J. Biochem. 126, 188-193 (1999)

Apolipoprotein B Production and Very Low Density Lipoprotein Secretion by Calf Liver Slices

Dominique Gruffat-Mouty,*,[1] Benoît Graulet,* Denys Durand,* Marie-Elisabeth Samson-Bouma,† and Dominique Bauchart*

**Laboratoire Croissance et Métabolismes des Herbivores, Unité Métabolismes Energétique et Lipidique, Institut National de la Recherche Agronomique, Centre de Recherches Clermont Ferrand-Theix, 63122 Saint Genès Champanelle, France; and* †*INSERM Unité 327, Faculté de Médecine X. Bichat, 16 rue Henri Huchard, BP 416, 75870 Paris Cedex 18, France*

Received March 15, 1999; accepted May 6, 1999

Secretion of triglycerides by the liver in ruminants as components of very low density lipoproteins particles is low as compared with that in primates or rodents. The rate-limiting steps for the hepatic export of very low density lipoproteins have been studied in liver slices to determine the origin of the low lipotropic capacity of calf liver compared to that of rat liver. The rates of production of apolipoprotein B (apo B) and albumin as well as the rate of secretion of VLDL-apolipoproteins were measured during 12-h incubation of liver slices in organo-culture using [^{35}S]methionine-cysteine labeling. Hepatic apo B production was similar in the two animal species but the VLDL-apolipoprotein secretion rate for calf liver slices amounted to only 20% of that observed for rat liver slices. Although calf and rat liver slices synthesized similar amounts of total protein, the hepatic production of albumin, measured in cells and media, was much higher in calf than rat liver slices (around 2.7-fold), whereas the rate secretion of albumin was similar in the two species. Our results showed that the slow rate of secretion of VLDL by calf liver cells was not consecutive to a low rate of synthesis of apo B but rather to a defect in VLDL assembly and/or secretion.

Key words: apolipoprotein B, calf, liver, rat, VLDL.

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012

Bovids are known to develop a fatty liver under different physiological or nutritional conditions such as high producing dairy cows in early lactation (*1*), or preruminant calves given a milk diet rich in *n*-6 polyunsaturated fatty acids (PUFA) derived from soybean oil (*2*) or saturated fatty acids (SFA) derived from coconut oil (*3*). *In vivo* and *in vitro* experiments have provided evidence that the relatively low plasma concentrations of very low density lipoproteins (VLDL) in ruminant animals are the result of a low rate of triglyceride (TG) export by the small intestine (as a consequence of the low lipid content of the diet) and by the liver (*4*). Indeed, studies carried out on goat hepatocytes in culture (*5*) and cow liver slices (*6*) clearly indicated that the secretion of TG as components of VLDL particles by the liver in ruminants is very lower as compared with that occurring in rodents. Moreover, we previously demonstrated that the slow rate of VLDL secretion by calf compared to rat liver slices is probably due in part to limited availability of TG for VLDL packaging (*7*). However, very little research has been conducted to determine whether or not the synthesis of other components of VLDL particles are involved in the rate-limiting steps for VLDL export.

[1] To whom correspondence should be addressed. Tel: +33-04-73-62-42-56, Fax: +33-04-73-62-46-39, E-mail: gruffat@clermont.inra.fr

Abbreviations: apo B, apolipoprotein B; ER, endoplasmic reticulum; LPL, lipoprotein lipase; MTP, microsomal TG transfer protein; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TG, triglycerides; VLDL, very low density lipoproteins.

© 1999 by The Japanese Biochemical Society.

The hepatic secretion of VLDL is a complex process requiring the co-ordinate synthesis of lipids and apolipoprotein B (apo B). Their assembly into lipoprotein particles needs the microsomal triglyceride transfer protein (MTP), which plays a key role in the early stages of lipoprotein assembly, most likely by transferring lipid to nascent apo B as it enters the lumen of the ER (*8*). In most animal species, the hepatic apo B level is essentially regulated postranscriptionally through intracellular degradation processing (*9*) which occurs in both ER and post-ER compartments (*10, 11*). These mechanisms of degradation involve both the ubiquitin-proteasome pathway (*12*) and ER-located cysteine proteases such as ER-60 (*13*). Moreover, apo B interacts with multiple molecular chaperone proteins in order to maintain the protein in a translocational competent conformation until translocation is completed (*14*). These mechanisms led to the idea that apo B is synthesized constitutively and in excess, and that it may offer some advantages by enabling the cells to rapidly adjust to the need to secrete TG into the circulation without invoking the translation of this enormous protein.

In contrast, it has been proposed for bovids, that the availability of apo B and its incorporation into VLDL particles might explain the low VLDL output in ruminants (*4, 15*). Indeed, in high producing dairy cows during early lactation, reductions of the hepatic contents and the plasma concentration of apo B100, the only form of apo B synthesized by the bovine liver (*16, 17*), were correlated with an increase in the liver TG content (*17, 18*). Similar induction of fatty liver was observed when ethionine (an inhibitor of

protein synthesis) was given to cows, suggesting that apolipoprotein availability is a limiting step for VLDL secretion (*19*).

The objective of this study was to identify the rate-limiting steps for the export of VLDL particles by the bovine liver by determining hepatic apo B synthesis and VLDL-apoprotein secretion in liver slices from calves. The results were compared to those obtained for rat liver slices, this animal being chosen as a model because it has been mainly used in research on the regulation of hepatic VLDL secretion and it did not appear to be particularly susceptible to fatty liver development except in the case of experimental fatty liver induction. Both calf and rat liver slices were incubated in the presence of 0.8 mM oleic acid to reproduce the nutritional conditions in the calf, which is usually fed a conventional milk diet rich in beef tallow.

The present study suggests that the slow rate of secretion of VLDL by calf liver cells is not consecutive to a low rate of synthesis of apo B but rather to a defect in VLDL assembly and/or secretion.

MATERIALS AND METHODS

Materials—Methionine/cysteine-free RPMI-1640, oleic acid, free fatty acid bovine serum albumin, L-glutamine, an antibiotic-antimytotic mixture and protease inhibitors were obtained from Sigma Chemical (St. Louis, MO, USA). A ^{35}S-protein labeling mix was obtained from du Pont de Nemours S.A. (Les Ulis, France). Organ culture dishes with center wells were obtained from Falcon, Division of Becton Dickinson (Cockeeysville, MD, USA). A Centrikon T-2060 ultracentrifuge equipped with a TST 41-14 swinging bucket rotor was obtained from Kontron Analysis Division (Zürich, Switzerland).

Hepatic Samples—The experiment was performed using 4 preruminant Holstein-Friesian male calves (1 month old) fed a conventional tallow-based milk diet and 4 young Sprague-Dawley male rats (6 weeks old) fed ad libitum a standard chow diet. Liver samples were obtained by puncture biopsy under general anaesthesia with isoflurane (2% in O_2, 0.5 liter/min) for calves and diethyl ether for rats. Tissue samples were placed immediately into an ice-cold saline solution comprising KCl 0.4 g/liter, NaCl 6 g/liter, $NaHPO_4$ 0.8 g/liter (pH 7.4) and D-glucose 2 g/liter, and used for metabolic assays within 30 min.

Liver Slice Incubation—Liver samples were metabolically labeled using a method developed for a previously described intestinal organ-culture (*20*). Liver samples from both calves and rats were sliced with a scalpel at around 0.5 mm thickness (20 to 40 mg). About 5 slices were placed on a stainless steel grid in an organ culture dish. Two dishes were used for each set of experimental conditions. They were incubated at 37°C under an atmosphere of 95% O_2-5% CO_2 in methionine/cysteine-free RPMI-1640 containing 0.8 mM sodium oleate (complexed with free fatty acid bovine serum albumin (0.2 mM) in a molar ratio of 4:1), 300 mg/liter L-glutamine, and the antibiotic-antimytotic mixture (100 U/ml penicillin, 0.1 mg/ml streptomycin, and 0.25 μg/ml amphotericin B). After 2 h depletion, biopsis were pulse labeled (3, 6, 9, or 12 h) with [^{35}S]-methionine/cysteine (1,175 Ci/mmol, 150 μCi/ml). At the end of the pulse labeling, the media were collected and supplemented with protease inhibitors (final concentrations: 0.31 μg/ml pepstatin, 1.56 μg/ml leupeptin, 1.56 μg/ml antipain, 0.31 μg/ml aprotinin, 31.2 μg/ml trypsin-chymotrypsin inhibitor, and 0.31 mM PMSF). The liver slices were washed twice with 1 ml ice-cold saline solution containing KCl 0.4 g/liter, NaCl 6 g/liter, $NaHPO_4$ 0.8 g/liter (pH 7.4), and D-glucose 2 g/liter. The washing solutions were pooled with the corresponding media. The liver slices were homogenised with a Dounce homogenizer in 2 ml Tris-HCl 25 mM (pH 8.0), NaCl 50 mM containing the same protease inhibitor mixture as above.

Total Neosynthesized Proteins—Proteins from a cell homogenate (100 μl) or the medium (100 μl) were precipitated with 2 ml ice-cold 10% trichloroacetic acid (TCA) after incubation 20 min at 4°C, and then pelleted by centrifugation for 5 min at $2,000 \times g$ and 4°C. The pellet was washed 3 times with 1 ml ice-cold TCA (5%) and then the proteins were solubilized by the addition of 0.5 ml NaOH 0.5 N. The protein solution was neutralized with 0.5 ml HCl 0.5 N, mixed with 4 ml scintillation fluid, and then counted for radioactivity.

Neosynthesized apo B and Albumin—Proteins from a cell homogenate (90 μl) or the medium (90 μl) were separated by electrophoresis on continuous polyacrylamide gradient, 2.5 to 7.5%, gel slabs under denaturing conditions according to Laemmli (*21*). The gels were stained with Coomassie Blue, and then the bands corresponding to apo B and albumin in the cell homogenate and medium were identified by comparison with those of standard bovine apo B100 and apo B48 proteins (prepared from delipidated calf LDL in our laboratory) and standard bovine serum albumin. Gels slices containing the separated proteins were excised and solubilized by incubation in 450 μl H_2O_2 30% and 50 μl ammoniac 15 N at 110°C for 2 h. The digests were mixed with 4 ml scintillation fluid and then counted for radioactivity.

VLDL Isolation—Aliquots of media (3 ml) were supplemented with a purified calf VLDL preparation (0.3 mg of TG-VLDL/tube) to remove [^{35}S]VLDL during VLDL isolation. The mixtures were then adjusted to the density of 1,063 g/liter with solid KBr and overlaid with 9 ml of a KBr solution with a density of 1,006 g/liter. VLDL were isolated by ultracentrifugal flotation at $100,000 \times g$ for 16 h at 15°C with no braking at the end of the run. The 2 ml fraction from the top of each tube containing floating VLDL was harvested by aspiration with a micropipette and then supplemented with an excess of bovine serum albumin (50 mg/tube) to dilute the [^{35}S]albumin which might be non-specifically absorbed on the VLDL particles. The mixtures were adjusted to a density of 1,063 g/liter with solid KBr and then washed under the same conditions as above. Five fractions of 500 μl were successively collected at the top of each tube by stepwise aspiration with a micropipette and then counted for radioactivity.

Other Measures—The dry matter contents of calf and rat livers was determined by oven-drying for 48 h at 80°C. The data were normalized as to the amount of dry matter to take variations in the hepatic content of water among species into account.

Statistical Analysis—All values are expressed as the means$\pm$SE of four independent experiments. The global responses of each group were analysed by repeated-measures analysis of variance (ANOVA) using the SAS program with time as the variable with repeated measures.

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012

The sources of variation in the model included species, time, and the interaction of species with time. When global responses exhibited significant differences, each point was analysed by means of the Bonferroni test with adjustment of the p value.

RESULTS

[^{35}S] Methionine-Cysteine Incorporation into Total Proteins—The incorporation of [^{35}S]methionine-cysteine into total TCA-precipitable cellular and medium proteins increased with the tissue incubation time for both calf and rat liver slices ($p<0.0007$), which indicated correct functioning of the liver slices during the incubation (Table I). However, this incorporation of ^{35}S-labeled amino acids was slightly higher calf than for rat liver slices after 9 h of labeling (+38%, $p<0.05$).

[^{35}S] Methionine-Cysteine Incorporation into Cellular apo B and Medium VLDL-Apolipoproteins—Apo B100, the only form of apo B synthesized in calf liver slices, and apo B100 plus apo B48 synthesized in rat liver slices were easily identifiable in homogenates after staining of the polyacrylamide gradient gels with Coomassie Blue (Fig. 1). In homogenates of rat liver slices, the incorporation of [^{35}S]-methionine-cysteine into apo B100 and apo B48 appeared to be parallel all during the labeling (Table II). The results were expressed as total apo B since both apo B100 and apo B48 were secreted as component of VLDL particles.

The total intracellular ^{35}S-labeled apo B content increased with the incubation time ($p<0.004$) but was not significantly different between calves and rats (Fig. 2A). In contrast, the rate of apo B synthesis, expressed as the ratio of ^{35}S-labeled apo B to total ^{35}S-labeled proteins $\times 100$, was 0.32 and 0.44% for calf and rat liver slices, respectively, on average.

After isolation of VLDL particles from the media, the amounts of ^{35}S-labeled VLDL-apoproteins were directly determined by counting of the VLDL fractions. Indeed, the amounts of VLDL secreted into the media, particularly in the case of calf liver slices, were too low to allow apo B identification on staining with Coomassie Blue of polyacrylamide gels, which meant it was impossible to measure apo B radioactivity in the aliquots used for electrophoresis. In contrast, the amounts of ^{35}S-labeled VLDL-apoproteins found in media increased with the incubation time ($p<0.0003$) for both calves and rats (Fig. 2B). However, the accumulation of ^{35}S-labeled VLDL-apoproteins in the media of calf liver slices was about fivefold lower than in the media of rat liver slices ($p<0.0002$) after 12 h labeling.

[^{35}S] Methionine-Cysteine Incorporation into Total Albumin—The albumin present in cell homogenates and media was easily identifiable on continuous polyacrylamide gradient gels stained with Coomassie blue (Fig. 1).

The incorporation of [^{35}S]methionine-cysteine into total albumin from cells and media increased linearly with the labeling time ($p<0.004$) for both animal species (Fig. 3A), but was 2.7-fold higher ($p<0.005$) in calf than rat liver slices. Total ^{35}S-labeled albumin from cells and media amounted to about 16.5 and 8.0% of the total ^{35}S-labeled proteins for calf and rat liver slices, respectively. The

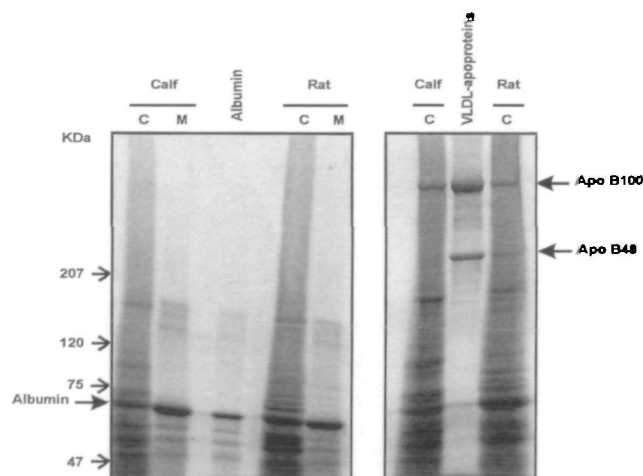

Fig. 1. Representative polyacrylamide gradient gels used for the detection of intracellular (C) albumin and apo B, and secreted (M) albumin in calf and rat liver slices. Calf and rat liver slices were incubated in the presence of [^{35}S]methionine/[^{35}S]cysteine (1,175 Ci/mmol) and 0.8 mM oleate for 12 h. At the end of the incubation, proteins in the media and cell lysates were separated by 2.5-7.5% linear gradient SDS-PAGE and then stained with Coomassie Blue. Molecular weight standards and bovine serum albumin were used to identify the bands corresponding to albumin, and delipidated calf LDL were used to identify the bands corresponding to apo B.

TABLE I. **Time-course of [^{35}S]methionine incorporation into total proteins of calf and rat liver slices on 12 h labeling.** Calf and rat liver slices were incubated in the presence of [^{35}S]methionine/[^{35}S]cysteine (1,175 Ci/mmol) and 0.8 mM oleate for 12 h. At the indicated times, total ^{35}S-labeled protein synthesis was estimated by determining the [^{35}S]methionine incorporation into trichloroacetic acid-precipitable cellular and medium proteins. Data are means±SE of four separate experiments. Significant effects of species and time were examined by repeated measures ANOVA. *Means in the same line were significantly different ($p<0.05$) with the Bonferroni test with examined adjustment of the p value.

Time of incubation (h)	Total protein radioactivity ($dpm \times 10^6$/g dry matter)	
	Calf	Rat
3	580±58	579±58
6	821±39	616±53
9	1,019±42*	740±68
12	1,103±184	758±152
Statistical effect of:		
–Species ($p<$)	0.05	
–Time ($p<$)	0.0007	

TABLE II. **Time-course of [^{35}S]methionine incorporation into cellular apo B100 and apo B48 of rat liver slices on 12 h labeling.** Rat liver slices were incubated in the presence of [^{35}S]-methionine/[^{35}S]cysteine (1,175 Ci/mmol) and 0.8 mM oleate. At the indicated times, proteins in cell lysates were separated by 2.5-7.5% linear gradient SDS-PAGE, and stained with Coomassie Blue to identify the apo B100 and apo B48 bands. The apo B bands were cut from the gels, the gels slices were solubilized, and the digests were counted for radioactivity. Data are means±SE of four separate experiments.

Time of incubation (h)	Rat apo B radioactivity ($dpm \times 10^6$/g dry matter)	
	apo B100	apo B48
3	1.04±0.07	1.35±0.32
6	1.53±0.16	1.52±0.15
9	1.48±0.24	1.69±0.25
12	1.51±0.23	2.00±0.39

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012

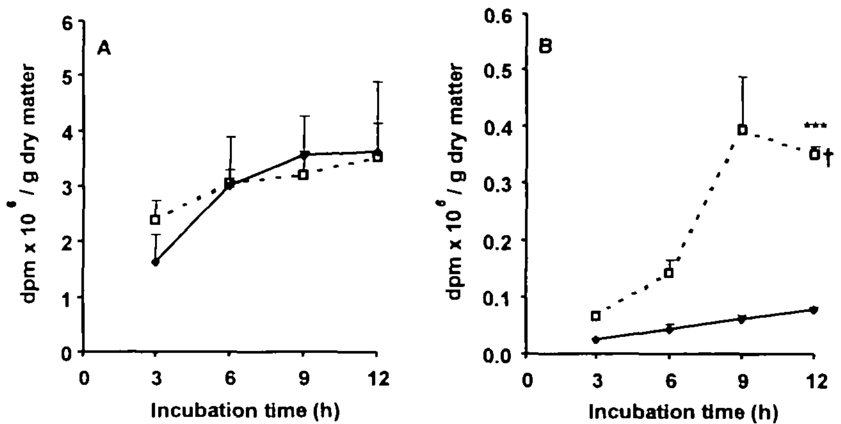

Fig. 2. **Time-courses of apo B production and VLDL secretion in calf and rat liver slices.** Calf ($n=4$, ◆) and rat ($n=4$, □) liver slices were incubated in the presence of [^{35}S]-methionine/[^{35}S]cysteine (1,175 Ci/mmol) and 0.8 mM oleate. At the indicated times, proteins in the cell lysates were separated by 2.5-7.5% linear gradient SDS-PAGE and stained with Coomassie Blue to identify the apo B bands. The apo B bands were cut from the gels, the gel slices were solubilized, and the digests were counted for radioactivity (A). Apo-VLDL (B) were isolated from media by ultracentrifugation at 40,000 rpm for 16 h. Five fractions from the top of each tube were counted for radioactivity. Data are means±SE of four separate experiments. Values denoted by *** are significantly different ($p<0.0002$) from the corresponding values obtained with rat liver slices with the Bonferroni test with adjustment of the p value. The curve denoted by † for calf liver slices was significantly different ($p<0.005$) from that for rat liver slices with repeated measures ANOVA.

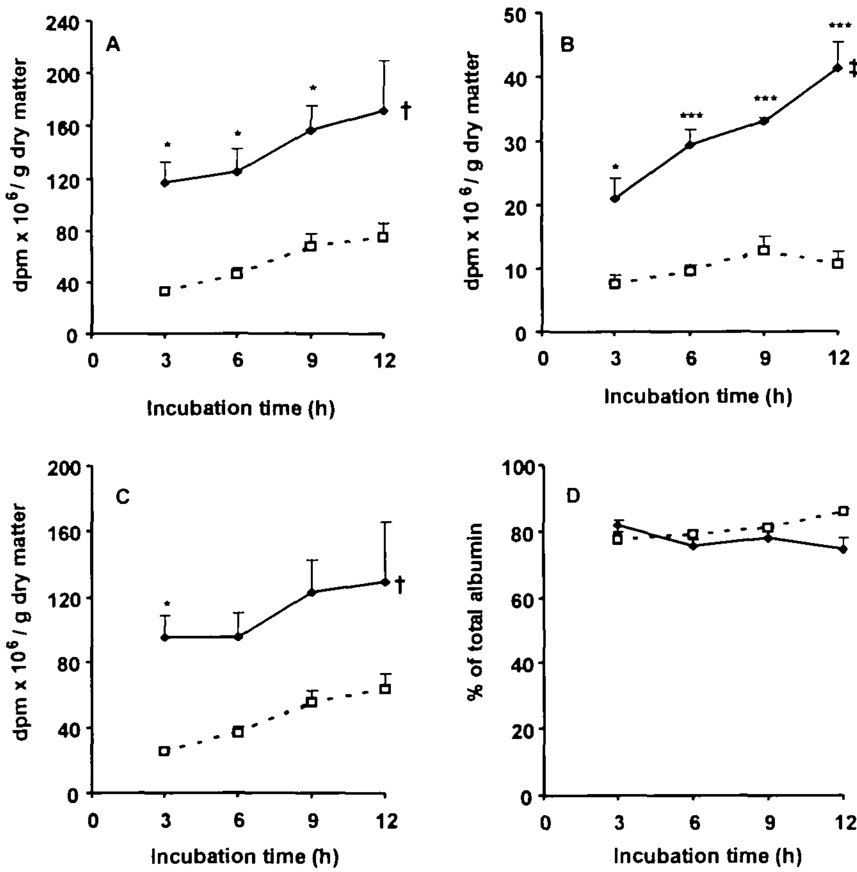

Fig. 3. **Time-courses of albumin production and secretion in calf and rat liver slices.** Calf ($n=4$, ◆) and rat ($n=4$, □) liver slices were incubated in the presence of [^{35}S]-methionine/[^{35}S]cysteine (1,175 Ci/mmol) and 0.8 mM oleate. At the indicated times, proteins is the media and cell lysates were separated by 2.5-7.5% linear gradient SDS-PAGE and stained with Coomassie Blue. The bands corresponding to albumin were cut from the gels and counted for radioactivity. Total albumin (A) corresponded to cellular albumin (B)+albumin in media (C). The rate of secretion of albumin (D) was calculated as the % of media albumin/total albumin. Data are means±SE of four separate experiments. Values denoted by * and *** are significantly different ($p<0.05$ and $p<0.005$, respectively) from the corresponding values obtained with rat liver slices with the Bonferroni test with adjustment of the p value. Curves denoted by † and ‡ for calf liver slices are significantly different at $p<0.005$ and $p<0.0001$, respectively, from those for liver slices with repeated measures ANOVA.

^{35}S-labeled albumin content of hepatocytes increased with the labeling time ($p<0.0003$) (Fig. 3B), but was 3-fold higher ($p<0.0001$) in calf compared to rat liver slices on average. In media of calf liver slices, the ^{35}S-labeled albumin content was around 2.6-fold higher than in media of rat liver slices ($p<0.005$) (Fig. 3C). The rate of secretion ^{35}S-labeled albumin remained constant with the labeling time (Fig. 3D), and similar in the two animal species (nearly 80% of total ^{35}S-labeled albumin).

DISCUSSION

Bovids generally exhibit low plasma concentrations of VLDL compared to other animal species (*22*), and are sensitive to fatty liver development (*4, 15*) under different physiological or nutritional conditions, such as high producing dairy cows in early lactation (*17*) or preruminant calves given a milk diet rich in n-6 PUFA (*2*) or SFA (*3*). In contrast to data obtained for man and rodent species, the availability of apo B and its incorporation into VLDL

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012

particles have been proposed to explain the low VLDL output in ruminants species (*4, 15*).

In order to determine the rate-limiting steps for hepatic VLDL export in bovids, we have measured apo B synthesis by calf liver using metabolic labeling of liver slices in organ culture, and compared the results with those obtained for rat liver slices. This *in vitro* system was chosen so as to retain the normal cellular architecture of the liver, to avoid the dedifferentiation of hepatocytes, and to conserve the regulatory effect under *in vivo* conditions (*23*), taking into account the difficulty in using isolated perfused livers or isolated hepatocytes from bovids. The viability of hepatocytes in this *in vitro* system was verified by determining albumin synthesis and secretion. Albumin synthesis appeared to be far higher (around 2.7-fold) in calf than rat liver slices. This difference was observed in cells and media since the albumin secretion rate was similar in the livers of the two species. In both species, albumin represented 10% of the total protein synthesized by the liver and 80% of it was secreted into the culture medium, these results being in complete agreement with data obtained through several *in vivo* and *in vitro* experiments on the rat (*24*). Although no previous study has been performed on albumin synthesis by the calf liver, it was shown that the level of albumin in the plasma of the Holstein heifer calf (3.37 g/dl; *25*) was 1.7-fold higher than in the young rat (2.06 g/dl; *26*).

Calf liver slices only synthesized the larger form of apo B, that is apo B100, as previously demonstrated by Greeve *et al.* (*16*) and Gruffat *et al.* (*17*), whereas rat liver slices produced two forms of apo B, apo B100 and apo B48. By determining the rates of incorporation of labeled amino acids into the two forms of apo B in isolated perfused rat livers, Sparks *et al.* (*27*) showed that rat hepatocytes synthesized equal amounts of apo B48 and B100, both being incorporated into nascent hepatic VLDL, although lipidation of apo B48-VLDL may occur *via* a different pathway to that of apo B100-VLDL (*28*). Under our experimental conditions, the incubation of liver slices for 12 h in the presence of ^{35}S amino acids gave steady-state data integrating the synthesis of apo B, its subsequent intracellular catabolism and its secretion. Under these conditions, the similar accumulation of apo B in calf liver slices (apo B100) and rat liver slices (apo B100+apo B48) strongly suggested that the same quantity of apo B was available for VLDL synthesis in the livers of the two animal species. The VLDL-apoproteins in bovine plasma mainly comprised apo B and, to a lesser extent, apo C (*29*), whereas, in rat, VLDL-apoproteins also contained apo E (*30*). Under our experimental conditions, the amounts ^{35}S-labeled VLDL-apoproteins present in the media of calf liver slices were about fivefold lower than those in the media of rat liver slices after 12 h labeling. This finding was in perfect agreement with previous data on liver slices of preruminant calves and young rats incubated in the presence of 0.8 mM [^{14}C]oleate for 7 h (*7*). Indeed, the results showed that the secretion of VLDL-lipids was 6-fold greater in rat than calf liver slices, although the incubation conditions were slightly different from those used in the present study.

Different possibilities can explain the low accumulation of VLDL in the media of calf liver slices: (i) rapid catabolism of VLDL immediately after their secretion, or (ii) a defect in the mechanisms of VLDL assembly and/or secretion.

VLDL newly secreted by calf liver slices might be immediately degraded, particularly under our experimental conditions where secreted VLDL stayed in contact with hepatocytes (lack of blood flow). The degradation might occur *via* a receptor pathway. However, a VLDL-receptor pathway is unlikely since this receptor is not detectable in the liver (*31*). Moreover, this receptor specifically binds the apo E present on VLDL and it has been demonstrated that lipoproteins of bovids do not contain apo E but another apoprotein probably corresponding to β2-glycoprotein-I, judging from its molecular weight (51 kDa), amphiphilicity and immunological reactivity (*4*). The other receptor pathway which might be involved is that of the LDL-receptor. The action of this receptor requires the activity of lipoprotein lipase (LPL), which plays a major role in the catabolism of VLDL into low density lipoproteins. However, it has been demonstrated that bovine liver is unable to synthesize LPL (*32*). Moreover, preliminary results obtained in our laboratory indicated that the LDL-receptor was expressed 4-fold less in the calf than the rat liver (Gruffat-Mouty *et al.*, unpublished data). Thus, all these data allowed us to eliminate the hypothesis of reuptake and degradation of newly secreted VLDL by the livers of calves.

Another possibility explaining the low accumulation of VLDL in the media of calf liver slices might be a defect in the mechanisms of VLDL assembly and/or secretion. From data for the rat liver, TG newly synthesized from exogenous fatty acids appeared to be a poor precursor for VLDL assembly compared to TG stored in the cytoplasmic storage pool (*33, 34*), and to be mobilized *via* the co-ordinated processes of lipolysis and re-esterification (*33, 35*). The rates of TG hydrolysis and re-esterification might determine the effective availability of TG at the site of VLDL assembly, which would partly explain the chronic and limited availability of TG for VLDL assembly in bovids. Moreover, it is clear that intracellular TG transfer needs the activity of the microsomal TG transfer protein (MTP) (*8, 36*). From the experimental data for the rat liver, MTP did not appear to be a regulatory target in short-to-medium terms (*37*). However, long-term dietary studies have revealed changes in MTP mRNA (*38, 39*). Very recently, preliminary results obtained in our laboratory indicated that MTP was expressed less the calf than in the rat liver, which suggests slower transfer of TG to the hepatic site of VLDL assembly (Graulet *et al.*, unpublished data). Thus, these results allowed us to speculate that the TG availability for VLDL assembly *via* MTP activity in the bovine liver might be a limiting step for VLDL secretion.

In conclusion, our results clearly showed that the rate of hepatic apo B synthesis by the calf liver was similar with that by the rat liver, suggesting that a defect in the processes of assembly and/or secretion of VLDL might be responsible for the low rate of secretion of VLDL by the calf liver rather than apo B production. The availability of TG at the site of VLDL assembly *via* the processes of lipolysis and re-esterification, and MTP synthesis is now under investigation.

We wish to thank C. Leoty and R. Souchet for the calf management, J. Lefaivre for the surgical preparation, and F. Duboisset, C. Legay and M. Martinaud for their technical assistance.

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012

REFERENCES

1. Gruffat, D., Durand, D., Graulet, B., and Bauchart, D. (1996) Regulation of VLDL synthesis and secretion in the liver. *Reprod. Nutr. Dev.* **36**, 375-389
2. Leplaix-Charlat, L., Durand, D., and Bauchart, D. (1996) Effects of diets containing tallow and soybean oil with and without cholesterol on hepatic metabolism of lipids and lipoproteins in the preruminant calf. *J. Dairy Sci.* **79**, 1826-1835
3. Bauchart, D., Durand, D., Picherit, C., Graulet, B., and Gruffat, D. (1998) Effects of dietary coconut oil on blood transport and in vivo hepatic metabolism of fatty acids in the preruminant calf. *Reprod. Nutr. Dev.* **38**, 203-204
4. Bauchart, D., Durand, D., and Gruffat, D. (1996) Lipid absorption and hepatic metabolism in ruminants. *Proc. Nutr. Soc.* **55**, 39-47
5. Kleppe, B.R., Aiello, R.J., Grummer, R.R., and Armentano, L.E. (1988) Triglyceride accumulation and very low-density lipoprotein secretion by rat and goat hepatocytes in vitro. *J. Dairy Sci.* **71**, 1813-1822
6. Pullen, D.L., Liesman, J.S., and Emery, R.S. (1990) A species comparison of liver slice synthesis and secretion of triacylglycerol from nonesterified fatty acids in media. *J. Anim. Sci.* **68**, 1395-1399
7. Graulet, B., Gruffat, D., Durand, D., and Bauchart, D. (1998) Fatty acid metabolism and very low density lipoprotein secretion in liver slices from rats and preruminant calves. *J. Biochem.* **124**, 1212-1219
8. Gordon, D.A. (1997) Recent advances in elucidating the role of the microsomal triglyceride transfer protein in apolipoprotein B lipoprotein assembly. *Curr. Opin. Lipidol.* **8**, 131-137
9. Yao, Z., Tran, K., and McLeod, R.S. (1997) Intracellular degradation of newly synthesized apolipoprotein B. *J. Lipid Res.* **38**, 1937-1953
10. Cartwright, I.J. and Higgins, J.A. (1996) Intracellular degradation in the regulation of secretion of apolipoprotein B-100 by rabbit hepatocytes. *Biochem. J.* **314**, 977-984
11. Wu, X., Sakata, N., Lele, K.M., Zhou, M., Jiang, H., and Ginsberg, H.N. (1997) A two-model for apo B degradation in HepG2 cells. *J. Biol. Chem.* **272**, 11575-11580
12. Liao, W., Yeung, S.C.J., and Chan, L. (1998) Protease-mediated degradation of apolipoprotein B targets both nascent peptides cotranslationally before translocation and full-length apolipoprotein B after translocation into the endoplasmic reticulum. *J. Biol. Chem.* **273**, 27225-27230
13. Adeli, K., Macri, J., Mohammadi, A., Kito, M., Urade, R., and Cavallo, D. (1997) Apolipoprotein B is intracellularly associated with ER-60 protease homologue in HepG2 cells. *J. Biol. Chem.* **272**, 22489-22494
14. Linnik, K.M. and Herscovitz, H. (1998) Multiple molecular chaperones interact with apolipoprotein B during maturation. *J. Biol. Chem.* **273**, 21368-21373
15. Grummer, R.R. (1993) Etiology of lipid-related metabolic disorders in periparturient dairy cows. *J. Dairy Sci.* **76**, 3882-3896
16. Greeve, J., Altkemper, I., Dieterich, J.H., Greten, H., and Windler, E. (1993) Apolipoprotein B mRNA editing in 12 different mammalian species: hepatic expression is reflected in low concentrations of apoB-containing plasma lipoproteins. *J. Lipid Res.* **34**, 1367-1383
17. Gruffat, D., Durand, D., Chilliard, Y., Williams, P., and Bauchart, D. (1996) Hepatic gene expression of apolipoprotein B100 during early lactation in underfed, high producing dairy cows. *J. Dairy Sci.* **80**, 657-666
18. Marcos, E., Mazur, A., Cardot, P., and Rayssiguier, Y. (1990) The effect of pregnancy and lactation on serum lipid and apolipoprotein B and A-I levels in dairy cows. *J. Anim. Physiol. Anim. Nutr.* **64**, 133-138
19. Ushida, E., Katoh, N., and Takahashi, K. (1992) Induction of fatty liver in cows by ethionine administration and concomitant decreases of serum apolipoproteins B-100 and A-I concentrations. *Am. J. Vet. Res.* **53**, 2035-2042
20. Bouma, M.E., Beucler, I., Pessah, M., Heinzmann, C., Lusis, A.J., Naim, H.Y., Ducastelle, T., Leluyer, B., Schmitz, J., Infante, R., and Aggerbeck, L.P. (1990) Description of two different patients with abetalipoproteinemia: synthesis of a normal-sized apolipoprotein B-48 in intestinal organ culture. *J. Lipid Res.* **31**, 1-15
21. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685
22. Bauchart, D. (1993) Lipid absorption and transport in ruminants. *J. Dairy Sci.* **76**, 3864-3881
23. Olinga, P., Meijer, D.K.F., Slooff, M.J.H., and Groothuis, G.M.M. (1997) Liver slices in *in vitro* pharmacotoxicology with special reference to the use of human liver tissue. *Toxicol. in vitro* **12**, 77-100
24. Waterlow, J.C., Garlick, P.J., and Millward, D.J. (1978) The effects of nutrition and hormones on protein turnover in the liver in *Protein Turnover in Mammalian Tissues and in the Whole Body*, pp. 697-746, North-Holland Publishing Company, Amsterdam, New York, Oxford
25. Laden, S.A., Wohlt, J.E., Zajac, P.K., and Carsia, R.V. (1984) Effects of stress from electrical dehorning on feed intake, growth, and blood constituents of Holstein heifer calves. *J. Dairy Sci.* **68**, 3062-3066
26. Martin, N.H. (1961) Chemical composition of animal tissues—plasma proteins in *Biochemists' Handbook* (Long, C., ed.) pp. 885-892, E. & F.N. Spon Ltd., London
27. Sparks, C.E., Hnatiuk, O., and Marsh, J.B. (1981) Hepatic and intestinal contribution of two forms of apolipoprotein B to plasma lipoprotein fraction in the rat. *Can. J. Biochem.* **59**, 693-699
28. Borén, J., Rustaeus, S., and Olofsson, S.O. (1994) Studies on the assembly of apolipoprotein B-100- and B-48-containing very low density lipoproteins in McA-RH7777 cells. *J. Biol. Chem.* **269**, 25879-25888
29. Bauchart, D., Durand, D., Laplaud, P.M., Forgez, P., Goulinet, S., and Chapman, M.J. (1989) Plasma lipoproteins and apolipoproteins in the preruminant calf, *Bos* ssp: density distribution, physicochemical properties, and the *in vivo* evaluation of the contribution of the liver to lipoprotein homeostasis. *J. Lipid Res.* **30**, 1499-1514
30. Krul, E.S., Dolphin, P.J., and Rubinstein, D. (1981) Secretion of nascent lipoproteins by isolated rat hepatocytes. *Can. J. Biochem.* **59**, 676-686
31. Lestavel, S. and Fruchart, J.C. (1994) Lipoprotein receptors. *Cell. Mol. Biol.* **40**, 461-481
32. Hocquette, J.F., Graulet, B., and Olivecrona, T. (1998) Lipoprotein lipase activity and mRNA levels in bovine tissues. *Comp. Biochem. Physiol.* (in press)
33. Gibbons, G.F. and Wiggins, D. (1995) Intracellular triacylglycerol lipase: its role in the assembly of hepatic very-low density lipoprotein (VLDL). *Adv. Enzyme Regul.* **35**, 179-198
34. Pease, R.J. and Leiper, J.M. (1996) Regulation of hepatic apolipoprotein-B-containing lipoprotein secretion. *Curr. Opin. Lipidol.* **7**, 132-138
35. Yang, L.Y., Kuksis, A., Myher, J.J., and Steiner, G. (1995) Origin of triacylglycerol moiety of plasma very-low density lipoproteins in the rat: structural studies. *J. Lipid Res.* **36**, 125-136
36. Wetterau, J.R., Lin, M.C.M., and Jamil, H. (1997) Microsomal triglyceride transfer protein. *Biochim. Biophys. Acta* **1345**, 136-150
37. Brett, D.J., Pease, R.J., Scott, J., and Gibbons, G.F. (1995) Microsomal triglyceride transfer protein activity remains unchanged in rat livers under conditions of altered very-low density lipoprotein secretion. *Biochem. J.* **310**, 11-14
38. Lin, M.C.M., Arbeeny, C., Bergquist, K., Gordon, D.A., and Wetterau, J.R. (1994) Cloning and regulation of hamster microsomal triglyceride transfer protein. *J. Biol. Chem.* **269**, 29138-29145
39. Bennett, A.J., Billett, M.A., Salter, A.M., and White, D.A. (1995) Regulation of hamster hepatic triglyceride transfer protein mRNA levels by dietary fats. *Biochim. Biophys. Res. Commun.* **212**, 473-478

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012