

## Weaning affects lipoprotein lipase activity and gene expression in adipose tissues and in *masseter* but not in other muscles of the calf

Jean-François Hocquette\*, Benoît Graulet, Michel Vermorel and Dominique Bauchart

Unité de Recherches sur les Herbivores, Institut National de la Recherche Agronomique, Centre de Recherches de Clermont Ferrand-Theix, 63122 Saint-Genès-Champanelle, France

(Received 9 November 2000 – Revised 8 May 2001 – Accepted 9 June 2001)

The nutritional and physiological modifications that occur during the weaning period induce adaptations of tissue metabolism in all mammal species. Among the adaptations due to weaning in ruminants, the regulation of lipoprotein lipase (LPL) activity, one of the rate-limiting steps of fatty acid utilization by tissues, was still unknown. The present study aimed at comparing LPL activity and gene expression in the heart, seven skeletal muscles and three adipose tissue sites between two groups of seven preruminant (PR) or ruminant (R) calves having a similar age (170 d), similar empty body weight (194 kg) at slaughter, and similar net energy intake from birth onwards. Triacylglycerol content of adipose tissues was 16% lower in R than in PR calves, ( $P < 0.01$ ). This could be partly the result from a lower LPL activity ( $-57\%$ ,  $P < 0.01$ ). LPL mRNA levels were also lower in R calves ( $-48\%$  to  $-68\%$ ,  $P < 0.01$ ) suggesting a pretranslational regulation of LPL activity. Activity and mRNA levels of LPL did not differ significantly in the heart and skeletal muscles except in the *masseter* in which LPL activity and mRNA levels were higher ( $+50\%$  and  $+120\%$  respectively,  $P < 0.01$ ) in the R calves. Regulation of LPL in *masseter* could be explained by the high contractile activity of this muscle after weaning due to solid food chewing. In conclusion, weaning in the calf affects LPL activity and expression in adipose tissues, but not in skeletal muscles except the *masseter*.

**Lipoprotein lipase: Muscle: Adipose tissue: Weaning: Bovine**

From birth to weaning, neonates suckle maternal milk or receive exclusively a milk-based diet to meet their energy needs. During the first weeks of life, feed intake must be sufficient and regular to allow for the rapid development of organs (such as digestive tract, brain, liver and kidney). This period is also characterised by a high rate of protein accretion in skeletal muscles that allows rapid body growth when the dietary energy and N supply is satisfactory.

Thereafter, the suckling–weaning transition is the most deep nutritional change that the animal has to face in its life. In the rat, the high-fat and low-carbohydrate milk diet is replaced by a carbohydrate-rich and low-fat diet. A great number of studies has been carried out in this model to demonstrate the regulation of tissue energy metabolism by nutritional changes (for review, see Girard *et al.* 1992).

However, the weaning–suckling transition differs markedly between ruminant animal species such as bovines, and single-stomached species such as man and rats. Indeed, at

weaning, fat and carbohydrates, the major energy-yielding substrates for the young calf, are replaced by volatile fatty acids, mainly acetate, and ketone bodies produced by the bacterial fermentation of solid foods in the rumen. Thus, weaning in ruminant animals is a specific and interesting model to study the nutritional regulation of energy metabolism. However, most of the experimental data published so far about weaning in ruminant animal species have concerned the development of the ruminal functions or the changes in plasma metabolites and hormones (Kouame *et al.* 1984; Quigley *et al.* 1991a,b). Only two studies in the bovine at weaning showed lower activities of glycolytic enzymes and glucose-6-phosphate dehydrogenase and higher activity of acetyl-CoA synthetase in the liver (Pearce & Unsworth, 1980) and lower activity of lactate dehydrogenase in skeletal muscles (Hocquette *et al.* 1997). However, no data are available about the regulation of the bovine lipoprotein lipase (LPL) activity at weaning, although this enzyme is considered as a key step for fatty

**Abbreviations:** LPL, lipoprotein lipase; OAT, omental adipose tissue; PAT, perirenal adipose tissue; PR, pre-ruminant; R, ruminant; SCAT, subcutaneous adipose tissue; TAG, triacylglycerol.

\* **Corresponding author:** Dr Jean-François Hocquette, fax +33 4 73 62 46 39, email hocquet@clermont.inra.fr

acid uptake by peripheral tissues (mainly adipose tissues and muscles) from circulating triacylglycerols (TAG) (for review, see Hocquette *et al.* 1998b). LPL activity is subject to complex hormonal and nutritional tissue-specific regulations that have been mainly studied in rodents (Olivecrona *et al.* 1991).

The cDNA of the bovine LPL has been cloned in the last decade (Senda *et al.* 1987). The expression of the bovine LPL gene has been studied in the mammary gland (Senda *et al.* 1987), in adipose tissues (Bonnet *et al.* 1998) and in skeletal muscles (Hocquette *et al.* 1998a). Meanwhile, a method to assay LPL activity was also optimized in bovine tissues by using rat serum as source of LPL activator (Hocquette *et al.* 1998a).

The aim of the present work was to investigate the regulation of LPL activity and gene expression in adipose tissues and skeletal muscles in the calf at weaning. Marked changes in LPL activity were firstly hypothesised as results of a lower dietary fat supply after weaning and of major differences in levels of plasma metabolites and hormones between milk-fed calves and weaned calves. However, we observed differences in LPL activity and expression in adipose tissues and in *masseter* muscle only, and not in other muscles of the carcass.

## Material and methods

### Reagents

[<sup>3</sup>H]Triolein (1.85–2.96 GBq/mmol) and Hyperfilms MP were supplied by Amersham International (Amersham, Bucks., UK). [ $\alpha$ -<sup>32</sup>P]deoxy-cytidine-5'-triphosphate (>111 TBq/mmol) and [ $\gamma$ -<sup>32</sup>P]deoxy-adenosine-3'-triphosphate (>111 TBq/mmol) were purchased from ICN Biochemicals (Irvine, CA, USA). Guanidium thiocyanate was obtained from Fluka (Ronkonkoma, NY, USA). Genescreen membranes were from New England Nuclear Life Science Products (Boston, MA, USA). T4 polynucleotide kinase was supplied by New England Biolabs Inc. (Beverly, MA, USA). Saturated phenol, chloroform-isoamyl alcohol (49:1, v/v), agarose and nonaprimer labelling kit were purchased from Appligène (Illkirch, France). RNA molecular-mass-markers were from Bethesda Research Laboratories (Bethesda, MD, USA). Other reagents were from Sigma (St Louis, MO, USA).

### Animals and experimental design

The fourteen Montbéliard male calves used were the animals described in a previous paper (Hocquette *et al.* 1997). They were tied and housed in individual stalls at ambient temperature in a room with natural lighting. Animals were allotted at the age of 88 d on the basis of live weight, average daily weight gain and average nutritional efficiency. From birth to 107 d of age, animals were maintained on a standard milk-substitute diet. From 107 d to slaughter, preruminant (PR) calves of the first group were fed individually on a commercial milk-substitute diet. Fat, carbohydrates and protein provided 35, 40 and 25 % absorbed energy respectively. Net energy intake was assessed from feed intake and net energy value of the

milk-substitute diet. The latter was calculated from chemical composition, metabolizability and the efficiency of metabolizable energy utilisation of similar diets in PR of similar body weight (Vermorel *et al.* 1974). Animals of the second group were progressively weaned from 107 to 128 d of age and became ruminant (R) calves. These calves were fed individually a mixed diet composed of 800 g concentrate and 200 g hay/kg, according to a feeding pattern designed to allow an average daily gain of 1300 g. Fat, carbohydrates, proteins and volatile fatty acids were assumed to provide approximately 8, 2, 25 and 65–70 % absorbed energy respectively, according to previous estimations (Hocquette *et al.* 1997). Net energy intake was calculated from feed intake and the tabulated net energy values of the feed ingredients (Jarrige, 1989). The rate of feeding was adjusted weekly so that net energy intakes from birth onwards were similar for the two groups of animals.

Calves were slaughtered at 170 d of age, after a 16 h overnight fast. Tissue samples (50–100 g) were always taken at the same site for all animals to minimise sampling error: heart, *masseter*, *diaphragma*, *rectus abdominis*, *longissimus thoracis* commonly designated *longissimus dorsi* (Kauffman *et al.* 1990), *semitendinosus* from the medial part, *tensor fasciae latae*, *cutaneus trunci* from the thick part, perirenal adipose tissue (PAT), omental adipose tissue (OAT) and subcutaneous adipose tissue (SCAT).

Muscle tissue samples were quickly trimmed of visible fat and connective tissue, cut into pieces, frozen in liquid N<sub>2</sub> in less than 10 min post-exsanguination and stored at –80°C for subsequent analysis. Adipose tissue samples were quickly cut into pieces, and then frozen at –80°C either in homogenization buffer for LPL assay (Hocquette *et al.* 1998a) or in liquid N<sub>2</sub> and stored at –80°C for subsequent analysis (Hocquette *et al.* 1997). Frozen samples were pulverized in liquid N<sub>2</sub> to get an homogeneous powder before any treatment.

### Analytical techniques

Blood samples were taken from the jugular vein after a 16 h overnight fast before slaughter. They were then centrifuged at 3500 g for 10 min at 15°C. Packed cell volume was measured after centrifugation of blood samples. Plasma samples were kept at –20°C until assayed. Commercial kits were used to determine plasma concentrations of insulin (INSIK-5 P2796; Sorin Biomedica, Saluggia, Italy), triiodothyronine (Amerlex – M T3 RIA; Amersham International), non-esterified fatty acids (Wako kit, Unipath S.A., Dardilly, France), glucose and TAG (PAP 250 ref 61271 and PAP 150 ref 61236 respectively; BioMérieux, Marcy-l'Etoile, France). Protein and DNA contents in muscles and in adipose tissues and activity of isocitrate dehydrogenase in muscles, were determined as previously described (Hocquette *et al.* 1996, 1997).

### Assay of lipoprotein lipase activity

LPL activity was assayed after detergent extraction as previously described (Hocquette *et al.* 1998a). Frozen muscle tissue powder or frozen adipose tissue pieces (approximately 4 g) were homogenized at 4°C in 9 ml

buffer/g (buffer was composed of ammonia-HCl (25 mM), pH 8.2 containing EDTA (5 mM), Triton X-100 (8 g/l), SDS (0.10 g/l), heparin (5000 IU/l) and peptidase inhibitors [pepstatin A (1 µg/ml), leupeptin (10 µg/ml) and aprotinin (0.017 TUI/ml)]. Insoluble material was discarded by centrifugation at 20 000 g for 20 min and at 4°C.

LPL activity was assayed with 4 µl tissue homogenate diluted 5-fold in water. The substrate emulsion was Intralipid (120 mM-TAG; Pharmacia & Upjohn, Stockholm, Sweden) into which a trace amount of [<sup>3</sup>H]triolein had been incorporated by sonication (75 W, 10 min) using a Vibra Cell apparatus (Bioblock Scientific, Illkirch, France). Incubation medium, in which 20 µl diluted tissue homogenate was added, was composed of 10 µl Intralipid emulsion, 10 µl heat-inactivated rat serum as a source of apolipoprotein C-II (the natural activator of LPL activity), 60 µl deionized water and 100 µl incubation buffer. This buffer contained fatty acid free bovine serum albumin (120 g/l), standard heparin (0.20 g/l), 0.2 M-NaCl and 0.3 M-Tris-HCl pH 8.5. The reaction was carried out for 60 min at 25°C in a shaking water bath. The reaction was stopped by addition of 500 µl distilled water and 2 ml isopropanol – heptane – H<sub>2</sub>SO<sub>4</sub> (48:48:3:1, by vol.). Total lipids were extracted and fatty acids were separated from TAG with alkaline ethanol and heptane as previously described (Hocquette *et al.* 1998a). An aliquot of the remaining alkaline ethanol phase containing <sup>3</sup>H-labelled fatty acids was counted by liquid scintillation.

LPL activity was the mean result from three incubations and was expressed in mU/g wet tissue weight (1 mU = 1 nmol fatty acids released/min). Results were converted to mU/mg tissue protein or mU/mg tissue DNA.

#### Quantification of lipoprotein lipase mRNA levels

Total RNA were isolated using guanidium thiocyanate as previously described (Hocquette *et al.* 1996). The amount of RNA was determined by absorbance at 260 nm. All samples had a 260:280 nm absorbance ratio of approximately 2.0. RNA were stored at –20°C precipitated by 0.1 volumes 2 M-sodium acetate pH 5.0 and 2.5 volumes ethanol. RNA aliquots (40 µg) were washed by diethylpyrocarbonate-treated water (250 ml/l) and ethanol (750 ml/l), then denatured in a solution containing 2.2 M-formaldehyde and formamide (500 ml/l) by heating at 65°C for 10 min. RNA were size-fractionated by 1.5% agarose gel electrophoresis. Their integrity was assessed by ethidium bromide staining before electrophoretic transfer to Genescreen membranes (New England Life Science Products) (Hocquette *et al.* 1996).

The bovine LPL probe (Senda *et al.* 1987) was kindly provided by Dr Y. Furuichi (Nippon Research Center, Kamakura, Japan) and it was labelled by random priming with <sup>32</sup>P using the nonaprimer labelling kit. Prehybridization and hybridization of blotted RNA were performed at 42°C in solutions containing formamide (450 ml/l) as previously described (Hocquette *et al.* 1996). Hyperfilm MP (Amersham International) were exposed to the membranes for 2–15 d at –80°C with two intensifying screens. Quantification of LPL mRNA levels was performed with scanning densitometry (Hoeffler, San Francisco, CA, USA).

Membranes were also hybridized with a probe for the rat 18S ribosomal RNA, to quantify precisely the relative levels of total RNA loaded (Hocquette *et al.* 1996). Levels of LPL mRNA were expressed in arbitrary densitometric units after correction for variations in loaded RNA.

#### Statistical analyses

ANOVA of the data was done using the GLM procedure of Statistical Analysis Systems (1987, version 6; SAS Institute Inc., Cary, NC, USA). For each variable, the effects tested in the model included group (G) of calves (PR, R), calf (C) nested within groups, tissue (i.e. muscle (M) or adipose tissue (AT)) and the interaction group×tissue (G×M or G×AT). The G factor was tested against C factor within groups. The residual mean square was used as the error term for other effects. Results are expressed as means values with their standard errors ( $SEM = \sqrt{\text{residual mean squares}/\text{number of observations per treatment}}$ ). Differences among groups (PR and R) or between muscles or adipose tissues inside a group (PR or R) were tested using the Student-Newman-Keuls test with a probability of 0.05.

## Results

### Experimental conditions and animal characteristics at slaughter

Calves were bred as previously described by Hocquette *et al.* (1997). Briefly, the live body weight was similar for PR and R calves (57.3 (SEM 1.3) and 58.0 (SEM 1.2) kg respectively) at the beginning of the experiment at 18 d of age. At slaughter at 170 d of age, live body weight was significantly higher for the R calves than for the PR calves (217 (SEM 2.7) v. 202 (SEM 4.3) kg respectively,  $P < 0.05$ ). Average daily weight gain was similar between the beginning of the experiment (18-d-old) and the mid period of weaning (118-d-old) for PR and R calves (1040 (SEM 28) and 990 (SEM 32) g respectively), but significantly higher for the R calves than for the PR calves (1280 (SEM 52) v. 950 (SEM 36) g respectively,  $P < 0.001$ ) between 118 and 170 d of age and slaughter. These differences were mainly due to a high rumen content in the weaned animals as indicated by the fact that the empty body weight at slaughter did not significantly differ between the two groups of calves (195 (SEM 5.0) and 193 (SEM 2.8) kg respectively). Total net energy intake during the experimental period did not significantly differ between PR and R calves. However, the carcass weight tended to be slightly lower for the R calves than for the PR calves (114 (SEM 1.8) v. 121 (SEM 3.0) kg respectively,  $P < 0.10$ ).

No significant differences between PR and R calves were found in packed cell volume. Plasma glucose and TAG levels were also similar in both calf groups (results not shown). However, the plasma non-esterified fatty acid level was higher in R than in PR calves (+45%,  $P < 0.05$ ). The plasma insulin level tended to be lower (–23%,  $P < 0.10$ ) and plasma triiodothyronine level was significantly lower (–40%,  $P < 0.001$ ) in R calves than in PR calves (results not shown).

### Biochemical characteristics of adipose tissues

Total protein and DNA contents were higher (+30% to +120%,  $P < 0.05$ ) in SCAT than in internal adipose tissues (PAT and OAT). In adipose tissues of R calves, mean total protein content tended to be higher (+18%) and mean DNA content was higher (+35%) than in adipose tissues of PR calves ( $P < 0.05$ ), the difference being the most significant in the SCAT (+25%,  $P < 0.05$ ). Total extractable RNA amounts did not differ significantly between adipose tissue sites of both calf groups ( $P = 0.26$ ) or between the two groups of calves for all adipose tissue sites ( $P = 0.12$ ): mean values were 73.9, 61.8 and 66.8  $\mu\text{g/g}$  fresh tissue in PAT, OAT and SCAT respectively from PR calves and 61.1, 56.8, and 51.0  $\mu\text{g/g}$  fresh tissue in PAT, OAT and SCAT respectively from R calves (SEM 7.37).

TAG contents were higher (+50% to +70%) in internal adipose tissues (PAT and OAT) than in SCAT ( $P < 0.05$ , Fig. 1A). They were lower in adipose tissues of R calves ( $P = 0.01$ , Fig. 1A), especially in the visceral adipose tissues (-15% in OAT,  $P < 0.01$ , and -13% in PAT,  $P < 0.10$ ).

### Lipoprotein lipase activity and mRNA level in adipose tissues

LPL activity was higher in internal adipose tissues than in SCAT (+130% to +170%,  $P < 0.05$ , Fig. 1B). Moreover, LPL activity was more than 2-fold lower in adipose tissues of R calves ( $P < 0.03$ , Fig. 1B) which may explain, at least in part, the lower TAG content of adipose tissue from R calves (Fig. 1A). Indeed, TAG content was positively correlated with LPL activity when data of the three adipose sites were analysed together ( $r = 0.51$ ,  $P < 0.02$ ). Northern blotting revealed two major LPL mRNA species in the bovine tissues of 3.5–3.8 and 3.2–3.4 kb respectively

(Fig. 2(A)). In some samples, a third species corresponding to a smaller LPL mRNA (around 1.7 kb) was detected (results not shown) as previously described (Kirchgesner *et al.* 1987; Senda *et al.* 1987; Hocquette *et al.* 1998a). However, this species was not detected in a majority of tissues and therefore, was not quantified. LPL mRNA levels significantly differed between adipose tissue sites ( $P = 0.0001$ ). Indeed, PAT contained the highest amount of LPL mRNA in arbitrary densitometric units per g fresh tissue ( $P < 0.05$ , Fig. 1C), per mg total extractable RNA or per  $\mu\text{g}$  DNA, when compared with OAT or SCAT. LPL mRNA levels differed also significantly between PR and R calves ( $P < 0.05$ , Fig. 1C). Indeed, mean values for LPL mRNA levels were 2-fold (for PAT and OAT,  $P < 0.10$ ) or 3-fold (for SCAT,  $P < 0.05$ ) higher for calves of the PR group than for those of the R group (Fig. 1C).

### Biochemical and metabolic characteristics of muscles

Isocitrate dehydrogenase activity, a characteristic of the oxidative muscle metabolism, total protein content, total DNA content and total extractable RNA (biochemical variables) were measured in samples of heart and seven skeletal muscles. Isocitrate dehydrogenase activity was the highest in oxidative muscles (heart, *masseter*, *diaphragma*) (Hocquette *et al.* 1997). Mean values of protein content ranged from 159 to 246 mg/g fresh tissue (SEM 7.8), the lowest values being observed in the heart and the highest values in the *rectus abdominis* (Table 1). Total DNA content and total extractable RNA of tissues (Table 1) were higher in oxidative muscles than in other skeletal muscles.

Isocitrate dehydrogenase activity was significantly higher in *masseter* from R than PR calves (+71%,  $P < 0.001$ ) and lower in *longissimus thoracis* from R than PR calves (-31%,  $P < 0.01$ ). Mean DNA content was 5% lower in

**Table 1.** Total protein, DNA and RNA contents in the heart and various skeletal muscles from preruminant (PR) and ruminant (R) calves† (Mean values with their standard errors for seven calves per group)

Biochemical variables...	Total protein (mg/g fresh tissue)		DNA ( $\mu\text{g/g}$ fresh tissue)		Total extractable RNA ( $\mu\text{g/g}$ fresh tissue)	
	PR	R	PR	R	PR	R
Calf group...						
H	169 <sup>B</sup>	159 <sup>c†</sup>	1978 <sup>A</sup>	1900 <sup>a</sup>	608 <sup>A</sup>	606 <sup>a</sup>
MA	184 <sup>B</sup>	178 <sup>c</sup>	1433 <sup>B</sup>	1423 <sup>b</sup>	363 <sup>B</sup>	447 <sup>b*</sup>
D	170 <sup>B</sup>	170 <sup>c</sup>	1445 <sup>B</sup>	1367 <sup>b</sup>	388 <sup>B</sup>	439 <sup>b</sup>
RA	231 <sup>A</sup>	246 <sup>a</sup>	1097 <sup>C</sup>	1049 <sup>c†</sup>	301 <sup>B</sup>	248 <sup>c</sup>
TFL	217 <sup>A</sup>	220 <sup>b</sup>	1108 <sup>C</sup>	1080 <sup>c</sup>	254 <sup>B</sup>	232 <sup>c</sup>
ST	179 <sup>B</sup>	167 <sup>c</sup>	1145 <sup>C</sup>	1073 <sup>c</sup>	218 <sup>B</sup>	251 <sup>c†</sup>
CT	223 <sup>A</sup>	226 <sup>b</sup>	1168 <sup>C</sup>	1115 <sup>c</sup>	302 <sup>B</sup>	250 <sup>c*</sup>
LT	192 <sup>B</sup>	174 <sup>c</sup>	924 <sup>D</sup>	871 <sup>d</sup>	303 <sup>B</sup>	282 <sup>c</sup>
SEM	7.8		36.9		40.2	
Statistical significance of effect of ( $P <$ ):§						
G	0.44		0.05		0.91	
C	0.35		0.10		0.71	
M	0.0001		0.0001		0.0001	
G × M	0.48		0.98		0.62	

H, heart; MA, *masseter*; D, *diaphragma*; RA, *rectus abdominis*; TFL, *tensor fasciae latae*; ST, *semitendinosus*; CT, *cutaneous trunci*; LT, *longissimus thoracis*.

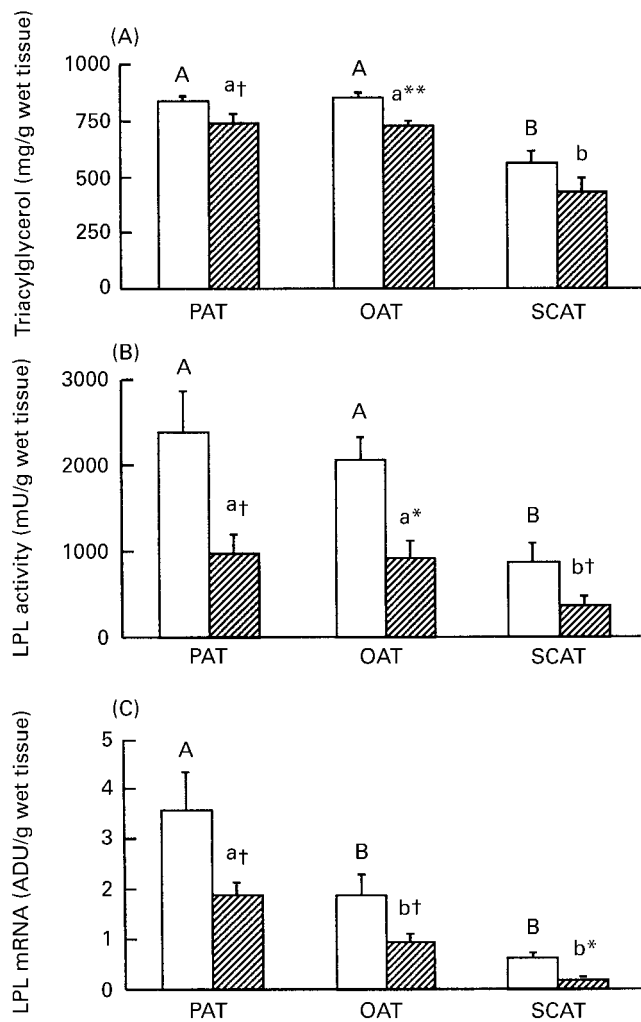
A, B, C, D, a, b, c, d Mean values within a row with unlike superscript letters were significantly different: ( $P < 0.05$ ).

Mean values were significantly different from those for PR calves: \*  $P < 0.05$ .

† For details of animals and procedures, see p. 434.

Mean values tended to be significantly different from those of R calves: †  $P < 0.10$ .

§ G, C, M, significant effects of group (G) tested against calves within group, of calf (C), of muscles (M) and of the group × muscle interaction (G × M).



**Fig. 1.** (A) Triacylglycerol (TAG) content, (B) lipoprotein lipase (LPL) activity and (C) LPL mRNA level in adipose tissues from pre-ruminant (□) and ruminant (▨) calves. PAT, perirenal adipose tissue; OAT, omental adipose tissue; SCAT, subcutaneous adipose tissue; ADU, arbitrary densitometric units. For details of diets and procedures, see pp. 434–435. Values are means for four to seven calves per group with their standard errors represented by vertical bars. <sup>A,B,a,b</sup>Mean values within a group (pre-ruminant or ruminant) with unlike superscript letters were significantly different ( $P < 0.05$ ). Mean values were significantly different from those of pre-ruminant calves: \*  $P < 0.05$ , \*\*  $P < 0.01$ . Mean values tended to be significantly different from those of pre-ruminant calves: †  $P < 0.10$ . The statistical significances ( $P$ -) of effect of group tested against calves within group, of calf, of adipose tissue and of the group  $\times$  adipose tissue interaction were: 0.01, 0.14, 0.0001 and 0.95 respectively for TAG content; 0.01, 0.18, 0.002 and 0.19 respectively for LPL activity; and 0.04, 0.11, 0.0001 and 0.25 respectively for LPL mRNA level.

muscles from R than PR calves ( $P < 0.05$ , Table 1). Total extractable RNA was significantly higher in *masseter* (+23%,  $P < 0.05$ ) and lower in *cutaneus trunci* (−17%,  $P < 0.05$ ) for R than for PR calves (Table 1). No significant differences in tissue protein content were found between PR and R calves (Table 1). Similarly, no significant differences in TAG, phospholipids and total cholesterol were found in *rectus abdominis* muscle between PR and R calves (results not shown).

#### Lipoprotein lipase activity and lipoprotein lipase mRNA level in muscles

LPL activity was higher in the heart and in *masseter* (the most oxidative muscles) than in other skeletal muscles (Fig. 3A). LPL activity was higher in *masseter* from R than PR calves (+50%,  $P < 0.01$ ). Inversely, LPL activity tended to be lower in the heart from the R compared with the PR calves (Fig. 3A).

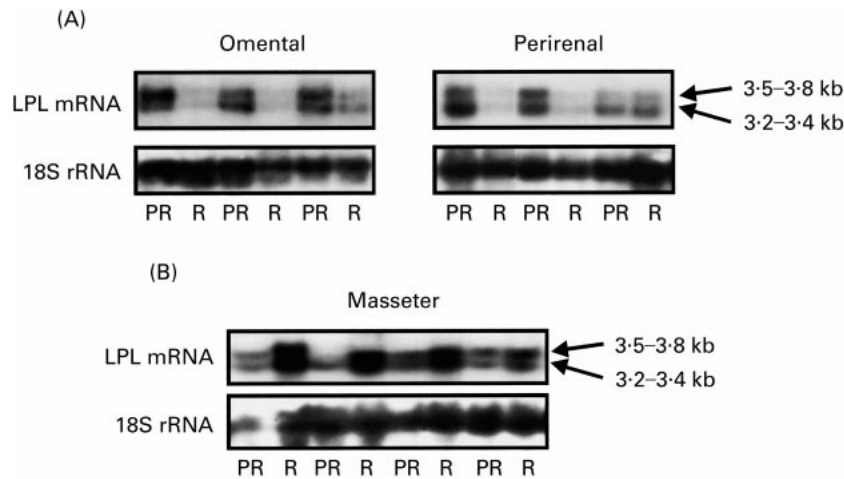
As for adipose tissues, Northern blot analysis revealed two major LPL mRNA species in muscles (Fig. 2B). As for LPL activity, LPL mRNA levels (Fig. 3B) significantly differed among muscles ( $P = 0.0001$ ). Indeed, LPL mRNA level in the heart was 10–>100-fold higher than in the studied skeletal muscles ( $P < 0.05$ ). No effect of weaning could be observed in levels of LPL mRNA from muscles except in *masseter*, of which the mean value was higher (+120%,  $P = 0.01$ ) for the R calves compared with the PR calves (Figs. 2B and 3B). The differences between muscles and between the two groups of calves were similar when values were expressed per  $\mu\text{g}$  total RNA or per  $\mu\text{g}$  DNA (results not shown).

#### Discussion

In ruminant animals at weaning, the liquid milk diet is replaced by solid foods based on cereals and hay. Studies on physiological consequences of this nutritional change have been focused on the development of ruminal fermentation, but to a limited extent on lipid metabolism in spite of its importance for the growth of the young calf (Hocquette & Bauchart, 1999). Thus, consequences of weaning on LPL activity and gene expression were investigated in the present study, since LPL is considered as one of the major rate-limiting steps of energy delivery from lipoproteins to tissues and of partitioning of fat between their site of storage (adipose tissues) and their site of oxidation (muscles) (Olivecrona *et al.* 1991; Pethick & Dunshea, 1993). The major result of this study is that LPL activity and expression were markedly reduced 63 d after the onset of weaning of the calf in adipose tissues but not in muscles.

#### Lipoprotein lipase activity and gene expression in adipose tissues

Adipose tissues are able to take up circulating fatty acids and to store them as TAG. One of the main sources of plasma fatty acids results from hydrolysis of the circulating TAG by LPL (Bauchart, 1993). Levels of LPL activity and gene expression are the highest in adipose tissues compared with other tissues in bovine animals (Hocquette *et al.* 1998a; present study), human subjects (Wion *et al.* 1987), rats (Semenkovich *et al.* 1989a), mice (Kirchgessner *et al.* 1987), guinea pigs (Braun & Severson, 1992) or chickens (Cooper *et al.* 1989). Moreover, in the pre-ruminant calf (Hocquette *et al.* 1998a) and in the adult cow (Chilliard & Robelin, 1985), LPL activity differs among adipose tissue sites, which could reflect differences in metabolic rates. Indeed, visceral adipose tissues (PAT and OAT) have a higher LPL activity than SCAT which could explain, at least in part, their higher TAG contents. These differences are



**Fig. 2.** Northern blot analysis of lipoprotein lipase (LPL) mRNA in (A) omental and perirenal adipose tissues and (B) *masseter* muscle from preruminant (R) or ruminant (R) calves. Total RNA was extracted from adipose tissue samples. RNA was then size-fractionated by 1.5% agarose gel electrophoresis and electrotransferred on a Genescreen membrane (New England Nuclear Life Science Products, Boston, MA, USA). The bovine LPL probe was labelled by random priming and hybridized before exposition to Hyperfilms MP (Amersham International, Amersham, Bucks, UK) with intensifying screens. Membranes were also hybridized with a probe for the rat 18S ribosomal RNA to take into account possible variations in the quantities of RNA loaded onto the gels. For further details of procedures, see p. 435.

partly associated with similar differences in LPL mRNA levels suggesting a pre-translational regulation of LPL expression.

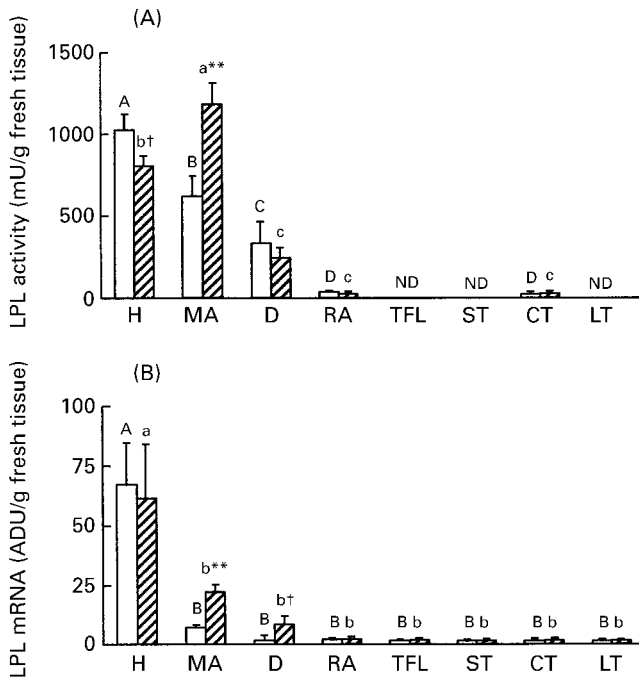
A novel observation in the present study is that, in spite of similar net energy intakes and empty body weight gains, LPL activity and gene expression were reduced after weaning in calf adipose tissues but not in muscles, except in *masseter*. The decrease in LPL activity and gene expression in adipose tissues may explain, at least in part, their lower TAG content in R compared with PR calves. However, a decrease in glucose transporter expression (Hocquette *et al.* 1997) may also contribute to the lower TAG content in adipose tissues after weaning. In addition, lipogenesis in adipose tissues results mainly from long-chain fatty acid uptake in PR calves, and from *de novo* synthesis from acetate in R calves. Whatever the molecular mechanisms, our results indicate that the proportion of dietary energy which was stored as TAG in adipose tissues was lower in R calves than in PR calves. This may result, at least in part, from the lower availability of energy for lipid accretion, due to the higher energy utilisation by the digestive tract in R calves because of its development, its metabolic rate and the greater work of digestion.

A differential regulation of LPL between adipose and muscle tissues has already been described in the literature. For instance, fasting or underfeeding markedly reduce LPL activity in adipose tissues in both single-stomached and ruminant animal species, whereas variable changes or no changes in LPL activity were observed in muscles in similar nutritional conditions (for review, see Faulconnier *et al.* 1999). However, in the present study, variations in LPL activity and gene expression in adipose tissues may be explained by qualitative or quantitative dietary changes related to weaning.

Although net energy intake from birth onwards was carefully matched between the two groups of calves, energy partitioning between tissues probably differed between PR and R calves due to the development of the activity of the digestive tract after weaning. The consequences may be a lower level of available energy for fat accretion in adipose tissue of R calves, which may be linked to a lower LPL activity in this tissue as discussed later.

Another physiological factor to take into account is the kinetics of nutrient absorption, and availability of nutrients 16 h after the last meal in the present study. In PR calves, total intestinal chylomicron and VLDL production reaches a maximum 8 h after intake (for review, see Hocquette & Bauchart, 1999) and TAG plasma concentration in the portal vein is still high 16 h after intake (Durand & Bauchart, 1986). For R calves, absorption of nutrients is more regular and anyway, at a high level, 16 h after the last meal, due to the slow process of digestion of food by the micro-organisms present in the rumen (van Houtert, 1993). Thus, in this experiment, PR and especially R calves, were unlikely to be either in a fasting state or in a postprandial state, suggesting a limited influence of the time spent after the last meal.

Weaning is characterized in the calf by a decreased dietary supply of carbohydrates and fat, and an increased supply of volatile fatty acids and ketone bodies produced by the fermentation of food in the rumen. Among these nutrients, it can be speculated, from data available in other species, that the decrease in the absorbed fat may be responsible for the decrease in LPL expression (Jump & Clark, 1999). Indeed, dietary fats were shown to modulate the expression of lipid-related genes in adipose cells (Ailhaud *et al.* 1996), such as the LPL gene (Auwerx *et al.* 1996). Activators of peroxisome proliferator-activated



**Fig. 3.** (A) Lipoprotein lipase (LPL) activity, and (B) LPL mRNA level in heart and various skeletal muscles from pruruminant (□) and ruminant (▨) calves. H, heart; MA, *masseter*; D, *diaphragma*; RA, *rectus abdominis*; LT, *longissimus thoracis*; ST, *semitendinosus*; TFL, *tensor fasciae latae*; CT, *cutaneous trunci*; ADU, arbitrary densitometric units; ND, not detectable. For details of diets and procedures, see pp. 434–435. Values are means for seven animals per group (except for LPL activity in RA and CT, for which it was three or four animals per group) with their standard errors represented by vertical bars. A,B,C,D,a,b,c,d Mean values within a group (pruruminant or ruminant) with unlike superscript letters were significantly different ( $P < 0.05$ ). Mean values were significantly different from those of pruruminant calves: \*\*  $P < 0.05$ . Mean values tended to be significantly different from those of pruruminant calves: †  $P < 0.10$ . The statistical significance ( $P=$ ) of effect of group tested against calves within group, of calf, of adipose tissue and of the group $\times$ adipose tissue interaction were 0.78, 0.0002, 0.0001 and 0.0001 respectively for LPL activity and 0.64, 0.43, 0.0001 and 0.93 respectively for LPL mRNA level.

receptors  $\gamma$  were indeed shown to direct a transcriptional response via a peroxisome proliferator-activated responsive element present in the LPL gene (Schoonjans *et al.* 1996). However, the regulation of LPL activity and expression by fatty acids is complex since it involves transcriptional and post-transcriptional mechanisms (Amri *et al.* 1996). Nevertheless, the direct regulation of LPL by fatty acids in cattle suggested in our present *in vivo* study needs to be fully analysed by *in vitro* approaches using bovine adipose tissue explants.

As previously described by Hugi & Blum (1997), insulinaemia tended to be higher in PR calves fed a lactose-rich milk substitute than in R calves fed a solid-based diet. Insulin is well known to stimulate LPL activity (Braun & Severson, 1992) by increasing LPL mRNA levels (Ong *et al.* 1988), especially by post-translational mechanisms (Semenkovich *et al.* 1989b; Reynolds *et al.* 1990). In the present study, the greater insulinaemia in PR calves may have stimulated LPL activity in adipose tissues by one of these mechanisms.

As previously described (Hugi & Blum, 1997), plasma triiodothyronine was lower in R calves than in PR calves. This decrease in plasma concentrations of thyroid hormones induced by weaning may regulate LPL activity in adipose tissue of R calves. Indeed, it was shown that heparin-elutable LPL was lower in subcutaneous adipose tissue from hypothyroid patients than from control human subjects, but total LPL activity was similar between the two groups (Pykalisto *et al.* 1976). Moreover, adipocytes from hypothyroid rats were shown to exhibit higher LPL activity and protein content than those from control rats (Kern *et al.* 1996).

Finally, in our present study, decrease in dietary glucose supply at weaning could down-regulate other genes coding for enzymes involved in lipid metabolism such as fatty acid synthase (for review, see Ferré, 1999) or hormone-sensitive lipase (Raclot *et al.* 1998). This might explain why differences in LPL activity between PR and R calves (+130% on average) did not match differences in TAG contents within adipose tissues (+20% on average). However, regulation of gene expression by glucose remains to be analysed further in ruminant animals.

#### *Lipoprotein lipase activity and gene expression in muscles*

LPL activity and gene expression have been quantified in the heart and in several skeletal muscles representing a panel of oxidative, glycolytic or mixed muscles. As previously observed (Hocquette *et al.* 1998a), LPL activity was higher in oxidative than in glycolytic muscles. Indeed, the ability of a muscle to hydrolyse plasma TAG by LPL would be, at least in part, co-ordinated with the ability of the muscle to completely oxidize the fatty acids within mitochondria. In the present study, the large differences in LPL activity between muscles were also observed for LPL mRNA levels. This suggests a major transcriptional regulation of the LPL gene, which does not exclude other mechanisms, such as intracellular activation (by dimerisation and glycosylation) and turnover (steady-state between synthesis, secretion into the extracellular medium or catabolism by the lysosomal pathway) of LPL (Braun & Severson, 1992; Kern *et al.* 1996).

Although fatty acids were shown to up-regulate transcription of genes coding for proteins involved in fat metabolism in myocytes (Van der Lee *et al.* 2000), our present *in vivo* experiment indicates that the reduction in dietary fat supply at weaning did not significantly affect LPL activity or expression in heart and most muscles in the calf. The significant increase in LPL activity and gene expression was only noted in *masseter* in R compared with PR calves. This change, associated with an increase in isocitrate dehydrogenase activity, confirms the positive metabolic relationships between the ability of a given muscle to take up fatty acids (a process controlled in part by LPL activity) and its ability to oxidize them by the Krebs cycle (assessed by isocitrate dehydrogenase activity). *Masseter*, located in the cheek, is increasingly active after weaning because it is strongly involved in chewing of solid feeds. This muscle-specific physiological change induces a late conversion of some type IIC muscular fibres into type I fibres, as an adaptive response to forage intake and

rumination (Picard *et al.* 1996). Thus, our results confirm the idea that local contractile activity is able to induce an increase in LPL expression (Hamilton *et al.* 1998).

In conclusion, our present study demonstrates that weaning reduces calf LPL activity in various adipose tissues, which may contribute to their lower TAG contents after weaning. The effects of weaning on LPL activity in the muscle tissues were not significant except in the *masseter*, probably because of its contractile activity for solid food chewing. All these results indicate an *in vivo* nutritional and/or hormonal regulation of LPL activity and expression in adipose tissues, but not in muscles.

### Acknowledgements

We thank Nicole Guivier for her skilled technical assistance, Robert Jailler, Sylvie Rudel and their group for the management of the animals, and Roland Jailler and his group for the management of the slaughterhouse.

### References

- Ailhaud G, Amri EZ & Grimaldi PA (1996) Fatty acids and expression of lipid-related genes in adipose cells. *Proceedings of the Nutrition Society* **55**, 151–154.
- Amri EZ, Teboul L, Vannier C, Grimaldi PA & Ailhaud G (1996) Fatty acids regulate the expression of lipoprotein lipase gene and activity in preadipose and adipose cells. *Biochemical Journal* **314**, 541–546.
- Auwerx J, Schoonjans K, Fruchart JC & Staels B (1996) Transcriptional control of triglyceride metabolism: fibrates and fatty acids change the expression of the LPL and apo C-III genes by activating the nuclear receptor PPAR. *Atherosclerosis* **124**, Suppl., S29–S37.
- Bauchart D (1993) Lipid absorption and transport in ruminants. *Journal of Dairy Science* **76**, 3864–3881.
- Bonnet M, Faulconnier Y, Fléchet J, Hocquette JF, Leroux C, Langin D, Martin P & Chilliard Y (1998) Messenger RNAs encoding lipoprotein lipase, fatty acid synthase and hormone-sensitive lipase in the adipose tissue of underfed-refed ewes and cows. *Reproduction Nutrition and Development* **38**, 297–307.
- Braun JEA & Severson DL (1992) Regulation of synthesis, processing and translocation of lipoprotein lipase. *Biochemical Journal* **287**, 337–347.
- Chilliard Y & Robelin J (1985) Activité lipoprotéine-lipase de différents dépôts adipeux et ses relations avec la taille des adipocytes chez la vache tarie en cours d'engraissement ou en début de lactation (Lipoprotein lipase activity in different adipose tissues and relations with the adipocyte size in the dry cow during fattening or at the beginning of the lactation). *Reproduction Nutrition Développement* **25**, 287–293.
- Cooper DA, Stein JC, Strielemann PJ & Bensadoun A (1989) Avian adipose lipoprotein lipase: cDNA sequence and reciprocal regulation of mRNA levels in adipose and heart. *Biochimica et Biophysica Acta* **1008**, 92–101.
- Durand D & Bauchart D (1986) Variations nyctémérales de la lipémie et de la glycémie au niveau des voies afférentes et efférentes du foie chez le veau préruminant (Nycthemeral variations in lipaemia and glycaemia in afferent and efferent hepatic vessels in the preruminant calf). *Reproduction Nutrition Développement* **26**, 371–372.
- Faulconnier Y, Bonnet M, Bocquier F, Leroux C, Hocquette JF, Martin P & Chilliard Y (1999) Régulation du métabolisme lipidique des tissus adipeux et musculaires chez le ruminant. Effet du niveau alimentaire et de la photopériode (Regulation of lipid metabolism of adipose tissue and muscle in ruminants. Effects of feeding level and photoperiod). *INRA Productions Animales* **12**, 287–300.
- Ferré P (1999) Regulation of gene expression by glucose. *Proceedings of the Nutrition Society* **58**, 621–623.
- Girard J, Ferré P, Pégurier JP & Duée PH (1992) Adaptations of glucose and fatty acid metabolism during perinatal period and suckling-weaning transition. *Physiological Reviews* **72**, 507–562.
- Hamilton MT, Etienne J, McClure W, Pavey BS & Holloway AK (1998) Role of local contractile activity and muscle fiber type on LPL regulation during exercise. *American Journal of Physiology* **275**, E1016–E1022.
- Hocquette JF & Bauchart D (1999) Intestinal absorption, blood transport and hepatic and muscle metabolism of fatty acids in preruminant and ruminant animals. *Reproduction Nutrition Développement* **39**, 27–48.
- Hocquette JF, Castiglia-Delavaud C, Graulet B, Ferré P, Picard B & Vermorel M (1997) Weaning marginally affects glucose transporter (GLUT4) expression in calf muscles and adipose tissues. *British Journal of Nutrition* **78**, 251–271.
- Hocquette JF, Graulet B, Castiglia-Delavaud C, Bornes F, Lepetit N & Ferré P (1996) Insulin-sensitive glucose transporter transcript levels in calf muscles assessed with a bovine GLUT4 cDNA fragment. *International Journal of Biochemistry and Cell Biology* **28**, 795–806.
- Hocquette JF, Graulet B & Olivecrona T (1998a) Lipoprotein lipase activity and mRNA levels in bovine tissues. *Comparative Biochemistry and Physiology B* **121**, 201–212.
- Hocquette JF, Ortigues-Marty I, Pethick DW, Herpin P & Fernandez X (1998b) Nutritional and hormonal regulation of energy metabolism in skeletal muscles of meat-producing animals. *Livestock Production Science* **56**, 115–143.
- Hugi D & Blum JW (1997) Changes of blood metabolites and hormones in breeding calves associated with weaning. *Journal of Veterinary Medicine - Series A* **44**, 99–108.
- Jarrige R (1989) *Ruminant Nutrition Recommended Allowances and Feed Tables*, Paris: Institut National de la Recherche Agronomique, pp. 389.
- Jump DB & Clarke SD (1999) Regulation of gene expression by dietary fat. *Annual Review of Nutrition* **19**, 63–90.
- Kauffman RG, Habel RE, Smulders FJM, Hartman W & Bergstrom PL (1990) Recommended terminology for the muscle commonly designated longissimus dorsi. *Meat Science* **28**, 259–265.
- Kern PA, Ranganathan G, Yukht A, Ong JM & Davis RC (1996) Translational regulation of lipoprotein lipase by thyroid hormone is via a cytoplasmic repressor that interacts with the 3' untranslated region. *Journal of Lipid Research* **37**, 2332–2340.
- Kirchgessner TG, Svenson KL, Lusi AJ & Schotz MC (1987) The sequence of cDNA encoding lipoprotein lipase. A member of a lipase gene family. *Journal of Biological Chemistry* **262**, 8463–8466.
- Kouame KG, Troccon JL, Patureau-Mirand P, Journet M & Pion R (1984) Nutrition des veaux au cours du sevrage. I Evolution de la consommation d'aliments et des concentrations sanguines de divers métabolites énergétiques (Calf nutrition during the weaning period. I. Variations in feed intake and blood levels of energetic metabolites). *Annales de Zootechnie* **33**, 427–444.
- Olivecrona T, Bengtsson-Olivecrona G, Chajek-Shaul T, Carpendier Y, Deckelbaum R, Hultin M, Peterson J, Patsch J & Vilaro S (1991) Lipoprotein lipase. Sites of synthesis and sites of action. *Atherosclerosis Review* **22**, 21–25.
- Ong JM, Kirchgessner TG, Schotz MC & Kern PA (1988) Insulin increases the synthetic rate and messenger RNA level of



- lipoprotein lipase in isolated rat adipocytes. *Journal of Biological Chemistry* **263**, 12933–12938.
- Pearce J & Unsworth EF (1980) The effects of diet on some hepatic enzyme activities in the pre-ruminant and ruminating calf. *Journal of Nutrition* **110**, 255–261.
- Pethick DW & Dunshea FR (1993) Fat metabolism and turnover. In *Quantitative Aspects of Ruminants Digestion and Metabolism*, pp. 291–311 [JM Forbes and J France, editors]. Wallingford: CAB International.
- Picard B, Gagnière H & Geay Y (1996) Contractile differentiation of bovine masseter muscle. *Basic and Applied Myology* **6**, 361–372.
- Pykalisto O, Goldberg AP & Brunzell JD (1976) Reversal of decreased human adipose tissue lipoprotein lipase and hypertriglyceridemia after treatment of hypothyroidism. *Journal of Clinical Endocrinology and Metabolism* **43**, 591–600.
- Quigley JD, Caldwell LA, Sinks GD & Heitmann RN (1991a) Changes in blood glucose, nonesterified fatty acids, and ketones in response to weaning and feed intake in young calves. *Journal of Dairy Science* **74**, 250–257.
- Quigley JD, Smith ZP & Heitmann RN (1991b) Changes in plasma volatile fatty acids in response to weaning and feed intake in young calves. *Journal of Dairy Science* **74**, 258–263.
- Raclot T, Dazats M & Langin D (1998) Regulation of hormone-sensitive lipase expression by glucose in 3T3-F442A adipocytes. *Biochemical and Biophysical Research Communications* **245**, 510–513.
- Raynolds MV, Awald PD, Gordon DF, Gutierrez-Hartmann A, Rule DC, Wood WM & Eckel RH (1990) Lipoprotein lipase gene expression in rat adipocytes is regulated by isoproterenol and insulin through different mechanisms. *Molecular Endocrinology* **4**, 1416–1422.
- Schoonjans K, Peinado-Onsurbe J, Lefebvre AM, Heyman RA, Briggs M, Deeb S, Staels B & Auwerk J (1996) PPAR $\alpha$  and PPAR $\gamma$  activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO Journal* **15**, 5336–5348.
- Semenkovich CF, Chen SH, Wims M, Luo CC, Li WH & Chan L (1989a) Lipoprotein lipase and hepatic lipase mRNA tissue specific expression, developmental regulation and evolution. *Journal of Lipid Research* **30**, 423–431.
- Semenkovich CF, Wims M, Noe L, Etienne J & Chan L (1989b) Insulin regulation of lipoprotein lipase activity in 3T3-L1 adipocytes is mediated at posttranscriptional and posttranslational levels. *Journal of Biological Chemistry* **264**, 9030–9038.
- Senda M, Oka K, Brown WV, Qasba PK & Furuichi Y (1987) Molecular cloning and sequence of a cDNA coding for bovine lipoprotein lipase. *Proceedings of the National Academy of Sciences, USA* **84**, 4369–4373.
- Van der Lee KAJM, Vork MM, De Vries JE, Willemsen PHM, Glatz JFC, Reneman RS, Van der Vusse GJ & Van Bilsen M (2000) Long-chain fatty acid-induced changes in gene expression in neonatal cardiac myocytes. *Journal of Lipid Research* **41**, 41–47.
- van Houtert MFJ (1993) The production and metabolism of volatile fatty acids by ruminants fed roughages: A review. *Animal Feed Science and Technology* **43**, 189–225.
- Vermorel M, Bouvier JC, Thivend P & Toullec R (1974) Utilisation énergétique des aliments d'allaitement par le veau pré-ruminant à l'engrais à différents poids (Energy utilization of liquid milk replacers by veal calves). In *Proceedings of the 6th Symposium on Energy Metabolism, Stuttgart (Germany)*, EAAP Publication no. 14, pp. 143–146 [KH Menke, H-J Lantzh and JR Reichl, editors]. Universität Hohenheim, Dokumentationsstelle, Stuttgart, Germany: EAAP.
- Wion KL, Kirchgessner TG, Lusi AJ, Schotz MC & Lawn RM (1987) Human lipoprotein lipase complementary DNA sequence. *Science* **235**, 1638–1641.