difference: TGFA derived from endogenous sources equals total TGFA minus TGFA derived from exogenous sources ([1-^{14}C]oleate). Each point is the mean ± SEM (n=4). Regression equations and significance of differences are: Panel A, $y = 1.92 + 0.10x$ (male), $y = 2.82 + 0.15x$ (female), $P < 0.02$; Panel B (TGFA derived from exogenous sources), $y = 0.65 + 0.17x$ (male), $y = 0.79 + 0.24x$ (female), $P < 0.05$; Panel B (TGFA derived from endogenous sources) $y = 1.29 - 0.07x$ (male), $y = 1.98 - 0.09x$ (female), $P < 0.05$.

DISCUSSION

The uptake of FFA by the livers from either male or female rats, as reported previously (34), was proportional to the concentration of FFA in the medium. When uptake was expressed per g of liver, the rate of uptake of FFA by livers from female rats exceeded that of the males for the same amount of FFA infused. These differences in uptake of FFA are minimized if expressed per liver or per 100 g body weight, because of the differences in liver weight relative to body weight (Table 1). Operationally, we have accepted that such differences exist and have compared all data per g of liver weight; for evaluation of rates of metabolism of the [1-^{14}C]oleate, the data have been calculated relative to the actual measured uptake of FFA. If FFA uptake is indeed sex dependent, it may be a function of transhepatic flow rates (35), which are higher in the female than in the male, per g of liver (Table 1). Whether any real physiological difference in rates of uptake of FFA exists between male and female in vivo is most difficult to answer.

The observation (1, 6, 7) that output of triglyceride by perfused livers from female rats exceeds that of the male animals was confirmed in the experiments reported here; our initial approach to a study of the mechanism(s) by which this difference is brought about was to compare metabolism of [1-^{14}C]oleate by

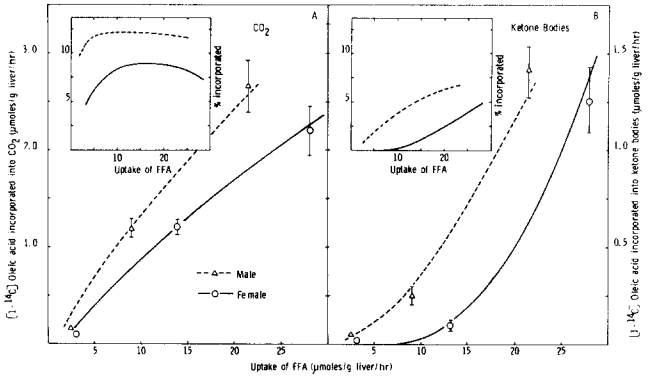

Fig. 8. Incorporation of [1-^{14}C]oleic acid into CO_2 (Panel A) and ketone bodies (Panel B). Calculations of rates of incorporation are similar to those described in the legend for Fig. 3. Each point is the mean ± SEM (n=4). Regression equations and significance of the differences are: Panel A, $y = -0.06 + 0.13x - 0.0004x^2$ (male), and $y = -0.17 + 0.11x - 0.0008x^2$ (female), $P < 0.02$; Panel B, $y = -0.05 + 0.02x + 0.002x^2$ (male) and $y = 0.11 - 0.04x + 0.003x^2$ (female). The insets indicate relative incorporation of oleic acid (% of ^{14}C taken up incorporated into products of oxidation) as a function of FFA uptake (μmoles/g liver per hr). The curves were calculated from the data generated by the regression equations.

livers from female and male animals, respectively. It is clear from this work that oleate is not metabolized identically by livers from the two sexes. The output of TG into the perfusate, the accumulation of TG in the liver, the synthesis of TG, the synthesis of PL, and the formation of oxidation products from oleate by the liver were proportional to the uptake of FFA in both sexes. However, the rate of conversion of [1-^{14}C]oleate to TG by the female exceeded that by the male, whereas conversion to PL and oxidation products by the male was more extensive than by the female. Since the ratio liver weight/body weight appears to change with sex, under our experimental conditions, the sex differences modulating the absolute amounts of FFA incorporated into metabolites may be minimized (e.g., incorporation into TG) or increased (e.g., incorporation into oxidation products) when expressed on the basis of total liver weight.

The lower rates of incorporation of exogenous oleic acid into PL by livers from female rats in comparison to males are consistent with the work reported by Bjørnstad and Bremer (11) and Lyman et al. (12); these workers demonstrated that the methylation of phosphatidylethanolamine was of quantitative importance in rat liver for the synthesis of phosphatidylcholine, and that this pathway was more active in the female rat than in the male. It is not known at this time, however, whether the specific incorporation of fatty acid during phosphatydate synthesis (36–38) is affected by sex or by sexual hormones.

As the quantity of FFA taken up by the liver increased, the percentage incorporated into products of esterification decreased, whereas the percentage incorporated into oxidation products increased. Thereafter, both percentages remained almost constant with increasing uptake of FFA by the liver. These data suggest that, as the supply of FFA to the liver is increased, FFA becomes the primary metabolic fuel, supplanting carbohydrate, which is the major source of substrate for oxidation by perfused livers from fed animals not infused with fatty acids (39). The observation that long chain acyl carnitines suppress the decarboxylation of pyruvate supports this contention (40, 41).

Regulation of rates of oxidation may be important primary mechanisms for the control of fatty acid metabolism by the liver, especially when the supply of FFA available to the liver is increased. Rates of oxidation of exogenous fatty acid may then modulate rates of esterification. This behavior was observed in experiments with livers from diabetic rats (42); it was shown in those experiments that the apparent depression of hepatic output of TG could be restored toward normal rates, providing much more exogenous FFA

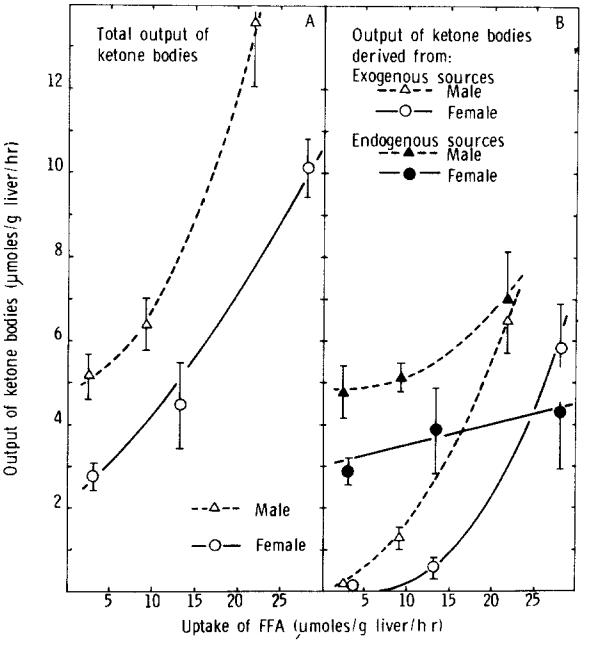

Fig. 9. Output of ketone bodies by the liver. Panel A shows the total mass of ketone bodies output. The derivation of ketone bodies from endogenous or exogenous sources is presented in Panel B. For calculations, see the legend for Fig. 7. To express the incorporation of [1-^{14}C]oleic acid in terms of ketone bodies, moles of oleic acid incorporated were multiplied by 4.5. Each point is the mean ± SEM (n=4). Regression equations and significance of differences are: Panel A, $y = 4.86 + 0.02x + 0.017x^2$ (male), and $y = 1.94 + 0.20x + 0.003x^2$ (female), $P < 0.01$; Panel B (ketone bodies derived from exogenous sources), $y = -0.21 + 0.09x + 0.010x^2$ (male), and $y = 0.47 - 0.16x + 0.013x^2$ (female), $P < 0.01$; Panel B (ketone bodies derived from endogenous sources), $y = 4.96 - 0.05x + 0.007x^2$ (male), and $y = 3.01 + 0.05x$ (female), $P < 0.05$.

TABLE 4. Output of glucose and urea by the liver

Groups	Glucose		Urea	
	Male	Female	Male	Female
	(μmoles/g liver per hr)		*(μmoles/g liver per hr)*	
I	14.2 ± 0.5	18.3 ± 3.7	10.2 ± 1.6	16.8 ± 0.7
II	20.5 ± 0.8	23.2 ± 2.9	9.7 ± 0.5	14.9 ± 1.9
III	43.6 ± 8.1	39.5 ± 7.8	9.2 ± 1.8	9.9 ± 1.4

Details of the conditions of perfusion and calculations are given in the section on Experimental Procedure. Values indicate means ± SEM (n = 4). Regression equations and significance of differences are: $y = 9.91 + 1.44x$ (male) and $y = 15.22 + 0.79x$ (female), $P > 0.05$ (NS), for output of glucose, where y is output of glucose (μmoles/g liver/hr) and x is hepatic uptake of FFA (μmoles/g liver/hr); and $y = 10.14 - 0.04x$ (male) and $y = 17.76 - 0.26x$ (female), $P < 0.001$, for output of urea, where y is output of urea (μmoles/g liver/hr) and x is hepatic uptake of FFA (μmoles/g liver/hr).

was available to the liver to satisfy simultaneous demands of substrate for oxidative (ketogenic) mechanisms. Similar conclusions were reached by McGarry, Meier, and Foster (43), and Ontko (44) in their studies on fasting. It is important in this regard that the reciprocal relationship between the percent incorporation of [1-^{14}C]oleic acid into oxidation products (CO_2 plus ketone bodies) and into triglyceride was observed with triglyceride secreted into the perfusate, while the proportion of [1-^{14}C]oleic acid retained in the liver remained almost unchanged (Fig. 6). In agreement with earlier observations, these data are suggestive of the existence of at least two relatively independent pools of hepatic triglyceride (22).

It is possible that the lower rate of oxidation of added oleic acid by livers from female rats reflects lower synthesis of acylcarnitine, the rate limiting step in the oxidation of long chain fatty acids (45–47), which may be responsible for the higher rates of esterification of fatty acids to TG by livers from female rats. However, the rate of output of TG by livers from female rats exceeded that from male animals even when the supply of exogenous FFA was small (Fig. 7) and oxidation of fatty acids was negligible (39); in these circumstances, it is unlikely that sex differences in rates of oxidation of fatty acids can account for increased output of triglyceride by livers from female rats. An alternative mechanism, such as direct stimulation of esterification of fatty acid to TG due to the enzyme activity involved in their biosynthesis, or to the supply of α-glycerophosphate must be considered.

It is important to determine whether the increased output of TG by livers from female animals in comparison to that in males is due to a difference in rates of biosynthesis of TG, to any inherent difference in ability to secrete TG (i.e., to form and secrete the very low density lipoprotein, VLDL, which is the carrier for TG), or to a combination of these factors. As a function of uptake of FFA, the ratio of incorporation of exogenous oleate into hepatic TG relative to perfusate TG was essentially similar in livers from both sexes. This observation leads us to postulate that regulation of esterification to form TG is a more important causal factor, although, clearly, this problem must be studied in considerable detail before any absolute conclusions can be reached.

The partition of fatty acid between oxidation to carbon dioxide and oxidation to ketone bodies, which is regulated in these livers from normal, fed rats by the quantity of FFA taken up by the liver, is modulated by sex. It has been suggested that the liver can change from oxidation through the citric acid cycle to ketogenesis by inhibition of citrate synthetase (E.C.4.1.3.7) by increased steady state concentrations of intramitochondrial ATP (48). Whether sex affects the oxidation of acetyl CoA through such a mechanism cannot be answered from the present experiments.

The rate of ketogenesis from endogenous and exogenous sources was less in livers from fed female rats than from male animals, regardless of the quantity of FFA presented to the livers. Sex differences in the relative concentrations of ketogenic and antiketogenic substrates might be factors determining the different rates of ketogenesis between livers from male and female rats. Finally, the low rate of ketogenesis in livers from female rats might be due to decreased activity of hydroxymethylglutaryl CoA synthetase, the rate-limiting enzyme of ketogenesis. With livers from both sexes, the nonparallelism between the net output of ketone bodies and the rate of incorporation of endogenous substrate into ketone bodies probably reflects a smaller proportion of acetyl CoA from endogenous sources preferentially channeled into ketogenic pathways when the rate of ketogenesis, depending primarily on the influx of exogenous fatty acid, is increased.

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