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Fatty Acid Metabolism and Very Low Density Lipoprotein Secretion in Liver Slices from Rats and Preruminant Calves

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The liver of bovine animals possesses a low ability to secrete triglycerides (TG) as part of the very low density lipoproteins (VLDL) compared with rat liver. We compared hepatic fatty acid (FA) metabolism between rat and calf in order to determine the limiting steps of TG-VLDL secretion in bovine animals. Liver slices from young Sprague-Dawley rats and preruminant Holstein×Friesian calves were incubated for 7 h with increasing concentrations (0.1, 0.2, 0.4, and 0.8 mM) of [^{14}C]oleate. The oxidation of oleate to CO_2 and acid-soluble products was 2- to 3-fold higher in rat than in calf liver slices. Since oleate uptake was 2-fold higher in rat than in calf, the oxidation rate represented 20-29% of oleate uptake in both animal species. Oleate was essentially incorporated into the neutral lipids (75-87% of total lipids) that were stored mainly in the cytosol in both animal species (81-90% of neutral lipids). The accumulation of neutral lipids in the cytosol was 3.4-fold higher while VLDL secretion was 6- to 18-fold more efficient in rat than in calf liver slices. Our results indicate that the slow rate of VLDL secretion by bovine liver is probably due to the limited availability of TG for VLDL packaging rather than to the preferential oxidation of FA.

Key words: calf, fatty acid metabolism, liver, rat, VLDL secretion.

In mammalian species, fatty acids (FA) present in the liver, can originate either from *de novo* lipogenesis or through uptake from the blood. In hepatocytes, FA may be oxidized completely to CO_2, partially oxidized to acetate and ketone bodies, or esterified to form phospholipids (PL), cholesteryl esters (CE), and especially triglycerides (TG) (*1*). While the products of FA oxidation are secreted easily because of their high solubility, TG secretion is a more complex process involving the formation of very low density lipoprotein particles (VLDL). Hepatic VLDL production is regulated by nutritional, hormonal, and physiological factors that are strongly dependent on the animal species (*1*).

Bovine animals are characterized by low levels of lipogenesis and a chronic low rate of secretion of VLDL by the liver (*2*). These findings are supported by *in vitro* studies demonstrating that TG secretion by hepatocytes is strongly limited in ruminants (bovine, sheep, and goat) compared to monogastric species, even when their rates of TG synthesis are similar (*3, 4*). This limited capacity of the bovine liver to secrete VLDL might induce lipid infiltration of the liver, especially under some physiological (*e.g.*, peripartum period) and nutritional (*e.g.*, high fat diet, fasting) conditions (*5*). The preruminant calf is a functional monogastric in which VLDL production by the liver has been shown to increase according to amount of dietary fat provided as beef tallow in the milk diet (*6*). However, milk-fed calves can develop TG infiltration in the liver when dietary lipids are rich in *n*-6 polyunsaturated FA (*7*) or medium chain FA (*8, 9*).

FA metabolism could explain the low rate of secretion of VLDL by the liver in bovine animals. Indeed, the partition of FA between oxidation and esterification pathways, which is the first potential limiting step in TG synthesis, has been shown to be regulated by fasting in the rat liver (*10*). Furthermore, in perfused rat liver, the inhibition of FA oxidation by a fungal metabolite, emeriamine, increases TG-VLDL secretion (*11*).

The availability of TG at the site of VLDL packaging might be another limiting step. Studies in cultured rat hepatocytes have shown that TG synthesis increases according to the concentration of free FA in the medium, but becomes saturated within the range of the physiological amount in the serum (*12*). Some recent data in rat hepatocytes indicate that neosynthesized TG are stored primary in cytosolic droplets from which they enter into a hydrolysis/esterification cycle to return to the endoplasmic reticulum (ER) for VLDL assembly (*1*). Thus, the partitioning of TG between the cytosolic and microsomal compartments seems to be important to the availability of TG for VLDL secretion. However, the regulation of these processes remains unknown in bovine species.

The aim of this work was to compare FA metabolism pathways (oxidation, esterification, and TG secretion as part of VLDL) between rat and calf liver to identify the

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Abbreviations: Apo B, apolipoprotein B; ASP, acid-soluble products; CE, cholesteryl esters; ER, endoplasmic reticulum; FA, fatty acids; MTP, microsomal triglyceride transfer protein; PL, phospholipids; TG, triglycerides; VLDL, very low density lipoproteins.

limiting steps that might explain the limited TG-VLDL secretion by bovine liver. For this purpose, we studied calf and rat liver slices to learn the effects of different concentrations of FA added to the medium on (a) the relative importance of the oxidation and esterification pathways of FA, (b) the subcellular distribution of synthesized TG between cytosolic droplets and the microsomal compartment, and (c) the secretion of VLDL.

MATERIALS AND METHODS

Chemicals and Materials—The medium used for liver slice incubation (RPMI-1640), bovine serum albumin (BSA) free of FA, oleic acid, and antibiotic-antimycotic cocktail were purchased from Sigma Chemical (St Louis, MO, USA). [1-^{14}C]Oleic acid (100 μCi/ml), [1-^{14}C]palmitic acid (200 μCi/ml), glycerol tri[9,10(*n*)-^{3}H]oleate (5 mCi/ml), and L-3-phosphatidyl[*N-methyl*-^{3}H]choline 1,2-dipalmitoyl (1.0 mCi/ml) were purchased from Amersham International (Bucks, UK). Hyamine hydroxyde was from ICN Biochemicals (Irvine, CA, USA). Perchloric acid and solvents (chloroform, methanol, propanol, diethyl ether, acetic acid) were from Prolabo (Paris, France). Ready Safe scintillation cocktail was from Beckman Instruments (Fullerton, CA, USA). Plastic organ culture Petri dishes were from Beckton Dickinson (Cockeeysville, MD, USA). Plastic center wells were from Kontes (Vineland, NJ, USA) and aminopropyl-activated silica Sep-Pak® cartridges were from Waters (Milford, MA, USA).

Tissue Preparation and Liver Slice Incubation—Three male Sprague-Dawley rats (6 weeks old; 200-220 g) were fed a standard chow diet *ad libitum* and allowed free access to water for the entire experimental period. Three preruminant Holstein × Friesian male calves (3 to 6 weeks old; 69-79 kg) were fed a conventional milk replacer from birth and fasted overnight on the day of the experiment. Liver tissue samples were obtained by surgical biopsy performed under general anesthesia (isoflurane 2% at 0.5 liter/min for calves; diethyl ether for rats) and prepared for metabolic labeling according to a method developed by Dr. M.E. Samson-Bouma (INSERM, U327, Paris, France). Biopsy samples were quickly rinsed in ice-cold saline solution (KCl 0.4 g/liter, NaCl 6 g/liter, and $NaHPO_4$ 0.8 g/liter pH 7.4) containing D-glucose (2 g/liter), trimmed of blood and connective tissue, and cut into 0.5 mm thick slices. Approximately 150 mg of fresh liver (*i.e.* 4 to 5 slices) were placed on stainless steel grids positioned either on a plastic organ culture Petri dish or in a 25-ml flask equipped with suspended plastic center wells (for specific CO_2 measurements) in the presence of RPMI-1640 medium free of FA. The samples were placed in an incubator for 2 h at 37°C under a water-saturated, 95% O_2-5% CO_2 atmosphere in order to deplete hepatocytes of intracellular FA. The medium was then replaced with fresh RPMI-1640 medium (0.9 ml in dishes and 1.4 ml in flasks) supplemented with an antibiotic-antimycotic cocktail (100 U/ml penicillin, 0.1 mg/ml streptomycin, and 0.25 μg/ml amphotericin B) and 0.1, 0.2, 0.4, or 0.8 mM [^{14}C]oleate (4 mCi/mmol) complexed to BSA (oleate:albumin molar ratio of 4:1). Liver slice incubations corresponding to a dish and a flask for each FA concentration tested were stopped after labeling for 7 h. Preliminary kinetic measurements after 3, 5, and 7 h of incubation demonstrated significant and reproducible VLDL production by liver slices from both calf and rat after 7 h of incubation. Moreover, all parameters (oleate uptake, CO_2 and ASP production, oleate esterification into lipids, VLDL production) increased linearly with incubation times from 3 and 7 h for both species and all oleate concentrations tested. Therefore, the medium was collected after 7 h of incubation, and the liver slices were washed with 2 ml of saline solution and homogenized in 2 ml of 25 mM Tris-HCl (pH 8.0), 50 mM NaCl buffer with a Dounce homogenizer.

Determination of FA Oxidation—The CO_2 produced by the liver slices was complexed with hyamine hydroxide (150 μl) introduced into the suspended plastic center wells inside flasks at the beginning of the time-course study and at 3 and 5 h of incubation. At the end of the incubation, the center wells were placed into scintillation vials containing 4 ml of Ready Safe scintillation cocktail and the radioactivity was counted. The production of ketone bodies was estimated by a modification of the method of Williamson and Mellanby (*13*). Briefly, at the end of the experiment, aliquots of the medium (500 μl) and the liver homogenates (250 μl) were treated for 20 min at 4°C with ice-cold perchloric acid (0.2 M final). The precipitated lipids and proteins were pelleted by centrifugation (4°C, 20 min, 1,850 × *g*). An aliquot of the supernatant containing the acid-soluble products (ASP, mainly ketone bodies) was introduced into a scintillation vial and the radioactivity was counted. The pellet, containing FA linked to BSA and lipids, was dissolved by adding 150 μl of 0.5 N NaOH and mixing vigorously. The mixture was equilibrated with 150 μl of 0.5 N HCl, placed in a scintillation vial, and the radioactivity was counted.

Purification of Cytosolic and Microsomal Fractions of Hepatocytes—Homogenates of liver slices were centrifuged for 5 min at 1,100 × *g* at 4°C to eliminate intact liver fragments, unbroken cells, and large debris. The supernatant was collected and the pellet was homogenized in 1 ml of 25 mM Tris-HCl (pH 8.0), 50 mM NaCl, and centrifuged under the same conditions as above. The supernatants were pooled and successively centrifuged for 5 min at 1,500 × *g*, 30 min at 10,000 × *g*, and 30 min at 18,000 × *g* to eliminate nuclei, mitochondria, and other large cell components, respectively. The residual supernatant was ultracentrifuged for 1 h at 100,000 × *g* at 4°C (Kontron Centrikon T-2060 ultracentrifuge with a TFT 65.13 rotor). The supernatant containing the floating fat (corresponding to the cytosolic fraction) was collected carefully as was the microsomal pellet which was suspended in 200 μl of 1 mM Tris-HCl (pH 7.4), 50 mM KCl, and 5 mM $MgCl_2$. The cytosolic and microsomal fractions of hepatocytes were kept at −20°C until subsequent analysis.

Determination of [^{14}C] Oleate Incorporation into Neutral and Polar Lipids in Microsomal and Cytosolic Fractions—Microsomal and cytosolic lipids were extracted according to the method of Folch *et al.* (*14*) after the addition of standard non-radioactive liver homogenate (850 μl containing approximately 10 mg of lipids) as a lipid carrier and [^{3}H]triolein (1.8 nCi) and [^{3}H]phosphatidylcholine (2.7 nCi) as internal TG and PL standards. After extraction, the volume of chloroform containing the total lipids was reduced to 400 μl on an air stream. The lipid classes were then separated by solid phase chromatography on an aminopropyl-activated silica Sep-Pak® cartridge according to the method of Kaluzny *et al.* (*15*). Briefly,

neutral lipids (mono-, di-, and triglycerides, free and esterified cholesterol) were eluted in 6 ml of chloroform-2 propanol (2:1, v/v). Free FA were then eluted in 6 ml of 2% acetic acid in diethyl ether. Finally, the polar lipids, mainly PL, were eluted in 6 ml of methanol. The lipid fractions were collected in scintillation vials, evaporated to dryness under an air stream, and counted for radioactivity. Preliminary experiments were carried out to determine the recovery of FA when [^{14}C]palmitate (3.2 nCi) was added to the homogenates as a lipid carrier. The average yields were 84.8, 84.5, and 84.2% for TG, FA, and PL, respectively.

Measurement of Secreted Very Low Density Lipoproteins—Three milliliters of medium supplemented with purified calf VLDL (0.3 mg of TG-VLDL/tube) as a carrier, were brought to a density of 1,063 g·liter^{-1} with potassium bromide and overlaid with 9 ml of KBr solution (density 1,006 g·liter^{-1}). VLDL were purified by ultracentrifugal flotation at 100,000×g for 16 h at 15°C in a Kontron Centrikon T-2060 ultracentrifuge with a TST 41-14 rotor. Two milliliters from the top of each tube were recentrifuged under the same conditions except that pure albumin (50 mg/tube) was added to remove traces of [^{14}C]FA adsorbed onto the VLDL particles. Finally, the purified VLDL were collected at the top of the tube (5 fractions of 500 μl) and counted for radioactivity in scintillation vials.

Statistical Analysis—Values are expressed as the means±SE of three independent experiments. The effects of animal species and oleate concentration in the medium were tested by ANOVA according to the GLM procedure of SAS (*16*). The effect of animal species was also tested for each oleate concentration by the Bonferroni test with an adjustment of the p values.

RESULTS

Oleate Uptake by Liver Slices—Oleate uptake by liver slices (expressed in nanomoles per g fresh liver) corresponded to the sum of the oleate incorporated into total lipids in the homogenate (neutral and polar lipids), oleate secreted into the medium as ASP, and oleate excreted as CO_2. After 7 h of incubation, the uptake of oleate by hepatocytes tended to increase in proportion to the initial amount added to the medium ($p<0.006$) for both animal

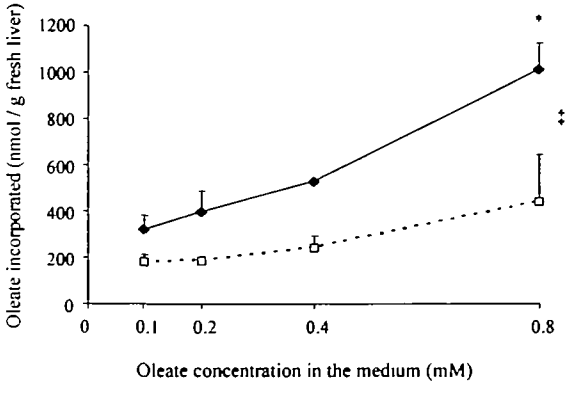

Fig. 1. **Uptake of oleate by liver slices.** Liver slices from rats (◆) and calves (□) were incubated in medium containing 0.1, 0.2, 0.4, or 0.8 mM [^{14}C]oleate for 7 h. Oleate uptake by the liver slices was calculated as the sum of oleate converted to CO_2 and ASP and that incorporated into total cellular lipids (neutral and polar lipids) after 7 h of incubation. Values are expressed per g of fresh liver and corrected for specific activity. Data are means±SE of 3 separate experiments. The significance of differences in species and oleate concentration in the medium was tested by ANOVA. ‡Curves tended to be significantly different between rat and calf ($p<0.10$) by ANOVA. *Means were significantly different between rat and calf ($p<0.05$) by the Bonferroni test with an adjustment of the p values.

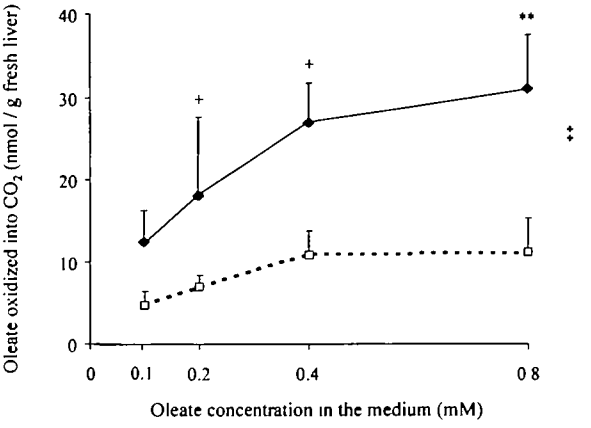

Fig. 2. **Oleate oxidation into CO_2 by liver slices.** Liver slices from rats (◆) and calves (□) were incubated in medium containing 0.1, 0.2, 0.4, or 0.8 mM [^{14}C]oleate for 7 h. Secreted CO_2 was complexed to hyamine hydroxyde and the radioactivity was measured in a scintillation counter. Values are expressed per g of fresh liver and corrected for specific activity. Data are means±SE of 3 separate experiments. The significance of differences in species and oleate concentration in the medium was tested by ANOVA. ‡Curves tended to be significantly different between rat and calf ($p<0.10$) by ANOVA. +Means tended to be significantly different between rat and calf ($p<0.10$) by the Bonferroni test with an adjustment of the p values. **Means were significantly different between rat and calf ($p<0.01$) by the Bonferroni test with an adjustment of the p values.

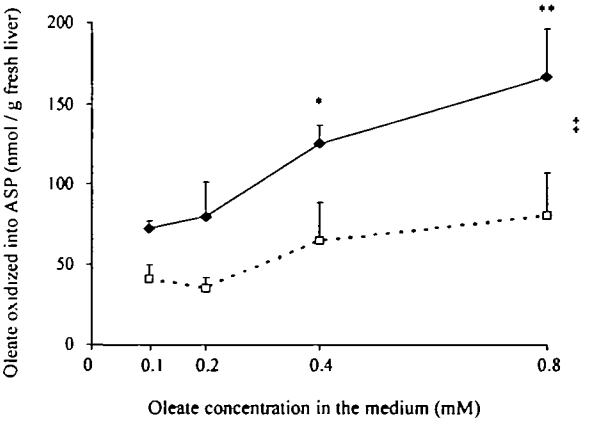

Fig. 3. **Oleate oxidation into ASP by liver slices.** Liver slices from rats (◆) and calves (□) were incubated in medium containing 0.1, 0.2, 0.4, or 0.8 mM [^{14}C]oleate for 7 h. The ASP produced were purified from cell homogenates and medium by perchloric acid treatment and radioactivity was measured in a scintillation counter. Values are expressed per g of fresh liver and corrected for specific activity. Data are means±SE of 3 separate experiments. The significance of differences in species and oleate concentration in the medium was tested by ANOVA. ‡Curves tended to be significantly different between rat and calf ($p<0.10$) by ANOVA. *,**Means were significantly different between rat and calf *($p<0.05$) and **($p<0.01$) by the Bonferroni test with an adjustment of the p values.

species (Fig. 1). The values ranged from 322 to 1,016 nmol of oleate per g of fresh liver from rats and 188 to 447 nmol per g of fresh liver from calves, for 0.1 to 0.8 mM oleate added to the medium. These values were not directly proportional to the amount of oleate added to the medium and tended to be higher for rat than for calf liver slices ($p < 0.055$), especially at high amounts of added oleate. At 0.8 mM oleate, oleate uptake by liver slices was significantly higher ($\times 2.3$, $p < 0.05$) in rats than in calves (Fig. 1).

Oleate Oxidation by Liver Slices—The total amount of CO_2 produced by the complete oxidation of oleate after 7 h of labeling (Fig. 2) increased with the amount of oleate added to the medium ($p < 0.017$). The production of CO_2 was maximal at 0.4 mM oleate and showed no further increase at 0.8 mM. Global statistical analysis (including all oleate concentrations tested) showed a tendency toward a significant difference between animal species ($p < 0.073$); however, the difference was highly significant ($p < 0.01$) at 0.8 mM oleate in medium, 36.3 and 13.0 nmol of oleate converted to CO_2 for rat and calf liver slices, respectively.

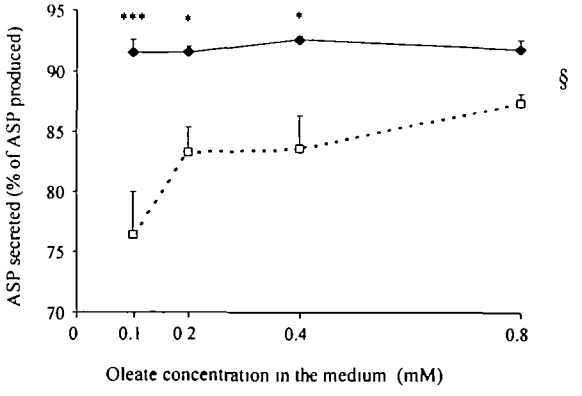

Fig. 4. **Rate of ASP secretion by liver slices.** Liver slices from rats (◆) and calves (□) were incubated in medium containing 0.1, 0.2, 0.4, or 0.8 mM [^{14}C]oleate for 7 h. The ASP produced were purified from cell homogenates and medium by perchloric acid treatment and their radioactivity was measured in a scintillation counter. Values are expressed per g of fresh liver and corrected for specific activity. The rate of ASP secretion corresponds to the ratio between the amount of ASP secreted into the medium and the amount of total ASP produced. Data are means ± SE of 3 separate experiments. The significance of differences in species and oleate concentration in the medium was tested by ANOVA. §Curves were significantly different between rat and calf ($p < 0.05$) by ANOVA. *,***Means were significantly different between rat and calf *($p < 0.05$) and ***($p < 0.001$) by the Bonferroni test with an adjustment of the p values.

The partial oxidation of FA was quantified by determining the amounts of of [^{14}C]ASP produced in homogenates of slices and secreted into the medium. After 7 h of incubation, the amounts of total ASP were significantly higher ($p < 0.001$) at 0.8 mM than at 0.1 mM oleate added to the medium ($\times 2.3$ and $\times 1.9$ for rat and calf, respectively) (Fig. 3). The difference in ASP production between rat and calf liver slices increases when the oleate concentration in the medium is raised to 0.8 mM (167 and 81 nmol of oleate converted to ASP per g of fresh liver for rat and calf, respectively; $p < 0.01$).

The secretion rate of ASP is expressed as the ratio of the amount of ASP secreted into the medium and the amount of total ASP produced by hepatocytes. In rats, the values obtained after 7 h of labelling were close to 92% of the total ASP produced regardless of the amount of oleate in the medium (Fig. 4). In calves, the values were lower than in rats ($p < 0.014$), ranging from 76.5 (at 0.1 mM oleate) to 87.4% (at 0.8 mM oleate) of total ASP produced. The secretion rate of ASP increased in calf liver slices according to the amount of oleate added to the medium, especially at the lowest values (0.1 to 0.4 mM), and tended to reach a plateau of close to 90% at oleate concentrations above 0.4 mM. The significant increase ($p < 0.037$) in the secretion rate of ASP (including both calf and rat data) is, therefore, mainly due to the increase observed in calf slices.

The oxidation rate of oleate (expressed as the percent of oleate converted to CO_2 and ASP out of the total oleate incorporated) was close to 24.5% for both animal species regardless of the amount of oleate added in the medium (Table I).

Oleate Incorporation into Microsomal and Cytosolic

TABLE I. **Oxidation rate of oleate in rat and calf liver slices.**

Oleate concentration (mM)	Oleate oxidation rate (percent of oleate incorporated into hepatocytes)	
	Rat	Calf
0.1	27.4±3.3	24.6±5.6
0.2	24.7±5.2	22.4±4.3
0.4	28.6±2.1	28.6±10.4
0.8	19.7±2.2	20.0±4.2
Statistical effect of		
— oleate concentration	NS	
— species	NS	

The oleate oxidation rate was calculated as the ratio of total oxidation products (sum of CO_2 and ASP) to total oleate incorporated into hepatocytes. Data are means ± SE of 3 separate experiments. The significance of differences in species and oleate concentration in the medium was tested by ANOVA.

TABLE II. **Incorporation of oleate into total lipids and their main constituents (neutral lipids and polar lipids) in rat and calf liver slices (microsomes plus cytosol).**

	Total lipids		Neutral lipids (nmol oleate incorporated per g of fresh liver)		Polar lipids	
Oleate concentration (mM)	Rat	Calf	Rat	Calf	Rat	Calf
0.1	26.8±10.4	7.6±2.8	23.3±8.4	6.2±2.5	3.5±2.0	1.4±0.4
0.2	23.4±5.4	10.8±2.9	19.4±4.2	7.9±2.2	4.0±1.4	2.9±0.6
0.4	44.9±12.4	14.9±3.1	38.5±12.4	10.9±2.6	6.4±0.1	4.0±0.5
0.8	53.5±12.4	20.0±3.0+	45.8±11.1	14.4±2.0+	7.7±1.8	5.6±1.6
Statistical effect of						
— oleate concentration	NS		NS		$p < 0.008$	
— species	$p < 0.001$		$p < 0.001$		NS	

Data are means ± SE of 3 separate experiments. The significance of differences in species and oleate concentration in the medium was tested by ANOVA. +Means tended to be significantly different between rat and calf ($p < 0.10$) by the Bonferroni test with an adjustment of the p values.

Lipids—Oleate incorporated into hepatocytes is distributed among oxidized products (CO_2 and ASP), esterified lipids (TG, PL, CE), and free oleate. Under our experimental conditions, the amount of free oleate in homogenates (results not shown) was higher than in isolated hepatocytes due to the apparent non-specific binding of FA to hepatic sinusoids and blood capillaries. Consequently, the amounts of free oleate were excluded from the lipid values determined in liver slices.

The incorporation of oleate into total microsomal and cytosolic lipids was 2.2- to 3.6-fold higher in rats than in calves ($p<0.001$) (Table II). Neutral lipids (the major lipid component of VLDL) averaged 75% of the microsomal plus cytosolic lipids in calf liver and 87% in rat liver (Table II). Moreover, the incorporation of oleate into neutral lipids was significantly higher ($\times 3.2$, $p<0.10$) in rat than in calf liver in the presence of 0.8 mM added oleate. The incorporation of oleate into polar lipids rose with the amount of exogenous oleate in the medium ($p<0.008$), but did not differ significantly between rat and calf (Table II). The relative incorporation of oleate into neutral lipids in the cytosol and microsomes is shown in Table III. Neutral lipids in the cytosolic fraction represent 81 to 90% of the neutral lipids present in the microsomal plus cytosolic fractions from both rat and calf liver slices. The values are 2.7- to 4.0-fold higher in rat than calf cytosol ($p<0.002$). Similarly, oleate incorporation into the neutral lipids of the microsomes tended to be higher in rat than calf, but a significant effect was obtained only at 0.8 mM oleate ($p<0.01$).

Oleate Incorporation into VLDL Lipids—Oleate incorporation into lipids secreted as part of the VLDL particles was 6- to 18-fold higher ($p<0.022$) in rat than in calf liver slices (Table IV). When expressed as a percentage of the lipids (neutral lipids+polar lipids) present in the cytosol and microsomes, lipids in VLDL in rat represent 9.4 to 14.7% of the lipid-precursor pools whereas the percentage in calf is only 3.6 to 4.9% (Table IV). Moreover, VLDL secretion does not appear in either animal species to be significantly modified by increasing the amount of oleate in the medium. When expressed as the percentage of oleate uptake, VLDL lipids represent 0.72 to 1.14% in rat, which are higher values than in calf which range from 0.13 to 0.30% ($p<0.008$).

TABLE III. **Incorporation of oleate into the neutral lipids of the microsomal and cytosolic fractions from rat and calf liver slices.**

	Cytosolic neutral lipids (nmol of oleate incorporated per g fresh liver)		Microsomal neutral lipids (nmol of oleate incorporated per g fresh liver)	
Oleate concentration (mM)	Rat	Calf	Rat	Calf
0.1	19.4±7.5	4.9±1.8	3.8±1.5	1.3±0.6
0.2	17.0±2.9	6.2±1.4	2.3±1.4	1.7±0.9
0.4	34.2±14.6	8.5±1.6	4.3±2.7	2.1±1.0
0.8	39.1±9.5	12.5±1.7	6.7±2.7	1.8±0.6**
Statistical effect of				
– oleate concentration	NS		$p<0.057$	
– species	$p<0.002$		NS	

Data are means±SE of 3 separate experiments. The significance of differences in species and oleate concentration in the medium was tested by ANOVA. **Means were significantly different between rat and calf ($p<0.01$) by the Bonferroni test with an adjustment of the p values.

DISCUSSION

In mammals, the production of VLDL by the liver results from complex processes involving coordinated mechanisms for the synthesis of both proteins and lipids, their subsequent packaging, and, finally, the intracellular migration of nascent VLDL into secretory vesicles leading to emission into the peripheral blood. Any alterations in these processes may cause a dysfunction in TG secretion leading to lipid infiltration into the liver (*1, 17*). In bovine animals, such as high yield dairy cows during early lactation and preruminant calves fed high fat milk diets containing coconut or soybean oil, insufficient secretion by the liver of TG as part of the VLDL particles leads to the development of steatosis (*7, 8, 18*). We studied possible causes to explain this deficiency by investigating (a) the relative importance of hepatic FA oxidation compared to FA esterification and (b) the relative importance of storage (cytosolic) and export (microsomal) pools of TG to the secretion rate of FA as part of VLDL-lipids in calf liver slices. The results were compared with those obtained in liver slices from rats, which are known for their high rate of VLDL production (*4*).

Oleate Uptake—Under our experimental conditions, oleate uptake by liver slices rises (but not in a directly proportional way) as the amount of oleate (from 0.1 to 0.8 mM) added to the medium is increased, in agreement with previous results in cultured hepatocytes from rats (*10*) and preruminant calves (*19*). The lack of proportionality is not explained by a saturation process since (i) the curves increase linearly, and (ii) the concentrations of oleate used

TABLE IV. **Secretion of oleate as part of the lipids in VLDL particles by rat and calf liver slices.**

	VLDL production (nmol of oleate per g fresh liver)		Lipid secretion rate (% of lipids in cytosol and microsomes)		(% of oleate uptake)	
Oleate concentration (mM)	Rat	Calf	Rat	Calf	Rat	Calf
0.1	3.9±1.9	0.2±0.1+	12.3±1.9	3.6±1.2+	1.14±0.44	0.13±0.05*
0.2	3.4±0.9	0.3±0.1	14.7±6.4	3.9±2.0*	1.10±0.10	0.18±0.05*
0.4	4.6±1.2	0.8±0.2+	9.4±1.3	4.9±1.2	0.87±0.23	0.30±0.07
0.8	7.2±2.3	0.9±0.3**	13.2±4.7	4.0±0.9+	0.72±0.24	0.20±0.04
Statistical effect of						
– oleate concentration	NS		NS		NS	
– species	$p<0.022$		$p<0.069$		$p<0.008$	

Data are means±SE of 3 separate experiments. The significance of differences in species and oleate concentration in the medium was tested by ANOVA. +Means tended to be significantly different between rat and calf ($p<0.10$) by the Bonferroni test with an adjustment of the p values. *,**Means were significantly different between rat and calf *($p<0.05$), **($p<0.01$) by the Bonferroni test with an adjustment of the p values.

in our experiments are close to physiological values and lower than the level of saturation (3 mM) of FA uptake reported in a perfused liver model (*20*).

Oleate uptake by our rat liver slices (20% of exogenous FA incorporated after 7 h) was lower than in perfused rat liver (about 90% of the perfused FA extracted by the liver after 2 h) (*20*) or isolated rat hepatocytes (50% of FA incorporated after 6 h) (*21*). However, it has been reported that, *in vivo*, bovine liver removes only 7 to 25% of the circulating non-esterified FA (*2*), a level comparable to our values (4 to 13% of exogenous FA incorporated by calf liver slices). Closer contact between cells and medium in the perfused liver model or in isolated hepatocytes might explain the higher values than those obtained in *in vivo* studies or in our liver slice model.

Nevertheless, the liver slice model shows a significant difference in oleate uptake by hepatocytes from rats and calves. This difference is not due to a methodologic artifact since parallel experiments using [^{35}S]methionine labeling revealed similar rates of protein synthesis in both rat and calf liver slices (Gruffat *et al.*, unpublished observations); however, the difference might be attributable to more effective or more numerous FA transporters (*22*) or to a higher rate of cellular FA utilization (oxidation or esterification) that stimulates uptake in rat hepatocytes (*23*).

Oleate Oxidation—The oxidation products of oleate produced by liver slices comprise 14 to 20% CO_2 and 80 to 86% ASP, as previously demonstrated in cultured rat hepatocytes (*3, 10*) and liver slices from adult bovines (*24*). In the latter study, the ASP were composed mainly of ketone bodies (63%), acetate (16%), and, as a minor part, intermediates of the Krebs cycle (*24*). The proportion of ketone bodies and acetate (79% of ASP), which represent the secreted ASP fraction, is similar to the proportion (76 to 87%) of ASP secreted by our calf liver slices. Indeed, the ASP secretion rate seems to reflect ASP composition. Consequently, the higher ASP secretion rate in rat than calf liver slices might be explained by the higher proportion of ketone bodies and acetate than Krebs intermediates.

The increase in oleate oxidation to ASP by rat and calf liver slices in conjunction with the increase in the amount of oleate added to the medium confirms previous results in isolated hepatocytes from calves (*19*) and rats (*25*). The complete oxidation of oleate to CO_2 also increases in both species, but only in the range of 0.1 to 0.4 mM added oleate. The steady-state level noted at oleate concentrations over 0.4 mM probably reflect a tendency of the Krebs cycle to saturate, since oxidation to ASP was still active. Such variations have been reported in rat liver homogenates by Ontko and Jackson (*26*) who noted a maximal FA oxidation at 0.5 and 0.65 mM of FA. The saturation of the oxidative process by exogenous FA has been also described in isolated mitochondria from rat and sheep livers (*27*).

The rate of FA oxidation to CO_2 is 2-fold higher in rats than in sheep (*27*). Moreover, it has been reported that the conversion of oleate to CO_2 and ASP is 3- to 4-fold lower in goat hepatocytes than in rat hepatocytes (*3*), in agreement with our results. All these results clearly indicate that hepatocytes from rats show a higher oxidative capacity than hepatocytes from ruminant species. However, when the amount of oleate oxidized is expressed as the percentage of oleate uptake, oleate oxidation is similar in calf and rat slices, which allows us to conclude that differences in the level of FA oxidation between the species can not explain the differences observed in TG-VLDL secretion.

Intracellular Localization of the Newly Synthesized Triglycerides—Another possible limiting step in VLDL secretion from calf hepatocytes might be the availability of TG for VLDL assembly. To examine this possibility, we compared the metabolism of lipids (esterified FA) produced from exogenous FA in rat and calf liver slices paying particular attention to their partition between their two main cellular localizations (cytosol and microsomes).

Neutral lipids are composed mainly of TG, which represent more than 90% of the neutral lipids in both rats and calves (*7, 29*), and, in minor proportions, di- and monoglycerides, which are intermediates in the synthesis of TG and PL, and CE. The rate of CE synthesis is low (*3, 28*) but not limiting for VLDL secretion since CE are poorly represented in the VLDL produced by the liver of calves fed milk containing beef tallow (*7*) or rats fed a standard chow diet (*29*). The levels of neutral lipids, which represent more than 75% of the newly synthesized lipids in both cytosol and microsomes, are higher in liver slices from rats than of calves. Most of these neutral lipids are located in the cytosol, in agreement with its capacity to store TG as lipid droplets (*30*), and with the fact that newly synthesized TG preferentially enter into this pool before being hydrolyzed and re-esterified for VLDL packaging and secretion (*31*). It was calculated that the *in vivo* half-life of TG in the hepatic cytosol averages 23 h in sheep (*30*). Such a relatively long TG storage time might explain the very low amount of oleate secreted into VLDL after 7 h of incubation. TG secretion rates represent 13% and 4% of the total newly synthesized TG in cultured hepatocytes from rats (*21*) and goats (*32*), respectively. These values, close to ours, confirm the correct purification of TG from the cytosol and microsomes under our experimental conditions. When expressed as percent of oleate uptake by the hepatocytes, oleate secretion as VLDL lipids was around 0.72-1.14 and 0.13-0.30% in rat and calf slices, respectively, results close to the values reported in sheep (*2*) and rats (*28*). In contrast with the oxidation rate, the difference between rat and calf in oleate secretion as part of VLDL lipids does not seem to be induced by the difference in oleate uptake by hepatocytes.

A positive relationship between the amount of intracellular TG and VLDL production rates has been noted in rats (*33*). However, comparisons between species have shown similar differences in VLDL secretion without any difference between rats and ruminant animals in FA incorporation into hepatic TG (*3, 4*). Nevertheless, these comparisons were made after the hepatocytes were incubated for only 4 h in the presence of radiolabeled FA (*3*). Moreover, Pullen *et al.* (*4*) used a FA concentration of 0.2 mM, at which we found no significant difference in neutral lipid content between rats and calves. Since the ratio of cellular TG content to VLDL production differed greatly between rats and calves in our experiments, we speculate that other regulatory mechanisms are also involved. The higher rate of TG secretion as part of the VLDL particles by rat than by calf liver slices could be due to (a) a shorter half-life of TG in the cytosolic pool in rat liver than in calf liver because of a more active cytosolic lipase that hydrolyzes stored TG and allows their return to the ER (*33*), or (b) the higher activity in rat than calf hepatocytes of the microsomal TG

transfer protein (MTP) needed for TG to move from the site of synthesis (at the ER surface) to the site of VLDL packaging (in the lumen of the ER) (*34*). These steps are thought to control the availability of TG for VLDL assembly. Other potential factors might include (c) a higher amount of hepatic apolipoprotein B (apo B), the major apoprotein of VLDL, in rat than in calf liver. The cellular apo B content results from a steady-state between synthesis and degradation by cellular proteases (*1*). Intracellular apo B catabolism is known to be regulated by the amount of TG in the ER, TG having a protective effect on apo B by forming an apo B-TG complex resistant to proteolysis, as observed in HepG2 cells (*35*). Thus, under our experimental conditions, the higher levels of TG in microsomes from rat than calf liver slices would favor more effective protection of the apo B used for VLDL assembly. Finally, it is possible that (d) a deficiency in secretory processes is also involved as reported in enterocytes from patients suffering from chylomicron retention disease (*36*).

In conclusion, the liver slice model allows us to compare FA metabolism and VLDL production between rats and calves with good efficiency. We have demonstrated clearly that the low rate of TG secretion as part of VLDL by calf liver compared with rat liver is not the result of different FA partitioning between the esterification and oxidation pathways. The determination of the subcellular distribution of newly synthesized lipids showed a higher level of TG synthesis in rat than in calf liver slices, with the TG being mainly stored in the cytosol of hepatocytes. However, the higher microsomal TG content in rats than calves leads us assume a greater availability of TG for VLDL synthesis. Nevertheless, the identification of the precise limiting steps in VLDL assembly and TG secretion in the bovine liver require further investigation of the characteristics of intracellular lipolysis, MTP activity, and apo B synthesis and degradation.

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