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 ($257 \%$, $P < 0.01$). LPL mRNA levels were also lower in R calves ($248 \%$ to $268 \%$, $P < 0.01$) 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 triglycerides (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

$[^3]H]$Triolein (1.85–2.96 GBq/mmol) and Hyperfilms MP were supplied by Amersham International (Amersham, Bucks., UK). [α-${}^{32}$P]deoxy-cytidine-5'-triphosphate ($>111$ TBq/mmol) and [γ-${}^{32}$P]deoxy-adenosine-s'-triphosphate ($>111$ TBq/mmol) were purchased from ICN Biochemicals (Irvine, CA, USA). Guanidine 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. Life Science Products (Boston, MA, USA). T4 polynucleotide kinase was supplied by New England Biolabs Inc. Life Science Products (Boston, MA, USA). T4 polynucleotide kinase was supplied by New England Biolabs Inc. Life Science Products (Boston, MA, USA). T4 polynucleotide kinase was supplied by New England Biolabs Inc. Life Science Products (Boston, MA, 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, ruminant (R) 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, metabolizibility 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, cutaneous 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$_2$ 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$_2$ and stored at −80°C for subsequent analysis (Hocquette et al. 1997). Frozen samples were pulverized in liquid N$_2$ 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 [pepsstatin A (1 μg/ml), leupeptin (10 μg/ml) and aprotonin (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 [3H]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 – H2SO4 (48:48:3, 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 3H-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 vol. of isopropanol and then dissolved in 30 μl distilled water. The RNA integrity was assessed by ethidium bromide staining and quantified by absorbance at 260 nm. All samples were size-fractionated by 1.8% agarose gel electrophoresis. The RNA was transferred to a nitrocellulose membrane and hybridized with a probe. The membranes 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 (Hoeffer, 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 = s/residual mean square/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 μg/g fresh tissue in PAT, OAT and SCAT respectively from PR calves and 61.1, 56.8, and 51.0 μg/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 (Kirchgessner 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 μg 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 (μg/g fresh tissue) | Total extractable RNA (μg/g fresh tissue) |
| Calf group... | PR | R | PR | R | PR | R |
| H | 169b | 159d | 1978a | 1900a | 608a | 606a |
| MA | 184b | 178c | 1433b | 1423b | 363b | 447b |
| D | 170b | 170c | 1445b | 1367b | 388b | 439b |
| RA | 231b | 246a | 1097c | 1049d | 301b | 248c |
| TFL | 217b | 220b | 1108c | 1080c | 254c | 232c |
| ST | 179b | 167c | 1145b | 1073c | 218b | 251c |
| CT | 223b | 226b | 1168c | 1115c | 302b | 250c |
| LT | 192b | 174c | 924b | 871d | 303b | 282c |
| 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.

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

‡ Significantly different from results for PR calves: †P < 0.05.

§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).
Lipoprotein lipase in calves

LPL 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 \( 10^{-3} \)-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 \( \mu g \) total RNA or per \( \mu g \) 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 & Dunseha, 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 lipoprotein lipase mRNA level 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 (Kirchgesner et al. 1987), guinea pigs (Braun & Severson, 1992) or chickens (Cooper et al. 1989). Moreover, in the preruminant calf (Hocquette et al. 1998a) and in the adult cow (Chiliard & 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...
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
In vitro suggested in our present nevertheless, the direct regulation of LPL by fatty acids in cattle post-transcriptional mechanisms (Amri et al. 1996). Fatty acids is complex since it involves transcriptional and regulatory elements present in the LPL gene (Schoonjans et al. 1996). Never-diacylglycerol receptors γ 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), insulin-induced conversion of some type IIC muscular fibres into type I fibres, as an adaptive response to forage intake and

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. 1998), 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

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.

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.
Ong JM, Kirchgessner TG, Schotz MC & Kern PA (1988) Insulin increases the synthetic rate and messenger RNA level of...


