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Effects of dietary fat and L-methionine on the hepatic metabolism of very low density lipoproteins in the preruminant calf, *Bos* spp

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Summary — The effects of triglycerides (TG) from tallow (1.21 and 2.13 g TG/kg of body weight (BW) per meal, diets R and B respectively) and from tallow plus cream (2.50 g TG/kg of BW per meal, diet L) with or without L-methionine (2.6 g/kg dry matter) on hepatic apparent secretion of very low density lipoproteins (VLDL) were investigated in 3 groups of 4 preruminant calves fitted with chronic catheters and with electromagnetic blood-flow probes implanted in their hepatic vessels. Increasing TG concentrations stimulated the apparent VLDL secretion by the liver (1.02, −0.36 and −1.51 mg VLDL mass/min per kg of BW in diets L, B and R, respectively). L-Methionine increased this secretion when associated with the lipid-restricted (diet R; 0.25 and −1.51 mg VLDL/min per kg of BW) and basal (diet B; 0.35 and −0.36 mg VLDL/min per kg of BW) diets (non-significant). However, the VLDL apparent secretion decreased with the lipid-enriched diet (diet L), which suggests an insufficient dose of L-methionine compared with the level of TG intake, and a possible competition between liver and intestine for utilization of L-methionine for the synthesis of TG-rich lipoproteins.

L-methionine / dietary triglycerides / VLDL / hepatic metabolism / preruminant calf

Résumé — Effets des matières grasses et de la L-méthionine sur le métabolisme hépatique des lipoprotéines de très basse densité chez le veau préruminant, *Bos* spp. *Les effets des triglycérides (TG) du suif (1,21 et 2,13 g de TG/kg de poids vif et par repas, lots R et B) et du suif addi-*

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Abbreviations: Apo B, apolipoprotein B; BW, body weight; CE, cholesteryl esters; EDTA, ethylenediaminetetraacetic acid; FC, free cholesterol; HV, hepatic vein; MA, mesenteric artery; NEFA, non-esterified fatty acids; PL, phospholipids; PV, portal vein; TC, total cholesterol; TG, triglycerides; VLDL, very low density lipoproteins.

tionné de crème (2,50 gTG/kg PV et par repas, lot L) associés ou non à de la L-méthionine (2,6 g/kg MS) sur la sécrétion hépatique apparente de lipoprotéines de très basse densité (VLDL), ont été étudiés sur 12 veaux préruminants équipés de cathéters et de sondes électromagnétiques implantés dans les vaisseaux hépatiques. La sécrétion apparente de VLDL par le foie s'accroît avec le taux de lipides du lait (+1,02 vs −0,36 vs −1,51 mg VLDL/min/kg PV avec les lots L, B et R). De même, la L-méthionine stimule cette sécrétion apparente mais non significativement avec les lots R (+0,25 vs −1,51 mg VLDL/min/kg PV) et B (+0,35 vs −0,36 mg/min/kg PV), mais la réduit avec le lot L suggérant que l'apport de L-méthionine est insuffisant à haut niveau d'apport en lipides. De plus, une possible compétition entre le foie et l'intestin pourrait limiter l'efficacité de la L-méthionine en situation de fort recyclage de lipides par le foie.

L-méthionine / triglycéride alimentaire / VLDL / métabolisme hépatique / veau préruminant

INTRODUCTION

During early lactation, high-producing dairy cows are in a state of negative energy balance, which is associated with a large increase in plasma non-esterified fatty acids (NEFA) mobilized from the body fat stores (Reid *et al,* 1983). In these animals, NEFA are mainly taken up by the liver and incorporated into stored triglycerides in the hepatocytes (Kleppe *et al,* 1988; Pullen *et al,* 1988; Bauchart, 1993). These animals are thus susceptible to having a fatty liver because bovine hepatocytes have a limited capacity to export triglyceride-rich lipoproteins such as very low density lipoproteins (VLDL) (Reid *et al,* 1979a; Herdt *et al,* 1988; Kleppe *et al,* 1988; Pullen *et al,* 1988; Bauchart *et al,* 1989). If lipid infiltration of the liver becomes severe, gluconeogenesis (a vital function of the liver) may be compromised (Armentano *et al,* 1991), predisposing the cows to ketosis (Zammit, 1990; Grummer, 1993). Under such conditions, high-producing dairy cows may exhibit increased susceptibility to infectious diseases and may manifest reproductive difficulties (Reid *et al,* 1979b, 1983).

Hepatic export of triglycerides (TG) *via* the secretion of VLDL may be influenced by a spectrum of stimuli that include genetic and dietary factors and a variety of hormones. Although chronic feeding of fatty acids or cholesterol does not stimulate VLDL apolipoprotein (apo) B synthesis in the liver of non-human primates (Sorci-Thomas *et al,* 1989), increasing the levels of plasma NEFA in the dairy cow (Pullen *et al,* 1989) and those of dietary cholesterol in the calf (Leplaix *et al,* 1992) led to a slight increase of VLDL secretion by the liver. Similarly, administration of estrogens, such as 17β-estradiol to preruminant calves (Auboiron *et al,* 1992) and dairy cows during early lactation (Grummer *et al,* 1989), stimulated hepatic secretion of VLDL. However, 17β-estradiol appeared to have distinct effects on the hepatic metabolism in the calves depending on the dose or the mode of administration (Auboiron *et al,* 1992).

Sulfur-containing amino acids, such as L-methionine or methionine derivates (methionine hydroxy analogs, MHA), have been extensively studied in bovines because they improve milk production and increase the milk fat and protein contents in lactating cows (Chow *et al,* 1990) and goats (Emmanuel and Kennelly, 1984). As suggested by Moore and Christie (1981), the increase in milk fat production induced by L-methionine might result from the enhanced synthesis of VLDL constituents (apolipoproteins, lecithin) by the liver, which then increased the availability of the lipids required for the synthesis of milk fat by the mammary gland. The hepatocytes of methionine-deficient rats secreted VLDL at only 30% of the normal rate, and the addition of L-methionine to the deficient hepatocytes

restored the secretion of VLDL to normal levels (Yao and Vance, 1988). However, feeding MHAs to dairy cows during early lactation did not increase the hepatic secretion of triglycerides (Pullen *et al,* 1989). This lack of effect might be explained by premature bacterial degradation of MHAs during the passage of the digestive contents in the rumen (Patterson and Kung, 1988).

The objective of the present study was to determine the specific effects of adding L-methionine to the milk diet of preruminant calves (a functional monogastric animal) on the *in vivo* apparent secretion of VLDL by the liver. The animals were divided into 3 groups and were adapted to a standard TG diet, a diet with restricted TG, or a TG-rich diet. The TG-rich diet is associated with potential impairment of hepatic metabolism. The effects of dietary TG and L-methionine on the physicochemical properties and metabolism of the main lipoprotein classes of peripheral blood in the preruminant calf have been presented elsewhere (Auboiron *et al,* 1994).

MATERIALS AND METHODS

Animals and diets

The experiments were conducted using 3 groups of 4 preruminant, crossbred Friesian-Holstein male calves. The animals in group 1 were 5 (±2) weeks old (57 ± 19 kg of BW). In group 2, animals were 3 (±0) weeks old (58 ± 6 kg BW) and those in group 3 were 4 (±1) weeks old (68 ± 5 kg BW). The animals were housed on a litter of wood shavings in an air-conditioned shed (average temperature: 20°C; hygrometric level: 80%).

The calves of the 3 groups were equipped with chronic catheters which were fitted in the hepatic afferent (portal vein and mesenteric artery) and efferent (hepatic vein) vessels, as described by Bauchart *et al* (1989). The blood-flow rates were determined by electromagnetic flowmetry (Gould Inc, Statham Instruments Div, Oxnard, CA, USA) permitting determination of net lipoprotein balance across the liver. Two probes were placed around the portal vein (15 mm id) and the hepatic artery (left branch; 3 mm id), as outlined by Durand *et al* (1988).

The basal diet was a milk replacer and was bucket-fed in 2 equal meals per day (08.00 and 16.00 h). It contained 16% dry matter (DM) which was composed of 68% spray-dried skim milk powder (*ie* 22.8% weight protein and 0.59% weight methionine), 23.0% tallow, 6.8% corn starch, and 2.2% vitamin and mineral mixture (Univor 22, Sodiavit, Montferrand, France). The total lipid and fatty acid content of the milk powder amounted to 24.1 and 22.0% of the DM, respectively.

The calves in group 1 were adapted over a 4 d period to a restrictive diet (diet R) which contained 30% of the DM of the basal conventional diet (Toullec, 1978) (1.21 ± 0.05 g of TG/kg of BW per meal) to which 2.3 g/kg of cystine (Sigma Chemical, Saint Louis, MO, USA) was added to the milk powder in order to compensate for the protein deficit. The calves in group 2 were similarly adapted to the basal diet (diet B; 2.13 ± 0.13 g of TG/kg of BW per meal), and those in group 3 were adapted to the basal diet to which 125 g cream/kg of diet DM (diet L, 2.50 ± 0.22 g of TG/kg of BW per meal) was added for 6 d. Finally, all the calves received the same respective diets to which L-methionine was added (2.6 g/kg of DM; Rhône Poulenc Animal Nutrition, Commentry, France) for 4 consecutive days (diets RM, BM and LM).

Blood samples

Blood (20 ml) was collected simultaneously from each of the 3 catheterized vessels (hepatic vein, portal vein and mesenteric artery) into Na_2-EDTA tubes (final concentration 1 mM) at peak lipid absorption (7 h after the morning meal). Plasma then was separated by centrifugation at 4 500 rpm for 10 min at 4°C and stored at 4°C until lipoprotein fractionation was initiated, typically within 24 h of its isolation.

Lipoprotein isolation

The different ultracentrifugation steps used for lipoprotein fractionation were performed in a Kontron model Centrikon T-2060 ultracentrifuge using a TST 41-14 swinging bucket rotor. Chylomicrons

(Sf flotation coefficient in Svedberg units > 400) were first removed from the plasma by ultracentrifugation flotation for 45 min at 20 000 rpm (52 000 *g*) and 15°C according to Zilversmit (1969). The VLDL (density < 1.006 g/ml) were then isolated from the chylomicron-free plasma by ultracentrifugation flotation for 16 h at 40 000 rpm (220 000 *g*) and 15°C (Bauchart *et al,* 1989).

Chemical analysis

The techniques used for measurements of the concentrations of the different classes of lipids, in both total plasma and lipoprotein fractions, have been described previously (Bauchart *et al,* 1989). Total cholesterol (TC) and free cholesterol (FC) were measured enzymatically using the reagent kit supplied by Merck (CHOD-iodide, Merckotest N14350, Darmstadt, Germany). Cholesteryl ester (CE) content was calculated using the relationship: CE = (TC – FC) x 1.68. Triglyceride content was estimated by the enzymatic method using Biomérieux reagent kit (PAP 100, No 6126.6, Biomérieux, Charbonnières-les-Bains, France) which determines total free glycerol content. Phospholipids (PL) were determined enzymatically involving use of Biomérieux kit (PAP 150, No 6149.1). The colorimetric method of Bicinchininic Acid Protein Assay Reagent (Pierce, Rockford, IL, USA) was used for the assay of protein concentrations.

Immunological analysis

The apo B content of the VLDL particles was determined by radial immunodiffusion according to the technique of Mancini *et al* (1965) adapted to the bovine apolipoprotein by Auboiron *et al* (1990) and using an antiserum to calf apo B (purified from bovine LDL) raised in rabbits (Auboiron *et al,* 1990).

Calculation of hepatic balance

The hepatic balance was determined from blood-flow measurement in the portal vein and mesenteric artery, and from the plasma concentrations of VLDL isolated by flotation ultracentrifugation from plasma drawn from the mesenteric artery, portal vein (afferent vessels) and hepatic vein (efferent vessel) (Bauchart *et al,* 1989). Plasma flow rates were corrected for packed-cell volume.

The hepatic afferent VLDL flow (mg/min per kg of BW) = $(C_{PV} \times F_{PV}) + (C_{MA} \times F_{HA})$, where F_{PV} and F_{HA} are the portal and hepatic arterial plasma flow rates (ml/min per kg of BW) respectively, and C_{PV} and C_{MA} are the portal and mesenteric arterial VLDL plasma concentrations (mg/dl) respectively. Hepatic efferent plasma flow was considered as being equal to hepatic afferent plasma flow which was calculated as: hepatic efferent VLDL flow = C_{HV} $(F_{PV} + F_{HA})$, where C_{HV} is the hepatic venous plasma VLDL concentration (mg/dl). We assumed that the hepatic balance corresponded to the amount of VLDL removed (negative balance) or produced (positive balance) by the liver. Thus, VLDL hepatic balances could be calculated from the following equations (Bauchart *et al,* 1989): VLDL hepatic balance = C_{HV} $(F_{PV} + F_{HA}) - (C_{PV} \times F_{PV}) + (C_{MA} \times F_{HA})$.

Statistical analysis

The nonparametric *U*-test of Mann and Whitney (1947) was used to estimate the statistical significance of the differences observed between the different parameters studied (plasma concentration, composition and hepatic balance of the VLDL) from the 3 groups of calves. The level of significance of the correlation coefficients *(r)* between the ingested amount of triglycerides *(x)* and VLDL plasma level *(y)* VLDL hepatic balance = C_{HV} $(F_{PV} + F_{HA}) - (C_{PV} \times F_{PV}) + ((C_{MA} \times F_{HA}))$ *(y)* for each set of data were determined from the table published by Snedecor and Cochran (1967).

RESULTS

The concentrations of the major plasma lipids in the mesenteric artery (peripheral blood) were determined in the preruminant calves fed the lipid-restricted diet (diet R, 1.21 g of TG/kg of BW per meal), the basal diet (diet B, 2.13 g of TG/kg of BW per meal) and the lipid-supplemented diet (diet L, 2.50 g of TG/kg of BW per meal) or these diets supplemented with L-methionine (diets

RM, BM and LM, 2.6 g/kg of DM intake) and are shown in table I. For all the diets used in this experiment, the plasma lipids were dominated by CE (35.7 – 41.9% of total lipids) and by phospholipids (38.0 – 42.2%). Triglycerides (9.0 – 14.4%), FC (1.9 – 3.4%) and NEFA (4.4 – 10.9%) were a minor plasma lipid component in these diets. Mean plasma concentrations of lipids were generally lower for diets R and RM than for the other diets, with the exception of NEFA in the 3 control diets (diets R, B and L) for which plasma levels were correlated inversely with the lipid intake (table I).

Addition of L-methionine to the control diet did not in general modify the plasma concentrations of the lipids irrespective of the quantity of fat ingested. It did lead, however, to a net decrease in plasma NEFA levels; the maximal effect was observed for diets RM (–56%) and BM (–55%; $P < 0.10$).

As shown in figure 1, the lipid distribution of plasma VLDL in the 3 hepatic vessels (mesenteric artery, portal and hepatic veins) of the calves fed control and methionine-supplemented diets was modified by the level of fat intake, but no marked difference was noted between the vessels. As compared to the basal diets (diets B and BM), and more particularly the lipid-supplemented diets (diets L and LM), the VLDL particles in calves fed lipid-restricted diets (diets R and RM) exhibited mean CE and FC contents which were 1.9–12.9 and 1.5–2.7 times higher, respectively, and which occurred to the detriment of the TG content (–20.7 to –31.0%) (table II). Similar decreases in apo B content of total VLDL particles (–57.7 to –53.4%) were observed on lipid-supplemented diets as compared to lipid-restricted diets (table III).

Plasma concentrations of VLDL increased with fat intake in the 3 hepatic vessels (table IV). The regression coefficient for the correlation between individual values of plasma VLDL concentrations in the mesenteric artery (y; mg/dl) and fat intake (x; g/kg of BW per meal) on the con-

Table I. Total concentration (mg/dl) of the major lipids in plasma from a mesenteric artery of preruminant calves fed lipid-restricted diet (diet R), basal diet (diet B) and lipid-enriched diet (diet L) or these diets supplemented with L-methionine (2.6 g/kg dry matter; diets RM, BM and LM).

	Plasma lipids (mg/dl) *					
	Lipid-restricted diet		*Basal diet*		*Lipid-enriched diet*	
	Control (diet R)	*Methionine (diet RM)*	*Control (diet B)*	*Methionine (diet BM)*	*Control (diet L)*	*Methionine (diet LM)*
FC	7.4 ± 4.0	7.9 ± 4.9	6.4 ± 3.2	5.3 ± 4.7	8.8 ± 1.1	10.7 ± 3.2
CE	98.3 ± 22.2	96.0 ± 20.6	123.2 ± 20.2	117.7 ± 27.2	116.2 ± 29.2	114.4 ± 35.7
TC **	65.9 ± 15.0	65.1 ± 15.4	79.7 ± 14.6	75.4 ± 20.3	78.0 ± 18.3	78.8 ± 23.8
TG	24.9 ± 17.9	21.5 ± 19.2	29.3 ± 10.9	26.4 ± 8.9	42.3 ± 9.2	45.0 ± 10.0
PL	105.0 ± 16.7^{a}	100.3 ± 22.4	129.0 ± 17.9^{b}	118.5 ± 29.0^{ab}	126.0 ± 24.3^{ab}	129.3 ± 33.0^{ab}
NEFA	30.0 ± 28.0^{ab}	13.2 ± 7.2^{b}	28.5 ± 13.0^{a}	12.8 ± 7.3^{b}	15.3 ± 6.6^{ab}	13.7 ± 13.5^{ab}

* Values are the mean ± SEM of duplicate analyses on plasma from 4 calves in each group. Plasma from these animals were used for TG-rich lipoprotein isolation by flotation ultracentrifugation. ** Values for TC represent the serum-free and esterified cholesterol. $^{a-b}$ Mean values with different superscript letters are significantly different ($P < 0.10$).

trol and methionine-supplemented diets are shown in figure 2. The relationship between these variables was linear and highly significant for the 2 groups of diets (control diets: $y = 7.70x - 5.54$; $r = 0.76$, $P < 0.01$; methionine-supplemented diets: $y = 14.73x - 15.83$; $r = 0.94$, $P < 0.001$). Calculation of the slope ratio (concentrations of VLDL in methionine-supplemented diets *versus* control diets) indicated that

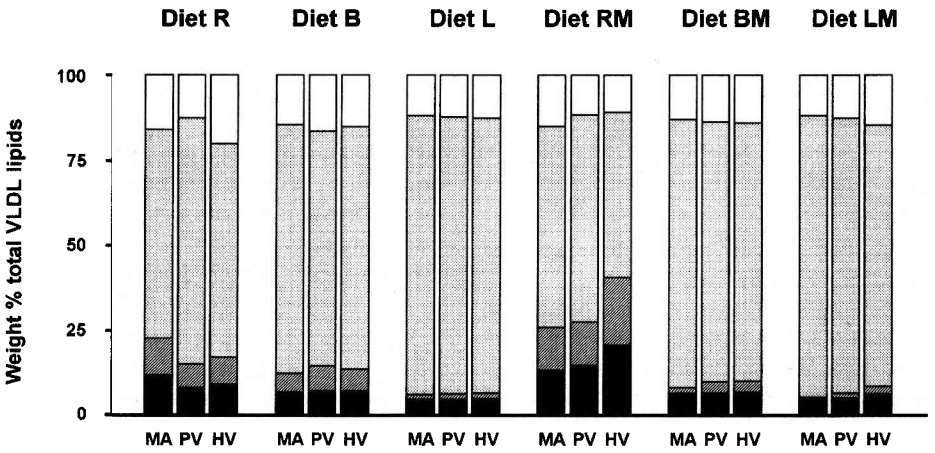

Fig 1. Mean lipid composition (free cholesterol, ☐; triglycerides, ■; cholesteryl esters, ▨; phospholipids, ■; mean weight percentage of total VLDL lipids) in plasma VLDL from the mesenteric artery (MA), hepatic vein (HV) and portal vein (PV) of preruminant calves fed the lipid-restricted diet (diet R, $n = 4$), basal diet (diet B, $n = 4$), and lipid-enriched diet (diet L, $n = 4$), and the same diets with L-methionine (2.6 g/kg of dry matter) (diets RM, BM and LM, $n = 4$ for each diet).

Table II. Mean chemical composition (% total lipoprotein mass) of VLDL ($d < 1.006$ g/ml) in plasma from a mesenteric artery of preruminant calves fed lipid-restricted diet (diet R), basal diet (diet B) and lipid-enriched diet (diet L) or these diets supplemented with L-methionine (2.6 g/kg dry matter; diets RM, BM and LM).

	VLDL chemical composition (%) *					
	Lipid-restricted diet		*Basal diet*		*Lipid-enriched diet*	
	Control (diet R)	*Methionine (diet RM)*	*Control (diet B)*	*Methionine (diet BM)*	*Control (diet L)*	*Methionine (diet LM)*
FC	8.9 ± 3.1^{ab}	10.7 ± 2.8^{a}	5.8 ± 0.3^{b}	5.4 ± 1.7^{b}	4.0 ± 0.8^{b}	4.2 ± 1.4^{b}
CE	8.6 ± 1.7^{a}	10.3 ± 4.7^{a}	4.5 ± 3.5^{ab}	1.3 ± 1.1^{b}	1.2 ± 0.9^{b}	0.8 ± 0.2^{b}
TG	48.3 ± 8.7^{a}	47.4 ± 11.9^{a}	60.9 ± 6.1^{ab}	65.2 ± 2.4^{b}	68.7 ± 1.8^{b}	67.8 ± 2.9^{b}
PL	13.9 ± 4.4^{ab}	15.5 ± 1.7^{a}	11.9 ± 1.9^{b}	10.7 ± 1.1^{b}	9.8 ± 1.2^{b}	9.6 ± 0.9^{b}
Proteins	20.3 ± 8.2	19.6 ± 5.5	16.9 ± 2.0	17.4 ± 0.7	16.4 ± 1.2	16.7 ± 0.6

* Values are the mean ± SEM of duplicate analyses on plasma from 4 calves in each group. $^{a\text{-}b}$ Means values with different superscript letters are significantly different ($P < 0.05$).

plasma VLDL was twice as high when L-methionine was added to these diets than to the control diets.

Addition of lipids to diets generally led to a stimulation of the *in vivo* apparent secretion of VLDL by the liver (table V), and especially in the case of the lipid-supplemented diet as compared to the lipid-restricted diet ($P < 0.10$). Increase in VLDL apparent secretion was positively correlated with

Table III. Apo B content (mean percentage VLDL weight) and apo B plasma level (mg/dl plasma) of VLDL ($d < 1.006$ g/ml) determined by radial immunodiffusion with antibody against bovine apo B.

	Lipid restricted diet		*Basal diet*		*Lipid-enriched diet*	
	Control (diet R)	*Methionine (diet RM)*	*Control (diet B)*	*Methionine (diet BM)*	*Control (diet L)*	*Methionine (diet LM)*
Apo B (% mean VLDL weight) *						
Mesenteric artery	2.7 ± 2.1	2.3 ± 2.7	1.2 ± 0.8	1.2 ± 0.3	1.1 ± 0.5	0.8 ± 0.1
Portal vein	2.5 ± 1.7^{a}	3.0 ± 2.1^{a}	1.4 ± 0.9^{ab}	1.1 ± 0.9^{ab}	1.1 ± 0.4^{b}	0.9 ± 0.2^{b}
Hepatic vein	2.6 ± 2.1	1.6 ± 2.5	1.0 ± 1.2	1.5 ± 0.7	1.1 ± 0.2	1.5 ± 1.0
Apo B (mg/dl)						
Mesenteric artery	0.15 ± 0.12	0.05 ± 0.07	0.12 ± 0.09	0.17 ± 0.03	0.17 ± 0.01	0.18 ± 0.05
Portal vein	0.19 ± 0.07^{c}	0.07 ± 0.05^{d}	0.09 ± 0.07^{d}	0.14 ± 0.09^{cd}	0.19 ± 0.04^{c}	0.17 ± 0.07^{c}
Hepatic vein	0.18 ± 0.14^{b}	0.05 ± 0.06^{a}	0.08 ± 0.09^{ab}	0.15 ± 0.03^{b}	0.20 ± 0.05^{b}	0.17 ± 0.07^{b}

VLDL were isolated by flotation ultracentrifugation in plasma from the mesenteric artery, hepatic vein and portal vein of preruminant calves fed lipid-restricted diet (diet R), basal diet (diet B) and lipid-enriched diet (diet L) or same diets supplemented with L-methionine (2.6 g/kg dry matter; diets RM, BM and LM). * Values are the mean ± SEM of duplicate analyses on plasma from 4 calves in each group. $^{a-d}$ Mean values with different superscript letters were significantly different: ^{a,b} ($P < 0.10$); ^{c,d} ($P < 0.05$).

Table IV. Concentration (mg/dl) of VLDL ($d < 1.006$ g/ml) isolated by flotation ultracentrifugation in plasma from the mesenteric artery, hepatic vein and portal vein of preruminant calves fed lipid-restricted diet (diet R), basal diet (diet B) and lipid-supplemented diet (diet L) or the same diets supplemented with L-methionine (2.6 g/kg dry matter; diets RM, BM and LM).

	Plasma concentration of VLDL (mg/dl) *					
	Lipid-restricted diet		*Basal diet*		*Lipid enriched diet*	
	Control (diet R)	*Methionine (diet RM)*	*Control (diet B)*	*Methionine (diet BM)*	*Control (diet L)*	*Methionine (diet LM)*
Mesenteric artery	5.3 ± 4.1^{a}	2.0 ± 1.0^{a}	8.4 ± 4.1^{b}	14.7 ± 3.6^{bc}	17.4 ± 5.7^{c}	23.3 ± 2.7^{c}
Portal vein	10.2 ± 6.4^{b}	2.0 ± 1.3^{a}	5.5 ± 3.0^{a}	11.2 ± 5.8^{ab}	17.1 ± 3.1^{b}	19.0 ± 8.9^{b}
Hepatic vein	6.0 ± 4.4^{a}	2.5 ± 2.1^{a}	4.9 ± 3.3^{a}	12.3 ± 5.8^{ab}	18.9 ± 5.4^{b}	14.7 ± 8.5^{b}

* Values are the mean ± SEM of duplicate analyses on plasma from 4 calves in each group. $^{a-c}$ Mean values with different superscript letters were significantly different ($P < 0.05$).

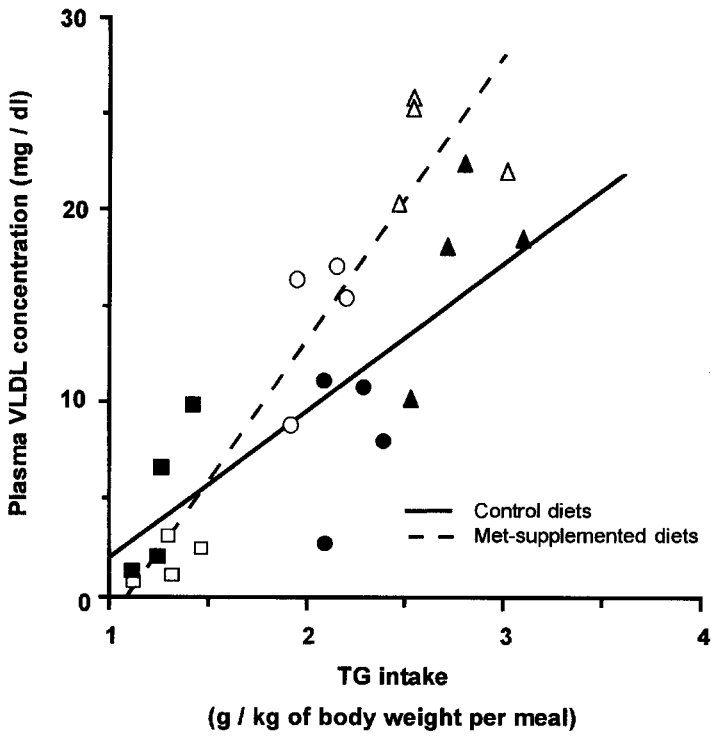

Fig 2. Individual plasma concentrations (mg/dl) of VLDL (d < 1.006 g/ml) isolated by flotation ultracentrifugation from the mesenteric artery of preruminant calves fed the lipid-restricted diet (diet R, ■), basal diet (diet B, ●), lipid-enriched diet (diet L, ▲) and the same diets (diet RM, □; diet BM, O; diet LM, Δ) with L-methionine (2.6 g/kg of dry matter).

increase in the hepatic apparent secretion of VLDL apo B (0.009 ± 0.030 *versus* 0.005 ± 0.011 mg/min per kg of BW for diets L and R, respectively). Addition of L-methionine to diets R and B enhanced the net apparent secretion of VLDL by the liver because the negative hepatic balance observed for the control diets became positive in the presence of L-methionine (table V). On the other hand, the negative and significant (P < 0.10) effects of L-methionine on the hepatic apparent secretion of VLDL was noted with the lipid-supplemented diet (diet LM).

DISCUSSION

In dairy cattle, VLDL secretion by the liver is considered to be low when compared to triglyceride synthesis (Herdt *et al*, 1988), which would explain the moderate to severe hepatic lipidosis noted in one-third of periparturient, high-producing cows (Reid and Roberts, 1983). In humans, VLDL production by the liver and the role of various factors in its regulation have been extensively studied in view of the identification of increase of plasma triglyceride levels as a major risk factor in heart disease (Castelli, 1986). Studies on hepatocytes in culture or on perfused liver in humans and rodents have indicated that cellular VLDL synthesis may be regulated at several levels, including the biosynthesis of the various lipid and protein constituents of these particles and the assembly and subsequent maturation of nascent VLDL (Yao and Vance, 1988; Gibbons, 1990; Vance, 1990). The level of fat intake appeared to be an important factor controlling the rate of VLDL secretion by both the liver and the intes-

Table V. Hepatic balance (mg/min/kg BW) of plasma VLDL ($d < 1.006$ g/ml) isolated by flotation ultracentrifugation in plasma from the mesenteric artery, hepatic vein and portal vein of preruminant calves fed lipid-restricted diet (diet R), basal diet (diet B) and lipid-supplemented diet (diet L) or the same diets supplemented with L-methionine (2.6 g/kg dry matter; diets RM, BM and LM).

Diet	*Hepatic balance of VLDL (mg/min/kg BW)* *
Diet R	
Control	-1.51 ± 1.77^{a}
Methionine	0.25 ± 0.86^{ab}
Diet B	
Control	-0.36 ± 0.94^{ab}
Methionine	0.35 ± 2.78^{ab}
Diet L	
Control	1.02 ± 2.13^{b}
Methionine	-2.16 ± 0.22^{a}

Hepatic balance is expressed as: (C_{HV} (F_{PV} + F_{HA}) − C_{PV} F_{PV} + C_{HA} F_{HA}) (for details, see *Materials and methods*). * Values are the means ± SEM of duplicate analyses on plasma from 4 calves in each group. [a–b] Mean values with different superscript letters were significantly different ($P < 0.10$).

tine. Under fasting conditions, secretion of VLDL by cultured hepatocytes in the rat (Gibbons and Burnham, 1991) and by the intact liver in the preruminant calf (Bauchart *et al,* 1989) was shown to be strongly depressed. Under such dietary conditions, the low secretion of VLDL in the rat concerned all lipid classes of these particles as well as apo B48 (Davis *et al,* 1985), whereas apo B100 secretion remained unchanged (Leighton *et al,* 1990).

The negative hepatic balance for VLDL determined under fasting conditions in the preruminant calf (Bauchart *et al,* 1989), in the dairy cow (Reid *et al,* 1979a), or with lipid-restricted or basal diets (diets R and B) in the preruminant calf in our experiment, indicated that rates of VLDL uptake exceeded VLDL secretion rates by the liver *in vivo*. However, the occurrence of a positive hepatic balance for VLDL in our calves which received a high fat diet (diet L) has been observed previously in cows (Reid *et al,* 1979a). Although the differences between lipid-restricted and lipid-enriched diets were significant only at $P < 0.10$, these results suggest a stimulation of VLDL synthesis by circulating dietary fatty acids administered as TG, in addition to a possible decrease of VLDL uptake. Similar observations have been reported for the intense uptake of circulating NEFA during the development of a fatty liver in the dairy cow (Herdt *et al,* 1983; Pullen *et al,* 1989).

Variations in the rate of VLDL secretion with the level of fat intake were associated with modification in the chemical composition of VLDL particles. The accumulation of cholesterol (mainly as CE) in the VLDL particles in TG-restricted diets confirmed the notion that *de novo* synthesis of long-chain fatty acids and their conversion into VLDL-TG occurs at a low rate in the liver of bovine species and is insufficient to compensate for the low supply of dietary fatty acids by these diets in our experiment.

A decrease in the apo B content (expressed as percentage weight of total VLDL particles) of VLDL particles coupled with an increase in fat intake has been described previously in rat hepatocytes (Patsch *et al,* 1983). It was explained on the basis of an increase in particle size resulting from preferential incorporation of triglycerides (Vance and Vance, 1990) and by the fact that, as in rat and humans, only one molecule of apo B is present in each VLDL particle (Elovson *et al,* 1988). However, possible regulation of apo B synthesis by dietary fatty acids should not be excluded, although it remains a much debated question (Vance and Vance, 1990). In the perfused rat liver, oleic acid did not

stimulate secretion of apo B (Salam *et al,* 1988), whereas it had a positive effect in HepG2 cell cultures (Moberly *et al,* 1990; Arrol *et al,* 1991; Dixon and Ginsberg, 1993). Hepatic TG are mainly implicated in the modulation of apo B secretion by oleate. Indeed, in the HepG2 cells, apo B secretion was stimulated by oleate with a short lag phase (40 min) and, conversely, this apo B secretion returned to the control rate with a similar lag phase when oleate was removed from the medium (Dixon and Ginsberg, 1993). However, treatment of primary cultures of hamster hepatocytes with oleate stimulated TG synthesis without modifying apo B secretion but the decrease in TG synthesis reduced the apo B secretion (Arbeeny *et al,* 1992).

Irrespective of the influence of dietary fatty acids on apo B secretion, it is clear that VLDL particles are not secreted without the presence of apo B (Vance and Vance, 1990). It is therefore probable that apo B production is one of the main factors limiting VLDL secretion in bovine species. It was demonstrated in goat hepatocytes that long-chain fatty acids were esterified with the same efficiency as in rat hepatocytes, but the rate of VLDL secretion was 20–25 times lower (Kleppe *et al,* 1988; Armentano *et al,* 1991). From studies in HepG2 cells (Pullinger *et al,* 1989), nutritional and hormonal regulations of hepatic apo B secretion did not seem to be modulated at transcription but rather at the translation or post-translation levels.

Enhancement by L-methionine of apparent VLDL secretion by the liver with diets R and B was not significant mainly because of a large between-animal variability. However, stimulating effects of L-methionine were observed for most of the animals in both groups. It seemed to indicate that this sulfur-containing amino acid is a limiting factor in hepatic TG secretion in bovine species, as has been proposed by Moore and Christie (1981). It is unclear whether this amino acid acts in the calf as a precursor for both lecithin and apo B synthesis (Moore and Christie, 1981). Similar stimulation of apparent VLDL secretion by the liver was observed in the early lactation in the dairy cow when infused with a mixture of L-methionine and L-lysine into the mesenteric vein (Durand *et al,* 1992). The lack of effect of L-methionine in association with our lipid-enriched diet (diet LM) was unexpected. This might result from an insufficient dose of L-methionine as compared to the high level of fat intake or from a possible competition between liver and intestine for L-methionine uptake. Indeed, recent studies of chylomicron metabolism in methionine-supplemented diet in the preruminant calf (Auboiron *et al,* 1994) show that L-methionine leads to a marked increase in plasma levels of chylomicrons in peripheral blood, indicating possible stimulation of the secretion of this TG-rich lipoprotein by the intestine.

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