Sex differences in hepatic uptake of long chain fatty acids in single-pass perfused rat liver

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Abstract The primary determinants of hepatic uptake of long chain fatty acids have been considered to be the plasma concentrations of fatty acid and albumin, with little or no intrinsic control by the hepatocyte itself. However, recent studies of liver cell suspensions have shown that in immature, adult, castrated, and hormone-treated rats, sex steroids exert striking effects on [^{14}C]oleate uptake and utilization (which were significantly increased by estradiol and diminished by testosterone). To determine whether these observed sex differences in fatty acid uptake also were present in the intact liver, single-pass [^{14}C]oleate uptake was measured in isolated perfused livers. Livers from sexually mature female and male rats were perfused single-pass with albumin-bound [^{14}C]oleate in Krebs-Ringer bicarbonate buffer. Net uptake, calculated as the product of the transhepatic difference in ^{14}C-labeled fatty acid concentration and perfusate flow rate, reached a steady-state within 1 min and remained constant throughout the 10-min perfusion period. At 0.17 mM [^{14}C]oleate and 0.15 mM albumin, extraction fraction and net uptake of [^{14}C]oleate per gram liver were more than twice as great in females as in male livers (0.33 ± 0.03 versus 0.15 ± 0.02, $P < 0.001$; and 218 ± 22 versus 101 ± 15 nmol/g liver, $P < 0.01$, with parallel differences in [^{14}C]oleate total utilization and incorporation into triglycerides. Significant differences in uptake also were observed at higher [^{14}C]oleate concentrations (0.34 and 0.68 mM). Under all conditions, oxidation of [^{14}C]oleate in female liver equaled or exceeded that in male liver, indicating that the increased incorporation into triglycerides and other glycerolipids was not simply the result of differences in the distribution of [^{14}C]oleate among cellular metabolic pathways. These studies demonstrate that in the intact liver, as in isolated hepatocytes, there are profound sex differences in the uptake of long chain fatty acids. This difference may account in part for the observed sex steroid effects on hepatic triglyceride biosynthesis and VLDL production. The mechanism of these uptake differences remains to be determined.—**Kushlan, M. C., J. L. Gollan, W-L. Ma, and R. K. Ockner.** Sex differences in hepatic uptake of long chain fatty acids in single-pass perfused rat liver. *J. Lipid Res.* 1981. **22:** 431–436.

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The fact that sex steroids significantly influence hepatic secretion of triglyceride-rich very low density lipoproteins (VLDL), is exemplified by the increased hepatic production rate and plasma concentration of VLDL-triglyceride associated with pregnancy (1–3) and the use of oral contraceptives (4), and the decrease caused by anabolic and progestational steroids (5, 6). However, the mechanisms of these sex steroid effects have remained unclear. Although there is evidence for a direct effect of estrogens on VLDL apolipoprotein synthesis in avian species (7–9), it is apparent that any steady-state increase in VLDL secretion must be accompanied by, and may indeed result from, increased availability of long chain fatty acids to the liver cell. Thus, when the supply of fatty acids exceeds the requirement of other pathways of utilization there is enhanced incorporation into glycerolipids (principally triglycerides) and consequently into VLDL.

Recently we demonstrated that in isolated hepatocyte suspensions prepared from sexually mature female rats, uptake, total utilization, and incorporation of [^{14}C]oleate into triglycerides was nearly twice that in cell suspensions from male livers (10). These differences largely reflected the effects of sex steroids, as demonstrated by studies of cells from immature, castrated, and hormone-treated animals (11). Whereas no significant sex differences were observed in the enzymes of fatty acid esterification, the concentration of fatty acid binding protein (FABP) in cytosol was approximately 50% greater in mature females than males, and correlated closely with total fatty acid utilization and incorporation into triglycerides (10, 11).

These findings suggest that the sex differences in hepatocyte fatty acid utilization reside primarily in the uptake process, rather than the cellular metabolic pathways. Implicit in this interpretation is the possibility that a sex steroid effect on the fatty acid uptake process may be a major determinant

Abbreviations: VLDL, very low density lipoproteins; FABP, fatty acid binding protein; FFA, free fatty acid.

of the overall availability of fatty acids in the hepatocyte and, therefore, of the rate of VLDL-triglyceride secretion.

Since it may not be valid to extrapolate results obtained in isolated hepatocytes to fatty acid uptake by the intact liver, it was essential that this process should be examined directly, particularly since the influence of sex differences and sex steroids on hepatic fatty acid uptake has been unclear (12, 13). This problem has been addressed using a non-recirculating liver perfusion system, which has enabled steady-state single-pass uptake rates to be determined. These studies demonstrate the existence of profound sex differences in fatty acid uptake by isolated perfused liver, and support the concept that sex steroid effects on hepatic VLDL-triglyceride production are mediated at least in part at this level. Portions of these studies have been reported in abstract form (14).

METHODS

Male and female Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) age 55 to 60 days, maintained on Purina Rat Chow ad libitum (Ralston Purina Co., St. Louis, MO), were used in all experiments. Previous studies showed that, at this age, sex differences in hepatocyte fatty acid utilization were fully developed and unrelated to sex differences in body weight (10, 11). [1-^{14}C]Oleate was obtained from New England Nuclear Corp. (Boston, MA), and unlabeled oleic acid and fatty acid-free bovine serum albumin were purchased from Calbiochem (San Diego, CA) and Sigma (St. Louis, MO), respectively. The bovine albumin contained <0.02 mol fatty acid per mol protein (15). Fluosol®, fluorocarbon emulsion was purchased from Grand Island Biological Co. (Grand Island, NY).

The perfusion circuit was housed in a thermostatically controlled lucite cabinet and the perfusate was circulated by means of a peristaltic pump (LKB Multiperpex 2115, Bromma, Sweden). The medium was passed in sequence through a silastic membrane oxygenator (16), a temperature-sensitive probe connected to the thermostat (maintained at 37°C), a stainless steel filter screen (Millipore), a bubble trap and pressure gauge, and eventually into the portal vein through a Teflon cannula. The perfusate was equilibrated at pH 7.4 with 95% O_2, 5% CO_2 (flow rate 0.4 liter/min). The operative technique for removal and preparation of the liver for perfusion was modified from that described by Hems et al. (17). The portal vein was cannulated with a 16-gauge catheter (Angiocath, Deseret Co., Sandy, UT), the liver was flushed with heparin (40 i.u. in 0.8 ml of 0.15 M NaCl), and perfusion commenced at about 8 ml/min with an oxygenated Krebs-Henseleit buffer containing 0.2% glucose. The inferior vena cava was then cannulated above the diaphragm and the liver dissected from the animal, transferred on nylon mesh to the perfusion chamber, and connected to the recirculating Fluosol®-43 medium.

Following a 30-min stabilization period, the fluorocarbon emulsion was replaced during a 4-min period of single-pass perfusion with Krebs-Henseleit buffer containing 0.2% glucose and 0.15 mM (1 g/dl) fatty acid-free albumin. The livers were then perfused, single-pass for a 10-min experimental period with the Krebs-Henseleit buffer containing 0.2% glucose, and 0.15 mM albumin with which the indicated concentration of [^{14}C]oleate had been complexed. Perfusion pressure was monitored and perfusate inflow and outflow P_{O_2} were measured and hepatic oxygen consumption was calculated. Perfusate flow rates and perfusion pressures were similar for females and males (**Table 1**). Oxygen consumption during perfusion with Fluosol® and Krebs-Henseleit buffer averaged 2.8 and 1.3 μmol/g liver per min, respectively. In addition, viability of perfused livers was satisfactory at the end of the perfusion period as documented by the normal appearance on electron microscopy and stable perfusate lactic dehydrogenase and transaminase activity. Bile flow during these experiments averaged 8.6 ± 1.2 μl/min, mean ± SEM (n = 11). Mean values for body weight and liver weight are shown in Table 1.

TABLE 1. Body weight, liver weight, and perfusion dynamics in single-pass perfused isolated rat liver[a]

	Body Weight	Liver Weight	Perfusate Flow Rate	Perfusion Pressure
	g	*g*	*(ml/g liver/min)*	*(cm H_2O)*
Female	185 ± 5.5[b]	6.20 ± 0.20	3.83 ± 0.08	2.5 ± 0.2
Male	266 ± 3.1	9.98 ± 0.44	3.93 ± 0.24	2.2 ± 0.4

[a] Isolated livers from sexually mature 55-day-old rats were perfused single-pass as described in Methods.
[b] Mean ± SEM; n = 5 for both groups.

During the 10-min experimental (single-pass perfusion) period, effluent samples were collected at 1-min intervals for analysis (see below). At the conclusion of the experiment the liver was removed immediately from the circuit, blotted dry, weighed, and homogenized in 0.89% (w/v) NaCl at 4°C.

Analysis of effluent samples and liver homogenate. Samples of effluent and homogenate were assayed for total ^{14}C. An additional aliquot of each sample was subjected to solvent extraction by the method of Folch, Lees, and Sloane Stanley (18); aliquots of the lower (chloroform) phase were assayed for ^{14}C and subjected to thin-layer chromatography on 0.25 mm silica gel 60 in a solvent system consisting of petroleum ether–ethyl ether–glacial acetic acid 90:15:1.5. Zones corresponding with authentic phospholipid, diglyceride, free fatty acid, triglycerides, and cholesteryl esters were identified, scraped from the plate, and analyzed for ^{14}C. In some experiments, free fatty acids in the effluent were analyzed by gas–liquid chromatography (15). Since effluent free fatty acid (FFA) consisted exclusively of oleate,[1] a contribution of endogenous hepatic FFA to the effluent is highly unlikely. This conclusion is consistent with the findings of other investigators (19, 20) and indicates that under these conditions oleate uptake is effectively a unidirectional process.

Total recoveries of perfusate ^{14}C in effluent and liver homogenate were 95,1 ± 0.8% for females and 97.1 ± 0.4% for males. Fractional hepatic extraction of perfusate oleate was calculated as the quotient of the mean steady-state oleate concentration drop across the liver, divided by perfusate oleate concentration. Net oleate uptake in nmol/min per g liver was calculated as the product of the extraction fraction, the perfusate oleate concentration in nmol/ml, and the perfusate flow rate in ml/min per g liver. In both effluent and liver homogenate, ^{14}C in oxidation products was taken as equivalent to that in the upper (H_2O-CH_3OH) phase of the Folch et al. extraction system (18), and was calculated as the difference between total and $CHCl_3$-extractable ^{14}C in each sample. Although some ^{14}C-labeled oleoyl CoA may be present in the upper phase after extraction of liver homogenate, [^{14}C]oleoyl CoA represents only a small fraction of total tissue ^{14}C; moreover, in separate experiments it was found that only 12% of ^{14}C-labeled acyl CoA was recoverable in the bulk upper phase of the extraction system.[2]

Samples were assayed for radioactivity in Liquifluor®-toluene (New England Nuclear Corp.) in a Beckman model LS-250 Liquid Scintillation System employing an external standard. For aqueous samples, 10% Biosolv-BBS3® (Beckman Instruments Corp.) was included in the counting mixture. Significance of differences between experimental groups was determined using the unpaired Student "*t*" test.

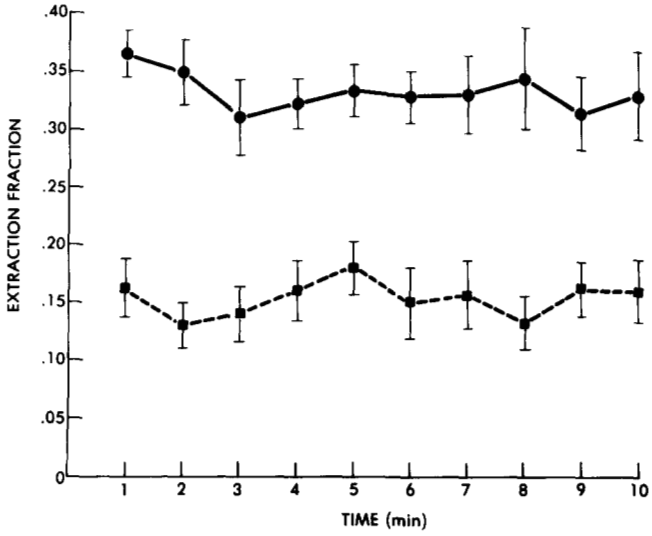

Fig. 1. Single-pass extraction of [^{14}C]oleate by perfused rat liver. Rat livers were perfused in a non-recirculating system, with Krebs-bicarbonate buffer containing 0.15 mM albumin and 0.17 mM [^{14}C]oleate. Effluent samples were obtained each minute for analysis of ^{14}C in fatty acids, lipids, and oxidation products as described in Methods. Extraction ratios (mean ± SEM) were 0.33 ± 0.02 for females (● —— ●) and 0.15 ± 0.02 for males (■ – – – ■); $P < 0.001$; n = 5 for both groups.

RESULTS

Mean fractional extraction of 0.17 mM [^{14}C]oleate from perfusate during a single-pass through the liver at each min for female and male livers is shown in **Fig. 1.** It is evident that steady-state hepatic uptake of [^{14}C]oleate was achieved within about 1 min and was maintained without significant fluctuation for the duration of the 10-min experimental period. A highly significant sex difference in fatty acid uptake was observed under these conditions. For female livers the extraction fraction (0.33 ± 0.02) was more than twice the value for males (0.15 ± 0.02, SEM, $P < 0.001$). Although mean liver weight was greater in males than in females (Table 1), in neither sex was there an apparent influence of liver weight on extraction fraction or net uptake of [^{14}C]oleate.[1] Moreover, despite the fact that male livers were larger, the sex difference in net oleate uptake per g was of such magnitude that, when calculated on the basis of total liver

[1] Kushlan, M. C., J. L. Gollan, W-L. Ma, and R. K. Ockner. Unpublished observations.
[2] Manning, J., and R. K. Ockner. Unpublished observations.

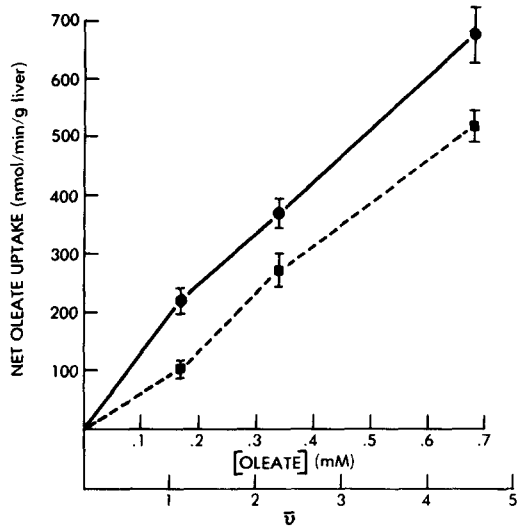

Fig. 2. Net single-pass uptake of [^{14}C]oleate by perfused rat liver. Livers were perfused as described in Methods, with medium containing [^{14}C]oleate at the indicated concentrations and albumin at 0.15 mM (1 g/dl). Oleate-albumin molar ratios ($\bar{v}$) are also indicated on the abscissa. Mean ± SEM; n = 5 (0.17 mM) or 4 (0.34 and 0.68 mM). Females (● —— ●); males (■ – – – ■).

weight, uptake was still 36.1% greater per liver in females, although the scatter was such that this difference failed to achieve statistical significance.

Fig. 2 shows the mean net oleate uptake per g liver at perfusate [^{14}C]oleate concentrations of 0.17 mM, 0.34 mM, and 0.68 mM, all at 0.15 mM albumin. It is evident that the sex differences in fatty acid uptake persist over a wide range of fatty acid concentrations and fatty acid:albumin molar ratios. Under these experimental conditions, uptake does not exhibit evidence of saturation.

In all experiments, more than 90% of the effluent ^{14}C was recovered as free fatty acid. A relatively minor fraction was accounted for by materials which were not extractable into the chloroform phase and which were regarded as oxidation products (including [^{14}C]HCO_3^- and labeled ketone bodies). It is important to note that throughout the 10-min perfusion essentially no ^{14}C appeared in effluent lipids other than fatty acid. This indicates that, to the extent that [^{14}C]oleate was incorporated into hepatic triglycerides and other fatty acid esters during the experiment, these products were retained within the liver.

In **Table 2** the results are shown for [^{14}C]oleate extraction fraction, net hepatic uptake, and incorporation into hepatic FFA, total oxidation products (hepatic plus effluent), all esters (including phospholipids, diglycerides, triglycerides, and cholesteryl esters) and triglycerides. The data indicate several important points. First, at all three perfusate substrate concentrations, oleate extraction and net uptake in female livers significantly exceeded those in males. Second, [^{14}C]oleate esterification and incorporation into triglycerides was also greater in females. Third, oxidation in female livers either equalled or exceeded that in male livers. Under all conditions, a relatively minor fraction of hepatic ^{14}C was present in FFA. These findings are qualitatively similar to those reported earlier in studies of isolated hepatocyte suspensions (10, 11), and indicate that the higher rate of incorporation of [^{14}C]oleate into triglycerides by

TABLE 2. Sex differences in hepatic uptake and metabolism of [^{14}C]oleate in single-pass rat liver perfusion: effect of perfusate oleate concentration

Oleate Concentration	Sex	Extraction Fraction	Net Uptake	Hepatic FFA	Oxidation	Esterification	Incorporation into Triglycerides
				(nmol/g liver/min)			
0.17 mM[b]	F	0.33 ± 0.03[a]	218 ± 22	3.9 ± 1.3	90.7 ± 12.0	80.7 ± 7.2	62.7 ± 6.3
	M	0.15 ± 0.02[d]	101 ± 15[e]	1.4 ± 0.5	37.4 ± 8.0[e]	54.1 ± 9.0[f]	30.2 ± 1.9[e]
0.34 mM[c]	F	0.27 ± 0.03	369 ± 26	1.9 ± 0.6	101 ± 15.4	210 ± 22	173 ± 18
	M	0.19 ± 0.03	271 ± 29[f]	1.0 ± 0.3	93.2 ± 19.5	129 ± 24[f]	97.5 ± 20.4[f]
0.68 mM[c]	F	0.26 ± 0.02	677 ± 48	9.4 ± 5.3	242 ± 20	327 ± 22	265 ± 16
	M	0.19 ± 0.01[f]	521 ± 28[f]	1.9 ± 0.5	234 ± 23	219 ± 23[g]	173 ± 20[g]

[a] Mean ± SEM.
[b] N = 5.
[c] N = 4.
[d] Versus corresponding female group, $P < 0.001$.
[e] Versus corresponding female group, $P < 0.01$.
[f] Versus corresponding female group, $P < 0.05$.
[g] Versus corresponding female group, $P < 0.02$.

Isolated livers were perfused as outlined in Methods. Immediately after the 10-min experimental period, livers were weighed, homogenized, and analyzed for radioactivity as described in Methods. Values shown for net uptake and oxidation include water-soluble oxidation products recovered in effluent. Esterification products, including triglycerides, were recovered in the liver homogenate only, and were not present in effluent.

female liver reflects sex differences in net FFA uptake rather than in distribution between oxidation and esterification pathways.

DISCUSSION

In these studies, the use of a non-recirculating isolated rat liver perfusion system has enabled single-pass hepatic extraction of [^{14}C]oleate to be measured under steady-state uptake conditions, and in the absence of significant hepatocellular secretion of ^{14}C in lipoprotein lipids. The results demonstrate that the rate of uptake and utilization of [^{14}C]oleate by female liver is significantly greater than that in male liver. These sex differences persisted over a wide range of oleate concentrations and showed no evidence of saturation under conditions in which perfusate albumin concentration was constant. The involvement of a saturable process for fatty acid uptake is not excluded, however, since the disproportionate increase in concentration of unbound fatty acid at higher fatty acid:albumin molar ratios could enhance uptake via a non-saturable diffusion mechanism and consequently obscure a saturable component.

These sex differences in hepatic uptake of oleate are consistent with earlier findings in isolated rat hepatocyte suspensions (10, 11). Soler-Argilaga and Heimberg (12) also observed sex differences in fatty acid uptake in recirculating perfused rat livers. However, because of differences in perfusate flow rates, these investigators expressed uncertainty as to the physiological significance of these findings, and later (13) concluded that fatty acid uptake by liver was not hormonally influenced. It is possible that differences in interpretation may reflect differences in experimental design. In their system, Soler-Argilaga and Heimberg (12) employed recirculating perfusion in which perfusate oleate concentration was maintained and continually replenished over a 3 to 4-hr period, and in which there were substantial sex differences in perfusate flow rate, thereby effectively precluding any direct quantitative assessment of the initial uptake process.

The mechanism of the observed sex differences in hepatic uptake and utilization of [^{14}C]oleate is unclear, although its elucidation may provide important insight into the nature and determinants of the uptake process(es). On the basis of previous data, it seems most unlikely that the differences in uptake could be attributed to the activity of enzymes in the fatty oxidation or esterification pathways (10) and hence, when considered in conjunction with the present findings, a primary effect on lipoprotein synthesis or secretion is also excluded. Furthermore, similar sex differences characterize the hepatic uptake of indocyanine green, a non-metabolized organic anion (21, 22), as well as that of sulfobromophthalein glutathione conjugate.[3] Thus, it may be inferred that the essential factor(s) underlying the sex differences in hepatocyte fatty acid utilization reside at the uptake level, and that these differences also characterize hepatic uptake of other albumin-bound amphipathic anions. (In this context, uptake is defined broadly as the sum of all aspects of fatty acid entry into the cell, up to the point at which it participates in the formation of a covalent bond.) Thus, it appears likely that the recognized effects of sex steroids on hepatic synthesis and secretion of lipoprotein triglyceride are due, at least in part, to their effects on the availability of plasma free fatty acids to the liver cell.

Although the process of fatty acid uptake is poorly undertood, present evidence indicates that it involves neither a specific receptor for fatty acids at the cell surface nor a saturable transmembrane carrier mechanism (15). Cytosolic fatty acid binding protein, which has been implicated in the cellular utilization of long chain fatty acids in liver, intestine, and other tissues (15, 23–25), is present in higher concentrations in the cytosol of female liver compared with male (10, 11), and may contribute to the observed uptake differences. However, while FABP stimulates the activity of a number of enzyme reactions in both oxidation and esterification pathways in which long chain fatty acids or acyl CoA thioesters are substrates (15), its potential role as an intracellular fatty acid carrier has been neither established nor excluded (15). Recent studies in our laboratory suggest that the uptake process may involve a specific and saturable interaction between albumin, to which plasma FFA are almost entirely bound, and a receptor for albumin and albumin-ligand complexes on the liver cell surface (26–28). Such an interaction could in part mediate the observed sex differences in fatty acid uptake (27). This intriguing concept and its implications for cellular uptake of a variety of albumin-bound ligands are under continuing investigation.

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