Expression and Nutritional Regulation of Lipogenic Genes in the Ruminant Lactating Mammary Gland

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Abstract The effect of nutrition on milk fat yield and composition has largely been investigated in cows and goats, with some differences for fatty acid (FA) composition responses and marked species differences in milk fat yield response. Recently, the characterization of lipogenic genes in ruminant species allowed *in vivo* studies focused on the effect of nutrition on mammary expression of these genes, in cows (mainly fed milk fat-depressing diets) and goats (fed lipid-supplemented diets). These few studies demonstrated some similarities in the regulation of gene expression between the two species, although the responses were not always in agreement with milk FA secretion responses. A central role for *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA as regulators of milk fat synthesis has been proposed. However, *trans*-10 C18:1 does not directly control milk fat synthesis in cows, despite the fact that it largely responds to dietary factors, with its concentration being negatively correlated with milk fat yield response in cows and, to a lesser extent, in goats. Milk *trans*-10, *cis*-12 CLA is often correlated with milk fat depression in cows but not in goats and, when postruminally infused, acts as an inhibitor of the expression of key lipogenic genes in cows. Recent evidence has also proven the inhibitory effect of the *trans*-9, *cis*-11 CLA isomer. The molecular mechanisms by which nutrients regulate lipogenic gene expression have yet to be well identified, but a central role for SREBP-1 has been outlined as mediator of FA effects, whereas the roles of PPARs and STAT5 need to be determined. It is expected that the development of *in vitro* functional systems for lipid synthesis and secretion will allow future progress toward (1) the identification of the inhibitors and activators of fat synthesis, (2) the knowledge of cellular mechanisms, and (3) the understanding of differences between ruminant species.

Keywords: nutrition · gene expression · lipogenesis · mammary gland · lactating ruminant

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Z. Bösze (ed.), *Bioactive Components of Milk*

Introduction

In ruminants, the major constituents of milk (lipids, proteins, carbohydrates, and salts) and their concentrations are linked to intrinsic or extrinsic (nutritional and environmental) factors (Coleman et al., 2000). Among these, the genetic factor, through the animal species, and the nutritional factor are the two major factors determining milk composition. Indeed, nutrition has a considerable effect on the composition of the lipids (Jensen, 2002), conversely to the protein fraction, which generally is only marginally affected by this factor (Coulon et al., 2001). Moreover, milk fat is an important component of the nutritional quality of dairy products, with the saturated fatty acids (FA) (mainly C12, C14, and C16) commonly considered to have a negative effect on human health when consumed in excess (Williams, 2000), whereas other FA, such as oleic and linolenic acids, have positive effects by a direct vascular antiatherogenic action (Massaro et al., 1999). Besides this, *cis*-9, *trans*-11 C18:2, the major conjugated linoleic acid (CLA) isomer found in ruminant products including milk, was shown both in animals studies and *in vitro* experiments to exert a number of advantageous physiological effects (Pariza et al., 2001). In addition, milk fat content and composition is one of the most important components of the technological and sensorial qualities of dairy products. Thus, modification of milk fat content and FA composition by dietary manipulation has been investigated in cows and goats, with particular attention on the effects of fat supplementation of the diet.

In bovines, the consequences of lipid supplementation on the milk yield and fat and protein contents have been well described, with an increase in milk production (for most lipid supplements) and a slight but systematic reduction in the protein and casein contents. In dairy cows, due to important interactions between dietary forages and concentrates and their components (fibers, starch, lipids), the supplements given do not all have the same efficiency regarding fat content modulation. Thus, concentrate-rich diets, concentrate-rich diets supplemented with vegetable oils, and diets supplemented with fish oil lead to a decrease in fat content, while encapsulated lipids lead to a large increase in fat content (Chilliard & Ferlay, 2004; Palmquist et al., 1993). Conversely, in goats almost all types of lipid supplements induce a marked increase in the milk fat content without systematic modification of milk production or protein content (Chilliard et al., 2003a). These modifications in the yield of fat are observed together with an important modification of milk FA composition, which is well documented both in cows (Chilliard et al., 2000, 2001; Palmquist et al., 1993) and in goats (Chilliard et al., 2003a, 2006a).

The mechanisms underlying these intra- and inter-species-specific responses are not yet well understood. Nevertheless, recently, thanks to the characterization of the lipogenic genes involved in milk synthesis and secretion and the development of molecular biology tools, few studies have been undertaken to relate the effects of diet on the milk FA profile to mammary gland lipid metabolism. These studies mainly considered lipogenic genes, in particular genes for the enzymes involved in the uptake, *de novo* synthesis, desaturation, and esterification of FA

in order to relate the effects of the diet on the abundance of their transcripts and/or enzymatic activities. This chapter reviews the present knowledge on the main lipogenic genes, in particular the known effects of nutritional factors, especially those of fat supplementation, on ruminant mammary lipogenic gene expression, together with milk fat content and FA composition. In addition, we present the putative molecular mechanisms underlying these regulations.

Milk Fatty Acid Origin

Milk fat is composed of ca. 98% triglycerides, of which ca. 95% is FA and less than 1% is phospholipids, with small amounts of cholesterol, 1,2-diacylglycerol, monoacylglycerol, and free FA. Milk FA have a dual origin: (1) They are either *de novo* synthesized in the mammary gland (Fig.1) from acetate and

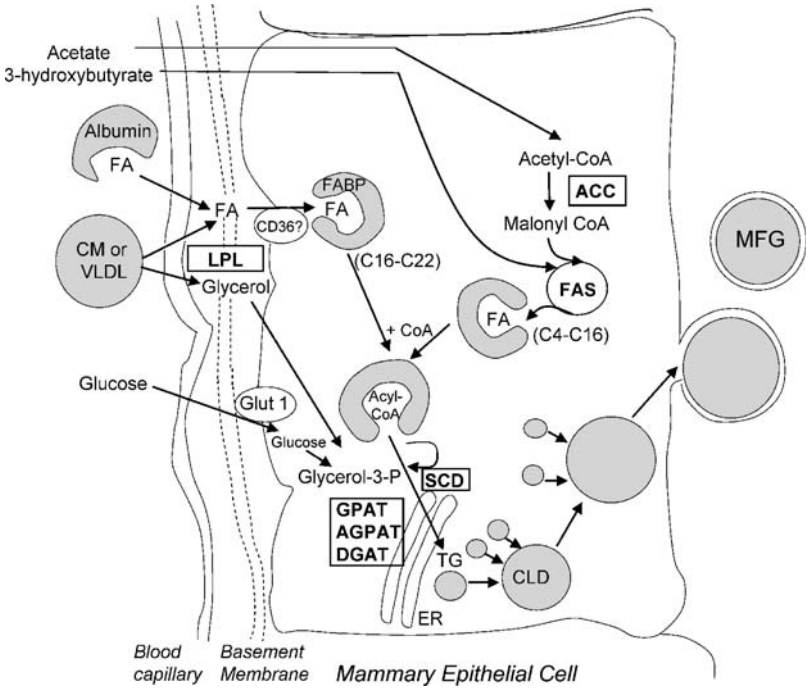

Fig. 1 Milk fat synthesis in the ruminant mammary epithelial cell. Abbrevations used: ACC = acetyl-CoA carboxylase; AGPAT = acyl glycerol phosphate acyl transferase; CD36 = cluster of differentiation 36; CLD = cytoplasmic lipid droplet; CoA = coenzyme A; CM = chylomicron; DGAT = diacyl glycerol acyl transferase; ER = endoplasmic reticulum; FA = fatty acid; FABP = fatty acid binding protein; FAS = fatty acid synthase; Glut 1 = glucose transporter1; GPAT = glycerol-3 phosphate acyl transferase; LPL = lipoprotein lipase; MFG = milk fat globule; SCD = stearoyl-CoA desaturase; TG = triglyceride; VLDL = very low density-lipoprotein

3-hydroxybutyrate, produced by ruminal fermentation of carbohydrates and by rumen epithelium from absorbed butyrate, respectively, thus resulting in short- and medium-chain FA (C4:0 to C16:0) that represent 40–50% of the FA secreted in milk, or (2) they are imported from the plasma, where they are either released by the enzyme lipoprotein lipase (LPL) (Barber et al., 1997) from triglycerides circulating in chylomicra or very low-density lipoprotein (VLDL), or derived from the plasma nonesterified fatty acids (NEFA) that circulate bound to albumin, for long-chain FA (≥C18) as well as ca. one-half of the C16:0, depending on the diet composition. These long-chain FA originate mainly from dietary lipid absorption from the digestive tract (with the dietary FA undergoing total or partial hydrogenation in the rumen) and from body reserves mobilization (especially at the beginning of lactation). Commonly, mobilization of body fat accounts for less than 10% of milk FA, with this proportion increasing in ruminants in negative energy balance in direct proportion to the extent of the energy deficit (Bauman & Griinari, 2001). Furthermore, FA may be desaturated, but not elongated, in the secretory mammary epithelial cells (MEC) (Chilliard et al., 2000).

Gene Characterization and Mechanisms of Mammary Lipogenesis

The acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) enzymes (encoded by the *ACACA* and *FASN* genes, respectively) are involved in the metabolic pathway for *de novo* FA synthesis in the mammary tissue, whereas the LPL enzyme is involved in the uptake of plasma FA. These FA could be desaturated by the stearoyl-CoA desaturase (SCD), resulting in synthesis of *cis*-9 unsaturated FA, and then esterified to glycerol sequentially via glycerol-3 phosphate acyl transferase (GPAT), acyl glycerol phosphate acyl transferase (AGPAT), and diacyl glycerol acyl transferase (DGAT). Then the triglycerides are secreted as milk fat globules (Fig. 1). The genes specifying these enzymes, implicated in the key processes of lipogenesis within the mammary gland, are candidate genes whose regulation has been studied first.

In ruminants, thanks to the recent knowledge of the cDNA sequences of several lipogenic genes (see Fig. 2)—*LPL* (Bonnet et al., 2000a; Senda et al., 1987), *ACACA* (Barber & Travers, 1998; Mao et al., 2001), *FASN* (Leroux et al., submitted; Roy et al., 2005), SCD (Bernard et al., 2001; Keating et al., 2005; Ward et al., 1998), *DGAT1* (Winter et al., 2002), *AGPAT* (Mistry & Medrano, 2002), and recently *GPAT* (Roy et al., 2006b)—molecular tools for studying their expression have been developed to allow the quantification of their mRNA by northern blot or real-time RT-PCR and/or the activity of the corresponding enzymes.

In addition, the recent development of high-throughput techniques such as microarrays allows us to complete this candidate gene approach. These new methods simultaneously provide data for the expression of thousands of genes

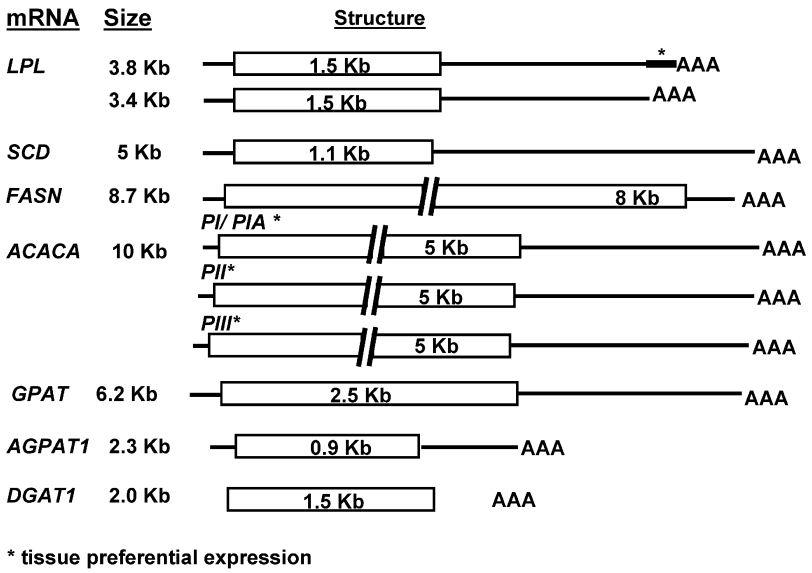

Fig. 2 Characterization (size and structure) of the transcripts of the main lipogenic genes in ruminant species, lipoprotein lipase (*LPL*; Senda et al., 1987; Bonnet et al., 2000), stearoyl-CoA desaturase (*SCD*; Bernard et al., 2001; Keating et al., 2005), fatty acid synthase (*FASN*; Roy et al., 2005; Leroux et al., in preparation), acetyl-CoA carboxylase (*ACACA*; Barber et al., 1998, 2005), glycerol-3 phosphate acyl transferase (*GPAT*; Roy et al., 2006b), acyl glycerol phosphate acyl transferase (*AGPAT*; Mistry & Medrano, 2002), and diacyl glycerol acyl transferase (*DGAT*; Grisart et al., 2002; Winter et al., 2002)

and allow a larger understanding of different mammary functions including milk lipid synthesis and secretion.

Uptake of Fatty Acids

The hydrolysis of lipoprotein triacylglycerol is catalyzed by LPL, which selectively releases FA esterified at the *sn*-1 (-3) position. In bovines, mammary tissue expresses three *LPL* transcripts, which are 1.7, 3.4, and 3.6 Kb in size (Bonnet et al., 2000a; Senda et al., 1987) due to the alternative use of the polyadenylation site. In this species, a predominance of the 3.4-Kb transcript in the mammary gland (Senda et al., 1987) as in ovine adipose tissue and of the 3.6-Kb transcript in muscles (Bonnet et al., 2000b) has been reported. In ovine species, the total cDNA sequence has been reported (Bonnet et al., 2000a). A complex regulation, by dietary and hormonal factors, modulates LPL activity via transcriptional, posttranscriptional, and posttranslational mechanisms. Immediately prior to parturition, mammary LPL activity increases markedly,

remains high throughout lactation, and is simultaneously downregulated in adipose tissue in cows (Shirley et al. 1973) and goats (Chilliard et al., 2003a).

From immunochemical and biochemical studies, it was shown that LPL is located both on (or near) the surface and within the cell of the major cell types of the different tissues as well as on the luminal surface of vascular endothelial cells. Mammary tissue contains various cell types in addition to parenchymal secretory MEC, including adipocytes, in varying proportions to MEC depending on the developmental and physiological status of the mammary gland. Discrepancies concerning LPL localization were reported from histological studies made on the rodent mammary gland. Thus, some studies (Camps et al., 1990) demonstrated the presence of LPL mRNA and protein in MEC using *in situ* hybridization and immunofluorescence techniques, respectively, and concluded that the origin of mammary LPL is the secretory MEC. Conversely, others (Jensen et al., 1991) demonstrated that LPL protein and mRNA are located in mammary depleted adipocytes or adipocyte precursors located in interstitial cells, suggesting that mammary LPL could originate partly from mammary adipocytes to be subsequently secreted and transported by cellular uptake and transcytosis, both to its final site of action on the capillary endothelial cell and through the secretory MEC into milk (Jensen et al., 1991; Neville & Picciano, 1997).

Arteriovenous difference measurements in lactating goats have shown that the utilization of triglycerides and NEFA for milk lipid synthesis is related to their plasma concentrations (Annison et al., 1968). Otherwise, experiments demonstrated that the availability of the substrate determines its utilization by the mammary gland with either a large NEFA utilization when plasma triglycerides are low and NEFA are high, as in fasting animals (West et al., 1972), or no net utilization of NEFA at plasma concentrations below 0.2 mM (Nielsen & Jakobsen, 1994). Similarly, triglyceride utilization increases when its plasma concentration increases. Thus, duodenal lipid infusion (Gagliostro et al., 1991) increased plasma triglyceride concentration and apparent mammary uptake, and there was simultaneously a net production of NEFA by the mammary gland, due to their release in the vascular bed during LPL action (Fig. 1).

The mechanism by which FA crosses the capillary endothelium and interstitial space to reach the MEC has not yet been identified. After arriving at the MEC, FA could cross the plasma membrane by diffusion or via a saturable transport system. In mammals, the acyl-CoA binding proteins (ACBP) (Knudsen et al., 2000) that bind long-chain acyl-CoA have an important role in regulating the FA transport and concentration in the cytosol. Nevertheless, in ruminants, Mikkelsen and Knudsen (1987) found lower concentrations of these ACBP in the mammary gland and muscles compared to the liver cytosol. Elsewhere it has been suggested in rodents and ruminants that another FA binding protein, CD36 (cluster of differentiation 36), expressed in the lactating MEC, and found in the milk fat globule membrane, heart, platelets, and adipocytes, may function as a transporter of long-chain FA (Abumrad et al., 2000). The detection of CD36 in mammary tissue could be linked to the

presence of adipocytes. Nevertheless, the presence of *CD36* mRNA in rodent MEC lines has been shown, with a slight enhancement of its gene expression after the addition of lactogenic hormones (Aoki et al., 1997).

Furthermore, fatty acid binding proteins (FABP; a family of intracellular lipid binding proteins found across numerous species) are involved in the uptake and intracellular trafficking of FA in many tissues (Lehner & Kuksis, 1996). In the bovine mammary gland, co-expression of FABP and CD36 has been shown, which increases during lactation and decreases during involution (Spitsberg et al., 1995), demonstrating that their expression is related to physiological variations of lipid transport and metabolism within the cell. In the same way, simultaneous elevations of *CD36* mRNA expression, of cytosolic TAG, and of lipid droplets were observed in primary bovine MEC (Yonezawa et al., 2004b). Barber et al. (1997) proposed a role for CD36 in the transport of FA across the secretory MEC membrane, working in conjunction with intracellular FABP. In the bovine lactating mammary gland, the presence of two forms of FABP has been demonstrated (Specht et al., 1996), identified as A-FABP and H-FABP, thus named according to the tissue of their first detection, adipose tissue and heart, respectively. Studying the proteins' cellular location, Specht et al. (1996) showed that A-FABP and H-FABP were present in myoepithelial cells and MEC, respectively. The expression of *FABP* types is generally interpreted in terms of specialized functions in FA metabolism, with *H-FABP* predominantly found in cells where FA are used as an energy source and probably involved in their β-oxidation. The significance of its abundance in mammary tissue in which active triglyceride synthesis and low FA oxidation occur during lactation remains to be understood.

In addition, ATP-binding cassette (ABC) transporters (a family of membrane proteins) are involved in the transport, against concentration gradients, of a wide variety of compounds, including ions, peptides, sugars, and lipids, at the cost of ATP energy (Klein et al., 1999). The *ABCG5* and *ABCG8* members of this family play an important role in cholesterol homeostasis and have been described specifically in intestine and liver cells in rodents (Mutch et al., 2004; Yu et al., 2002). The recent identification and expression of *ABCG5* and *ABCG8* transporters in the bovine mammary gland open a wide range of future investigations on their potential role in lipid trafficking and excretion during lactation and control of sterol concentrations in milk (Viturro et al., 2006).

De Novo Fatty Acid Synthesis

Acetyl-CoA Carboxylase Gene (*ACAC*)

The *ACACA* and *ACACB* genes are distinct genes that respectively encode the isoenzymic ACC proteins ACCα and ACCβ. The *ACACA* gene is expressed in all cell types but is found at its highest levels in the lipogenic tissues

(Lopez-Casillas et al., 1991), where its protein product ACCα provides cytoplasmic malonyl-CoA for FA synthesis. The *ACACB* gene is the major form expressed in heart and skeletal muscles (Abu-Elheiga et al., 1997), where its protein product ACCβ is implicated in the regulation of the β-oxidation of FA in the mitochondria (Abu-Elheiga et al., 2000). Expression of the ACCα isoenzyme is regulated in a complex fashion in the short term, through allosteric mechanisms with cellular metabolites possessing a positive (citrate) or negative effect (malonyl-CoA and long-chain acyl-CoA), and reversible phosphorylation on a number of specific serine residues, as well as chronically, through the regulation of transcription of the gene (Kim, 1997).

In ruminants, the *ACACA* cDNA sequence was first reported by Barber and Travers (1995) and corresponds to the synthesis of a protein with 2,346 amino acids. More recently, the *ACACA* gene was characterized in sheep (Barber & Travers, 1998) and cattle (Mao et al., 2001). Initially, the existence of three promoters, PI, PII, and PIII, was demonstrated, and their use together with alternative splicing of the primary transcripts from promoters I and II results in the generation of a heterogeneous population of transcripts differing in the sequence of their 5'UTR. These promoters are used in tissue-differential fashion. PIII use is limited to lung, liver, kidney, brain, and predominantly the lactating mammary gland in bovine (Mao et al., 2002) and ovine (Barber et al., 2003) species. PII expression is ubiquitous, with an elevated expression in the lactating mammary gland. PI is preferentially used in adipose tissue and liver under lipogenic conditions and in lactating bovine mammary gland. PI generate ~30% of *ACACA* mRNA (Mao et al., 2001), while in the ovine mammary gland, its contribution is low (2%; Molenaar et al., 2003). The reason for this difference of promoter usage between the bovine and the ovine is unknown. In addition, Barber et al. (2005) demonstrated the existence of a fourth promoter in human, rodent, and ruminant species and mainly expressed in brain, which again underlines the complexity of the structure and regulation of the *ACACA* gene.

Fatty Acid Synthase Gene (*FASN*)

The *FASN* gene encodes the protein FAS, which, under a complex homodimeric form, is responsible for the synthesis of short- and medium-chain FA (C4-C16) in the mammary gland during lactation (Wakil, 1989). In ruminants, the FAS enzyme contains six catalytic activity domains on a single protein of 2,513 amino acids. Contrary to what is observed in rodent mammary gland and duck uropygial gland, ruminant FAS synthesizes medium-chain FA without the implication of a thioesterase II (Barber et al., 1997). In addition to being able to load acetyl-CoA, malonyl-CoA, and butyryl-CoA, ruminant FAS contains a loading acyltransferase whose substrate specificity extends to up to C12, with the result that it is able to load and also release these medium-chain FA (Knudsen & Grunnet, 1982). This way of medium-chain FA synthesis is specific to the

lactating ruminant mammary gland, whereas the product of FAS in other ruminant tissues is predominantly C16:0, as in non-ruminant tissues (Christie, 1979). *FASN* mRNA ranges in size from 8.4 to 9.3 Kb, depending on the species: In several human tissues (Jayakumar et al., 1995), bovine mammary gland (Beswick & Kennelly, 1998), and ovine (Bonnet et al., 1998) and porcine (Ding et al., 2000) adipose tissues, only one transcript has been detected by northern blot. Conversely, two mRNA, generated by the use of two alternative polyadenylation signals, have been detected in rat adipose tissue (Guichard et al., 1992) and mammary gland (Schweizer et al., 1989). The gene, termed *FASN,* has recently been cloned in bovine (Roy et al., 2005), and an alternate transcript was discovered without part of exon 9 (minus 358 bp). In caprine, the cDNA was recently characterized with only one transcript described (Leroux et al., submitted). In addition, Roy et al. (2006c) identified several single-nucleotide polymorphisms (SNPs) in the bovine *FASN* gene, and the analysis of two of them, located respectively in exon1 and 34, suggested an association of these polymorphisms with variations in milk fat content.

Stearoyl-CoA Desaturase

The *SCD* gene encodes a protein of 359 amino acid residues located in the endoplasmic reticulum that catalyzes the Δ-9 desaturation, introducing a *cis* double bond, of a spectrum of fatty acyl-CoA substrates, mainly from C14 to C19. In rodents, *SCD* relies on different genes whose expression and regulation by polyunsaturated FA (PUFA) are tissue-specific (Ntambi, 1999). Conversely, in ruminants there is only one *SCD* gene (Bernard et al., 2001), generating a 5-Kb transcript that was characterized in sheep (Ward et al., 1998), cows (Chung et al., 2000), and goats (Bernard et al., 2001). In goats, the 3'-UTR sequence derives from a single exon and is unusually long (3.8 Kb), as observed for humans (Zhang et al., 1999), rats (Mihara, 1990), and mice (Ntambi et al., 1988). In addition, the caprine 3'-UTR is characterized by the presence of several AU-rich elements, which could be mRNA destabilization sequences, and presents a genetic polymorphism with the presence or absence of a triplet nucleotide (TGT) in position 3178-3180 (Bernard et al., 2001, and GenBank accession number AF325499). Immediately after parturition, *SCD* mRNA in ovine (Ward et al., 1998) and activity in bovine (Kinsella, 1970) increased in the mammary gland. In lactating goats, the *SCD* gene is highly expressed in the mammary gland and subcutaneous adipose tissue, compared to perirenal adipose tissue (Bernard et al., 2005b). In the lactating mammary gland, palmitoleoyl-CoA and oleyl-CoA are synthesized from palmitoyl-CoA and stearoyl-CoA by the action of the SCD enzyme (Enoch et al., 1976). In addition, in bovine mammary gland, SCD is responsible for the synthesis of the major part of *cis*-9, *trans*-11- (Corl et al., 2001; Griinari et al., 2000; Loor et al., 2005d; Shingfield et al., 2003) and of *trans*-7, *cis*-9- (Corl et al., 2002) CLA isomers.

The promoter region of the bovine *SCD* gene has recently been characterized (Keating et al., 2006), and a region of critical importance, designated stearoyl-CoA desaturase transcriptional enhancer element (STE) and containing three binding complexes, was identified in the MAC-T cell. In addition, this STE region was shown to play a key role in the inhibitory effect on *SCD* gene transcription of *trans*-10, *cis*-12 CLA and, to a lesser extent, of *cis*-9, *trans*-11 CLA (Keating et al., 2006).

Esterification of FA to Glycerol

In mammals, FA are not distributed randomly on the *sn*-1, *sn*-2, and *sn*-3 positions of the glycerol backbone of the milk triglycerides; this nonrandom distribution determines functional and nutritional attributes (German et al., 1997). In bovine, a description of the stereospecific position of the major FA in TAG has been reviewed by Jensen (2002). A high proportion (56–62%) of FA esterified at positions *sn*-1 and *sn*-2 of the glycerol backbone are medium- and long-chain saturated FA (C10:0 to C18:0), with C16:0 equally distributed among *sn*-1 and *sn*-2, C8:0, C10:0, C12:0, and C14:0 more located at *sn*-2, and C18:0 more located at *sn*-1. In addition, about 24% of FA esterified at position *sn*-1 is C18:1. Finally, a high proportion of FA esterified at position *sn*-3 is short-chain FA (C4:0, C6:0, C8:0; 44% on a molar basis) and oleic acid (27%). When consumed by humans, milk TAG are hydrolyzed by pancreatic lipase specifically in the *sn*-1 and *sn*-3 positions, allowing the FA present at position *sn*-2 to be preferentially absorbed because they remain in the monoacyl glycerol form (Small, 1991). The first step in triglyceride biosynthesis is the esterification of glycerol-3-phosphate in the *sn*-1 position, which is catalyzed by the glycerol-3 phosphate acyl transferase (GPAT). Two isoforms of GPAT have been identified in mammals, which can be distinguished by subcellular localization (mitochondrial vs. endoplasmic reticulum) and sensitivity to sulfhydryl group modifying agent N-ethylmalmeimide (NEM). The mitochondrial isoform is resistant to NEM, and the endoplasmic reticulum isoform is sensitive to NEM. In rodents, both isoforms have a role in the TAG synthesis in the liver and adipose tissue (Coleman & Lee, 2004). Regarding the mitochondrial *GPAT* gene, the genomic structure and cDNA sequence were recently determined in ruminants, with the presence of two transcripts differing in their 5'-UTR (Roy et al., 2006b).

The second step of triglyceride synthesis is committed by AGPAT (or lysophosphatidic acid acyltransferase, LPAAT). AGPAT has a greater affinity for saturated fatty acyl-CoA (Mistry & Medrano, 2002) in the order C16 > C14 > C12 > C10 > C8 (Marshall & Knudsen, 1977), which is in accordance with the observed high proportion of medium- and long-chain saturated FA at the *sn*-2 position in milk, with palmitate as the major FA (representing 43% of the total palmitate found in triacylglycerol). Consequently, a possible regulation of

substrate specificity of the enzymes of FA esterification should be of major importance for both the mammary cell and human nutrition. In addition, substrate availability in the bovine and ovine mammary gland is also a factor for the *sn*-2 position FA composition, allowing its manipulation to some extent by nutritional factors, in interaction with the aforementioned substrate affinity. Bovine and ovine *AGPAT* genes were characterized, cloned, and located on bovine chromosome 23 (Mistry & Medrano, 2002). Bovine and ovine AGPAT are proteins made up of 287 amino acids that differ by only one amino acid residue.

The third enzyme, DGAT, is located on the endoplasmic reticulum membrane. DGAT is the only protein that is specific to triacylglycerol synthesis and therefore may play an important regulatory role (Mayorek et al., 1989). However, little is known about the regulation of *DGAT* expression, whereas its gene has been particularly well studied in ruminants due to its genetic variability. The complete bovine *DGAT1* gene (Grisart et al., 2002; Winter et al., 2002) and the near-complete coding region of the caprine *DGAT1* gene (Angiolillo et al., 2006) have been sequenced. A quantitive triat loci (QTL) QTL for milk fat content has been detected in the centromeric region of cattle chromosome 14, and *DGAT1* was proposed as a positional and functional candidate for this trait (Winter et al., 2002). These studies found a nonconservative substitution of lysine by alanine (Grisart et al., 2004) in *DGAT1* caused by AA to GC dinucleotide substitution at position 10434 of the gene sequence, in exon 8 (GenBank accession number AY065621). This polymorphism was related to milk composition and yield variations. The K allele was recently shown *in vitro* to be associated with increased activity of the enzyme in agreement with its positive link with bovine milk fat percentage (Grisart et al., 2004). In addition, in the German Holstein population, Kühn et al. (2004) described five alleles at a variable number tandem repeat (VNTR) polymorphism in the *DGAT1* promoter, which showed an effect on fat content additional to the *DGAT1 K232A* mutation. Due to the presence of a potential transcription factor binding site in the 18nt element of the VNTR, the variation in the number of tandem repeats of the 18nt element might be causal for the variability in the transcription level of the *DGAT1* gene. In sheep, *DGAT1* is an obvious candidate gene for milk fat content, as a QTL was detected in chromosome 9, which is homologous to the bovine chromosome 14 region (Barillet et al., 2005).

Regulation of Mammary Lipogenic Gene Expression by Dietary Factors

The response of gene expression to nutrient changes involves the control of events that could occur at transcriptional (e.g., through transcription factors), posttranscriptional (e.g., such as mRNA stability), translational (e.g., its initiation, etc.), and posttranslational (e.g., via turnover or activation of enzymatic protein) levels. However, it is often unclear whether the regulatory factors are

the dietary components themselves or their metabolites or are hormonal changes produced in response to the nutritional changes. Moreover, in most of the studies, as in those reported in this chapter, it is difficult to determine the level of regulation involved since, for a given gene, measurements of the relevant mRNA, the enzyme protein content, and the activity are not often studied simultaneously. Only few data are indeed available in ruminants on the nutritional regulation of mammary lipogenic gene expression either *in vivo* or *in vitro*.

The *in vivo* trials have been carried out in midlactation cows and goats. Data in cows come from five studies that were undertaken mainly (four of the five studies) with milk fat-depressing (MFD) diets:

- **Study 1**: a high level of concentrate (containing 76% cracked corn, 19% heat-treated soybean meal, 1% sunflower oil) with a forage- (alfalfa hay) to-concentrate ratio of 16/84, compared to a control diet with a high-forage (composed of 83% corn silage and 17% alfalfa hay) content and a forage-to-concentrate ratio of 53/47 (Peterson et al., 2003; 3 cows),
- **Study 2**: a high level of concentrate (containing 52% ground corn and 15% soybean meal) with a forage- (corn silage) to-concentrate ratio of 25/70, supplemented with 5% of soybean oil, compared to a high level of forage (containing 76% corn silage and 24% alfalfa hay) with a forage-to-concentrate (containing mainly of 57% ground corn and 34% soybean meal) ratio of 60/40 (Piperova et al., 2000; 10 cows),
- **Study 3**: a diet based on grass silage (19%), corn silage (19%), and rolled barley (44%) supplemented, or not, with either 1.7% of glutaraldehyde-protected fish oil or 2.7% unprotected fish oil (Ahnadi et al., 2002; 16 cows),
- **Study 4**: a diet based on grass (27%), corn silage (30%), and rolled barley (22%) supplemented with 3.3% unprotected canola seeds plus 1.5% canola meal, or 4.8% formaldehyde-protected canola seeds, compared to the same diet with 4.8% of canola meal, which, for the two treatment diets, represents a supply of 0.7% and 0.6% of extra lipids compared to the control diet, respectively (Delbecchi et al., 2001; 6 cows),
- **Study 5**: a forage-based diet (containing 25% corn silage, 17% alfalfa silage, and 3% alfalfa hay) with a forage-to-concentrate (22% of ground corn and 28% of grain mix) ratio of 45/55 and supplemented with 3% of soybean oil and 1.5% of fish oil, compared to a forage-based diet (containing 35% corn silage, 23% alfalfa silage, and 5% alfalfa hay) with a forage-to-concentrate (15% of ground corn and 20% of grain mix) ratio of 65/35 (Harvatine & Bauman, 2006; 16 cows).

In goats, data are from two nutritional studies undertaken with dietary lipid supplementations varying in their nature, form of presentation, and dose:

- **Study 6**: a 54% hay diet supplemented, or not, with 3.6% of lipids from oleic sunflower oil or formaldehyde-treated linseeds, with the concentrate fraction containing 40% rolled barley, 17% dehydrated sugar beet pulp, 10% pelleted dehydrated Lucerne, and 17% soybean meal (Bernard et al., 2005c; 14 goats),

- **Study 7**: two diets with 47% of either hay (H;13 goats) or corn silage (CS;14 goats) supplemented, or not, with 5.8% of either linseed or linoleic sunflower oil, with the concentrate fraction containing 24(H)–24(CS)% rolled barley, 39(H)–34(CS) dehydrated sugar beet pulp, 12(H)–0(CS) pelleted dehydrated Lucerne, and 25(H)–42(CS)% soybean meal, respectively (Bernard et al., unpublished 27 goats).

In these two goat studies, conversely to what is generally observed in dairy cows (Bauman & Griinari, 2003), dietary lipid supplementation always increased the milk fat content and did not increase (or only slightly increased) milk yield, in accordance with previously published goat studies (Chilliard et al., 2003a, 2006a). The seven cow and goat studies presented above have been used as a database to allow us to evaluate how dietary factors may change metabolic pathways and lipogenic gene expression, in interaction with animal species peculiarities.

Regulation of Lipoprotein Lipase

Studies in cows on MFD diets reported either no change in the abundance of mammary *LPL* mRNA (study 3 with 1.7% "protected" fish oil ; Ahnadi et al., 2002) or a tendency to decrease (study 1; Peterson et al., 2003) or a strong decrease (study 5; Harvatine & Bauman, 2006, and study 3 with 2.7% unprotected fish oil; Ahnadi et al., 2002). In addition, still with an MFD diet, no change in the abundance of *FABP* has been observed (Peterson et al., 2003). However, in the two former studies (1 and 3), milk long-chain FA (> C16) yield (g/day) was significantly decreased, by 43% after "protected" fish oil supplementation (study 3) and by 28% with a high-concentrate diet (study 1). Elsewhere, in suckling beef cows fed hay supplemented with 14% high-linoleate safflower seeds, compared to 12% corn and 6% safflower seed meal, a trend toward a greater *LPL* mRNA was observed ($P = 0.09$; Murrieta et al., 2006) together with an increase in long-chain FA percentage in milk fat. Furthermore, in goats fed a hay-based diet supplemented with oleic sunflower oil (study 6; Bernard et al., 2005c), no significant effect was observed on mammary LPL activity, whereas *LPL* mRNA content was increased, together with a large increase (83%) in long-chain FA (C18) secretion. In goats fed with hay or corn silage supplemented with either linseed or sunflower oil (study 7), a large increase in the secretion of milk long-chain FA (> 100%) was observed without any effect on mammary LPL activity and mRNA except when corn silage was supplemented with sunflower oil, in which case LPL activity increased. In addition, in late-lactating goats fed an alfalfa hay-based diet, supplementation with 3.8% lipids from soybeans had no effect on mammary *LPL* mRNA abundance, while the secretion of long-chain FA (C18) in milk was increased by 58% (Bernard et al., 2005b). Thus, most of the results available in ruminants are not in agreement with those reported in rodents, where a very high dietary

lipid intake (20%) enhanced both mammary gland LPL activity and lipid uptake (Del Prado et al., 1999).

The aforementioned results led to the hypothesis that either *LPL* mRNA or LPL activity measured *in vitro* in optimal conditions generally does not limit the uptake of long-chain FA by the mammary gland in ruminants. Other factors such as the availability of plasma triglyceride FA (Gagliostro et al., 1991) and the location of LPL (capillary endothelial cells, MEC, or depleted adipocytes) could play an important role. These results are also in accordance with those from previous studies in lactating goats, demonstrating that mammary utilization of plasma triglycerides and NEFA is related to their arterial concentration (see earlier section). Moreover, the existence of positive correlations between milk stearic acid percentage and milk fat content was observed (Chilliard et al., 2003b), related both to the response to dietary lipids and to individual variations within each dietary treatment (Bernard et al., 2006). This suggests that a significant availability and uptake of this FA is an important factor for milk fat secretion in goats, as in cows (Loor et al., 2005a, b).

Regulation of Genes Involved in de Novo Lipid Synthesis (ACCA and FASN)

In cows, an MFD diet induced joint reductions in *ACACA* mRNA abundance and ACC activity and in FAS activity in mammary tissue, together with a dramatic 60% decrease in C4–C16 FA secretion (g/day) (study 2; Piperova et al., 2000). Furthermore, Ahnadi et al. (2002; study 3) observed a decrease in mammary *ACACA* and *FASN* mRNA levels along with a 38% decrease in C4–C16 FA secretion with fish oil-supplemented diets. Moreover, in cows with a high-concentrate diet (study 1; Peterson et al., 2003), the observed reductions of milk fat secretion (27% decrease) and of C4–C16 (30% decrease) match a reduction of mammary mRNA abundance of several genes involved in milk lipid synthesis pathways, in particular *ACACA* and *FASN*, whereas no effect on milk κ-*casein* mRNA was observed. In addition, in cows fed an MFD diet, a downregulation of *FASN* and *ACACA* as well as other lipogenic genes such as *SCD, LPL,* and *FABP3*, was observed using a bovine oligonucleotide microarray (Loor et al., 2005c). Similarly, in cows fed a low-forage diet supplemented with soybean and fish oils, Harvatine and Bauman (2006; study 5) observed a decrease in the yield of milk fat (38%) and short- and medium chain-FA (50%), together with a significant reduction in the expression of *FASN* and other genes involved in the regulation of lipid metabolism (*SREBP1*, *INSIG-1*, *S14*). However, in suckling beef cows, Murrieta et al. (2006) observed no effect from a diet supplemented with high-linoleate safflower seeds on mammary *ACACA* and *FASN* mRNA levels, despite a 33% decrease in the weight percentage of C10–C14 FA.

Elsewhere, food deprivation for 48 hours in dairy goats was shown to change mammary transcriptome profil, using a bovine 8,379-gene microarray (Ollier et al., 2007): in particular, six genes involved in lipid metabolism and transport such as *FASN*, *ACSBG1* (acyl-CoA synthetase "bubblegum"), and *AZGP1* (zinc-alpha-2-glycoprotein precursor) were downregulated simultaneously with a decrease in milk lactose, protein, and fat secretion. The regulation of these last two genes needs further studies to evaluate their impact on mammary lipogenesis.

In goats (studies 6 and 7), the observed slight decrease of 15–22% in milk C4–C16 FA secretion (g/day) after supplementation of hay or corn silage diets with vegetable lipids was not accompanied by any significant variation of *ACACA* and *FASN* mRNA levels or activities. However, a positive relationship ($r = +0.62$) was observed (Fig. 3a) between *ACACA* mRNA variation of and C4–C16 secretion response to lipid supplementation, calculated from 81 individual values obtained by biopsies from goats fed hay or corn silage diets supplemented with 5.8% of either linseed or sunflower oil (study 7). Altogether, results from cow and goat studies demonstrated a positive relationship between both *ACACA* ($r = +0.66$; Fig. 4a) and *FASN* ($r = +0.73$; Fig. 4b) mRNA abundance and C4–C16 secretion responses to dietary treatment. These results suggest that variations of *ACACA* and *FASN* mRNA abundance play a role in the response of milk short- and medium-chain FA to dietary manipulation, especially the addition of lipids to the diet. Furthermore, the responses to lipid supplementation of *ACACA* and *FASN* mRNA were correlated ($r = +0.56$; Fig. 3b) in study 7, suggesting the existence of a similar regulatory mechanism for these genes.

The differences observed between cows (Ahnadi et al., 2002; Piperova et al., 2000) and goats (Bernard et al., 2005c) in the magnitude of responses of *ACACA* (Fig. 4a) and *FASN* (Fig. 4b) mRNA and/or activity to dietary PUFA supplementation may be partly explained by factors linked to the diet, such as the level of starchy concentrate in the diet, the nature (fish oil vs. vegetable oil) and presentation (seeds vs. free oil) of the lipid supplements, as well as species differences. The fact that lipid supplementation in goats always induces an increase in milk fat content and secretion whereas in cows it generally decreases them (see earlier section) supports species-differences implications.

Altogether, from *in vivo* studies where *ACACA*and *FASN* mRNA levels and activities in the mammary gland were measured (studies 6 and 7 and study 2 for ACC in cows), positive relationships between mRNA and activities variations in response to dietary treatment were observed for *ACACA* and *FASN* (Bernard et al., 2006). This suggests a regulation at a transcriptional level, at least, for these two genes in the ruminant mammary gland, which is in accordance with data obtained in rat adipose tissue (Girard et al., 1997) and liver (Girard et al., 1994) in response to nutritional factors. For *ACACA,* this level of regulation occurs in addition to the well-documented posttranslational

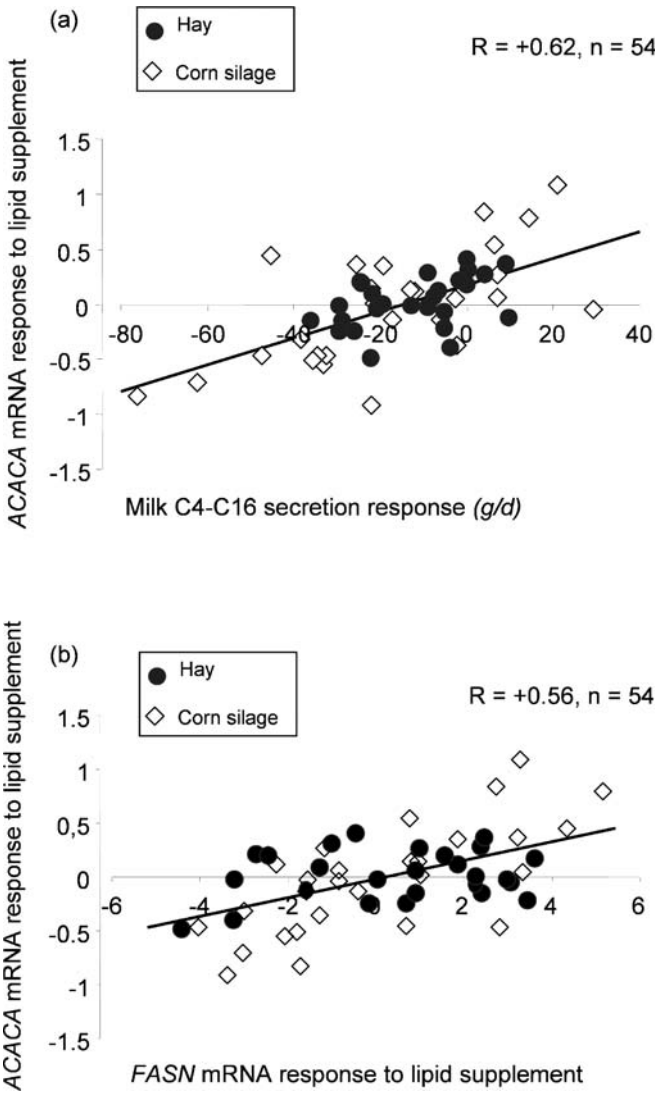

Fig. 3 Relationships between (a) milk short- and medium-chain fatty acid secretion and mammary acetyl-CoA carboxylase (*ACACA*) mRNA level responses to different dietary treatments (adapted from Bernard et al., 2006), and (b) mammary fatty acid synthase (*FASN*) and acetyl-CoA carboxylase (*ACACA*) mRNA-level responses to different dietary treatments. Results from 54 individual responses (lipid-supplemented = control) from 81 mammary biopsies in two 3 × 3 Latin squares, in 27 goats (study 7; see text). Lipid supplements were linseed oil or sunflower oil

regulation, in particular through covalent modification via phosphorylation/dephosphorylation under hormonal control as well as by allosteric activation or inhibition by cellular metabolites (see earlier section).

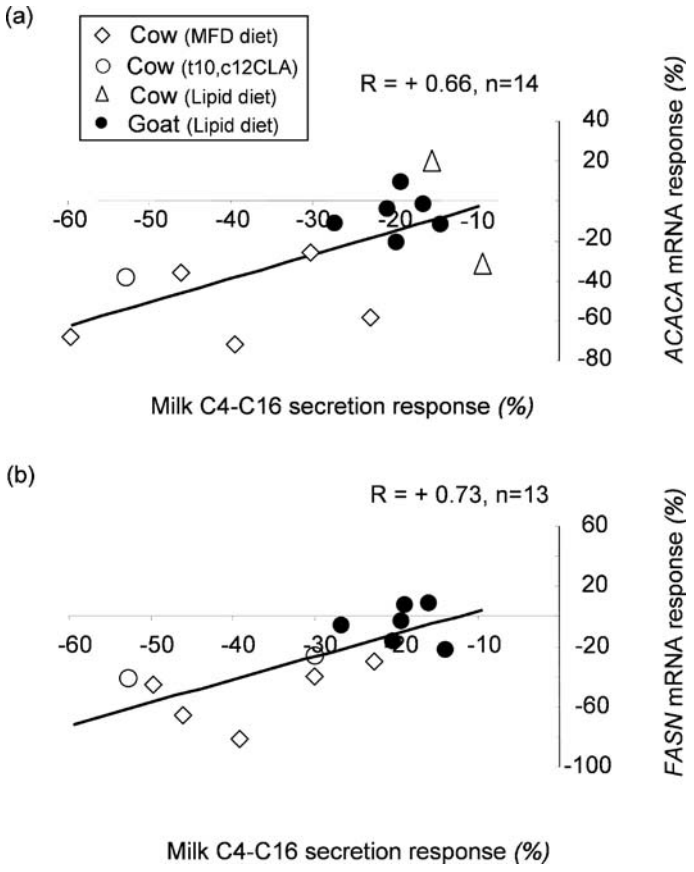

Fig. 4 Relationships between milk short- and medium-chain fatty acid secretion and mammary acetyl-CoA carboxylase (*ACACA*) (a) and fatty acid synthase (*FASN*) and (b) mRNA-level responses to different dietary treatments in goats and cows. Results are expressed as treatment mean response (vs. control group). Dietary treatments are described in the text and consisted of either lipid supplementation in goats (●; studies 6 and 7; $n = 41$; Bernard et al., 2005c, unpublished) and cows (△; study 4; Delbecchi et al., 2001) or milk fat-depressed (MFD) diets (◇ studies 1–3 and study 5; Peterson et al., 2003; Piperova et al., 2000; Ahnadi et al., 2002; Harvatine & Bauman, 2006), and postruminal *trans*-10, *cis*-12 CLA infusion (○ 13.6 g/day and 10 g/day, respectively, in Baumgard et al., 2002, and Harvatine & Bauman, 2006) in cows

Regulation of Stearoyl-CoA Desaturase

In rodents, nutritional regulation of SCD activity mainly occurs in the liver and has been studied extensively (Ntambi, 1999). Conversely, in the ruminant lactating mammary gland, only a few studies have investigated the nutritional regulation of *SCD* mRNA abundance and/or protein activity.

From the five nutritional studies performed in cows, four reported mammary *SCD* expression (studies 1, 3–5), and only feeding 1.7% of protected

fish oil supplement (rich in long-chain *n*-3 FA) (Ahnadi et al., 2002) significantly decreased ($P < 0.05$) *SCD* mRNA abundance.

In goats fed hay-based diets supplemented with lipids, mammary *SCD* mRNA abundance decreased with formaldehyde-treated linseed and enzyme activity decreased with oleic sunflower oil, linseed oil, and sunflower oil, whereas lipid supplementation on *SCD* mRNA or activity had no effect on a corn silage diet (studies 6 and 7). Elsewhere, in late-lactating goats fed an alfalfa hay-based diet, mammary *SCD* mRNA was not affected by the dietary addition of 3.8% lipids from soybeans (Bernard et al., 2005b). Altogether, results from goats suggest an interaction between the basal diet and the dietary lipids used and predict a negative transcriptional or posttranscriptional regulation by dietary PUFA and/or by their ruminal biohydrogenation products may occur.

In vivo △-9 desaturase activity has often been estimated by the milk ratios for the pairs of FA that represent a product/substrate relationship for SCD (*cis*-9 C14:1/C14:0, *cis*-9 C16:1/C16:0, *cis*-9 C18:1/C18:0, *cis*-9, *trans*-11 CLA/*trans*-11 C18:1; Bauman et al., 2001) due to the fact that the *in vitro* activity assay needs fresh materials, is laborious (Legrand et al., 1997), and is done in optimal conditions (pH, substrate, cofactors) that differ from *in vivo* conditions.

From the goat studies, we saw that the four FA pair ratios that represent a proxy for SCD activity were more or less related to the SCD activity itself, across 10 dietary groups (Bernard et al., 2005c; Chilliard et al., 2006b; Fig. 5). However, in terms of response to dietary lipids (six comparisons), the milk ratio of myristoleic acid to myristic acid (*cis*-9 C14:1/C14:0) gave the best estimation for the response of mammary SCD activity. This is due to the fact that almost all the myristoleic acid present in the milk is likely to be synthesized in the mammary gland by SCD. Indeed, myristic acid originates almost exclusively from *de novo* synthesis within the mammary gland (C14:0 is poorly represented in feedstuffs used for ruminants, including lipid supplements). Conversely, variable proportions of palmitic, palmitoleic, stearic, oleic, vaccenic, and rumenic acids come from absorption from the digestive tract and/or mobilization of body fat reserves. Then the ratios that involve these latter FA are less indicative of SCD activity. In addition, differential uptake, turnover, and use of the different FA of the four pair ratios by the mammary tissue itself may occur. Moreover, the four FA pair ratios could be influenced by other factors than SCD activity, such as a differential accuracy in the quantification of the *cis*-9 isomers in milk as well as, as stated above, by differences between *in vivo* (effective) and *in vitro* (potential) SCD activity.

Regulation of Acyltransferases

Only one study in cows reported data on genes encoding acyltransferases with an observed reduction of mammary *GPAT* and *AGPAT* mRNA abundance

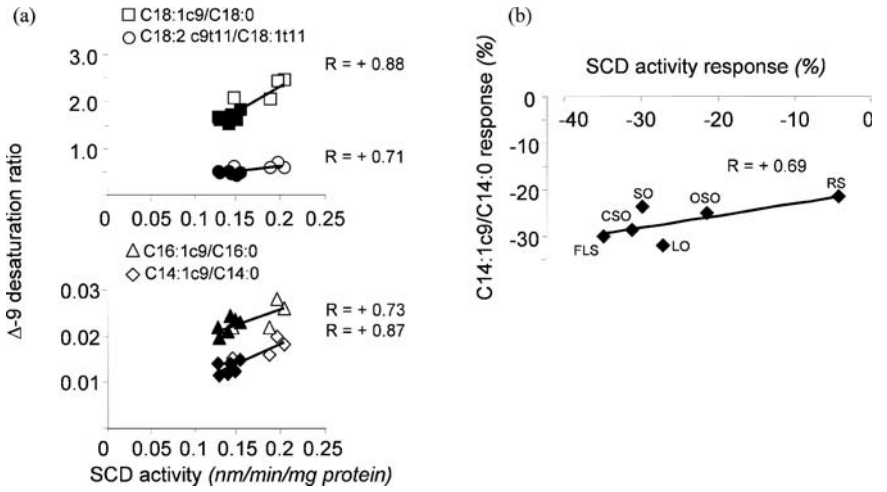

Fig. 5 Relationships between milk FA desaturation ratios and stearoyl-CoA desaturase (SCD) activity in 43 goats fed hay-based diet supplemented, or not, with lipids. The lipid supplements were either 3.6% of lipids from oleic sunflower oil (OSO) or formaldehyde-treated linseed (FLS) (study 6; Bernard et al., 2005c), or 5.8% lipids from linseed oil (LO) or sunflower oil (SO) (study 7; Bernard et al., unpublished), or 6.5% whole rapeseed (RS) or 4.5% of sunflower oil (CSO) (Chilliard et al., 2006b). (a) Results are means of the six lipid-supplemented groups (black symbols) and the four control groups (white symbols). (b) Relationship between the responses (% of the control group) of SCD activity and milk *cis*-9 C14:1/C14:0 to the six lipid supplements

together with a reduction of milk fat yield (by 27%) with a high-concentrate diet (Peterson et al., 2003). In goats, food deprivation (FD) 48 hours before slaughter was shown to increase mammary *AGPAT* and *DGAT1* mRNA content, whereas milk fat secretion decreased (Ollier et al., 2006). This apparent discrepancy could be due to a known posttranscriptional regulation of these two genes. Indeed, phosphorylation/dephosphorylation mechanisms have been suggested for AGPAT activity by Mistry and Medrano (2002), whereas a translational control has been reported for DGAT1 expression (Yu et al., 2002).

Molecular Mechanisms Involved in the Regulation of Mammary Lipogenesis by Nutrition

A Key Regulatory Role for Specific Fatty Acids

In Vivo Results in Nutritional Studies

From earlier *in vivo* studies with postruminal infusions of plant oils (e.g., Chilliard et al., 1991), a role for long-chain saturated FA and/or PUFA in decreasing

mammary *de novo* FA synthesis has been suggested. Nevertheless, only recently was a central role proposed for specific *trans*-FA as potent inhibitors of mammary lipid synthesis, from studies with specific dietary conditions inducing a dramatic MFD (Bauman & Griinari, 2003). In the same way, an impairment of mammary lipid synthesis has been observed in rats fed a diet containing a mixture of *trans*-isomers (Assumpcao et al., 2002).

Indeed, the so-called low-milk fat syndrome in cows seems to be mainly due to specific PUFA biohydrogenation products formed in the rumen. Diets that induce MFD belong to three groups:

1. Diets rich in readily digestible carbohydrates and poor in fibrous components, without the addition of lipid supplements (e.g., high-grain/low-forage diets; Peterson et al., 2003), but containing a minimal amount of PUFA in dietary feedstuffs (Griinari et al., 1998),
2. Low-fiber diets associated with supplemental PUFA of plant origin (Piperova et al., 2000),
3. Diets associated with dietary supplements of marine oils (fish oils, fish meals, oils from marine mammals and/or algae) that induce MFD regardless of the level of starch or fiber in the diet (Ahnadi et al., 2002; Chilliard et al., 2001).

In the past, a number of theories have been proposed to explain diet-induced MFD, with the starting point for all these theories being an alteration in ruminal fermentations (reviews by Bauman & Griinari, 2001, 2003). One of them is that rumen production of acetate and butyrate was too low to support milk fat synthesis. Another one is that ruminal production of propionate increased, enhancing the hepatic rate of gluconeogenesis and the levels of circulating glucose and insulin and adipose tissue lipogenesis, thus inducing a shortage of nutrients available for the mammary gland. Another theory that now prevails was first proposed by Davis and Brown (1970) and, as mentioned above, suggests that mammary fat synthesis is inhibited by specific *trans*-FA, which results from alterations in rumen PUFA biohydrogenation.

In cows, *in vivo* trials support the *trans*-FA theory because a wide range of MFD diets are accompanied by an increase in the *trans*-C18:1 percentage of milk fat (review by Bauman & Griinari, 2003). An important development of this theory was the discovery that MFD was associated with a specific increase in *trans*-10 C18:1 rather than total *trans*-C18:1 isomer (Griinari et al., 1998). This finding was confirmed in several studies in cows from which a curvilinear response curve between *trans*-10 C18:1 and fat yield responses (Fig. 6) was evidenced (Loor et al., 2005a). Elsewhere, a curvilinear relationship between the decrease in milk fat percentage and small increases in milk *trans*-10, *cis*-12 CLA (Bauman & Griinari, 2003) was also observed in some, but not all, studies. For example, the reduction of milk fat (27% decrease) and of C4–C16 (30% decrease) secretion observed in cows with a high-concentrate diet (study 1; Peterson et al., 2003) was accompanied by a small but significant increase in milk fat *trans*-10, *cis*-12 CLA secretion (+0.5 g/day).

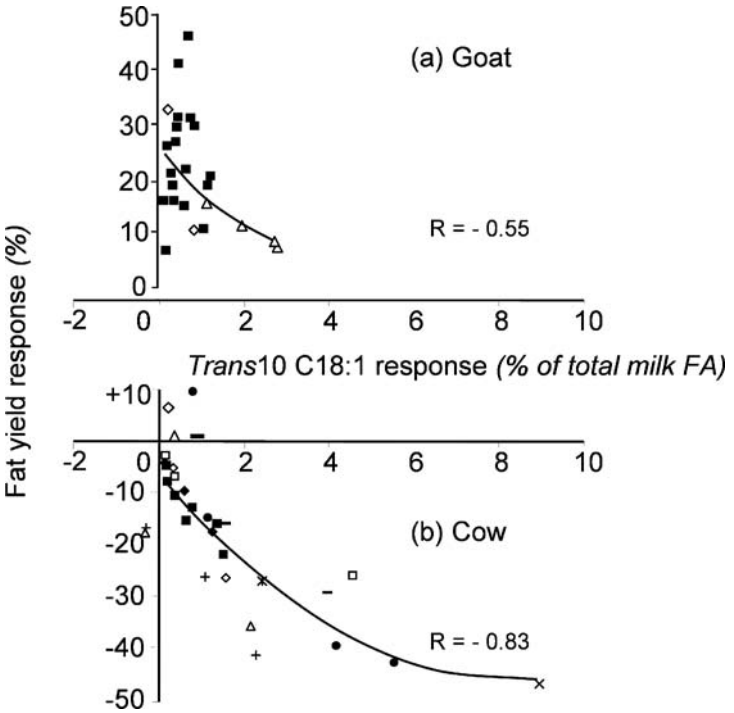

Fig. 6 Relationships between milk *trans*-10 C18:1 and fat yield responses in goats and cows. (a) Goat studies: data from 24 lipid-supplemented groups compared to 12 control groups (353 goats). The forages were either hay (■), fresh grass (◇), or corn silage (△). The lipids supplements were either sunflower oil, oleic sunflower oil, linseed oil, extruded linseeds, or rapeseeds (4–6% lipids in diet DM) and were given for 3 to 5 weeks (adapted from Chilliard et al., 2006a, b). (b) Cow studies: data from 31 lipid- or concentrate-supplemented groups, compared to control groups (13 studies) (adapted from Loor et al., 2005a)

The *trans*-10 C18:1 isomer synthesis in the rumen is probably due to the reduction of *trans*-10, *cis*-12 CLA resulting from an alternative pathway for biohydrogenation of linoleic acid, which increases when rumen pH decreases. Indeed, this "*trans*-10 pathway" seems to increase with diets rich in concentrate and/or corn silage and to occur after a one- to two-week period of latency after the start of dietary PUFA supplementation, following an earlier but transient increase in the "*trans*-11 pathway" (Chilliard & Ferlay, 2004; Roy et al., 2006a; Shingfield et al., 2006).

We must emphasize that in goats as in cows, a negative and curvilinear relationship between the responses of milk *trans*-10 C18:1 percentage and milk fat yield is observed (Chilliard et al., 2006a; Fig. 6), despite the fact that the fat yield response was always positive in goats, but always negative or null in cows. Then, in goats, the highest increases in *trans*-10 C18:1 were observed with either corn silage or fresh grass diets (Fig. 6) and matched with

the lowest increases in milk fat yield. In addition, the maximum observed value of milk *trans*-10 C18:1 in goats was much lower than in cows, and no significant increases in goat milk *trans*-10, *cis*-12 CLA occurred in the 24 diet comparisons in Fig. 6. These observed similarities and differences among ruminants suggest a species specificity of FA ruminal and/or mammary metabolism. The positive effect of lipid supplementation on goat milk fat yield could be due in part to the mammary sensitivity to increased availability of stearic acid arising from dietary PUFA biohydrogenation in the rumen (Bernard et al., 2006).

Regarding the impact of PUFA on lipogenic gene expression and, in particular, on SCD, still little is known in ruminants. Conversely, in rodents the downregulation of *SCD* gene expression by (*n*-6) and (*n*-3) PUFA has been largely described in liver and AT (Ntambi, 1999), whereas little is known in the lactating mammary gland (Lin et al., 2004; Singh et al., 2004). Indeed, Singh et al. (2004) observed negative effects from olive oil or safflower oil fed to lactating mice on both *SCD* mRNA and activity in the mammary gland and *SCD* mRNA in the liver. Similarly, in the mouse mammary gland, Lin et al. (2004) reported a decrease in *SCD, ACACA,* and *FASN* mRNA abundance and SCD activity by the dietary addition of either *trans*-11 C18:1, *cis*-9, *trans*-11 CLA, or *trans*-10, *cis*-12 CLA, whereas these treatments had no effect on these transcripts in liver. These results on lactating mice indicate a tissue-specific regulation of lipogenic gene expression by *trans*-FA and outline the possibility to manipulate mammary *SCD* gene expression by nutrition.

In goats, the observed negative effect of the addition of oleic sunflower oil, sunflower oil, and linseed oil to hay diet (trials 6 and 7) on SCD activity (Fig. 5b) and that of formaldehyde-treated linseed on *SCD* mRNA might be partly attributed to dietary *cis*-9 C18:1, C18:2 (*n*-6), and/or C18:3 (*n*-3) escaping from the rumen and/or to *trans*-isomers formed during ruminal metabolism of these three FA (Chilliard et al., 2003c; Ferlay et al., 2003; Rouel et al., 2004). In addition, besides PUFA biohydrogenation processes, oleic acid could be isomerized in several *trans*-C18:1 isomers, including *trans*-10, as shown in microbial cultures from bovine rumen (Mosley et al., 2002). This finding is in agreement with the observed increase of several *trans*-C18:1 isomers in milk from goats fed oleic sunflower oil (Bernard et al., 2005c; Ferlay et al., 2003; Chilliard et al., 2006a). Nevertheless, data in Fig. 5b suggest that α-linolenic acid from formaldehyde-treated linseeds should be more efficient than dietary oleic acid-rich oil to decrease SCD activity.

Duodenal or Intravenous Infusion of Specific Fatty Acids

Duodenal infusion trials of pure-CLA isomers demonstrated that *trans*-10, *cis*-12 CLA inhibits milk fat synthesis in dairy cows, whereas the *cis*-9, *trans*-11 CLA isomer has no effect (Baumgard et al., 2000; Loor & Herbein, 2003). The severe reduction (48%) in milk fat yield due to the infusion of a high

dose (13.6 g/day) of *trans*-10, *cis*-12 CLA (Baumgard et al., 2000) was accompanied by a dramatic reduction (> 35%) of mRNA abundance of enzymes involved in mammary uptake and intracellular trafficking of FA (*LPL* and *FABP*), *de novo* FA synthesis (*ACACA* and *FASN*), desaturation (*SCD*), and esterification (*GPAT* and *AGPAT*). Similarly, intravenous administration of *trans*-10, *cis*-12 CLA, either from 2 to up to 6 g/day (Viswanadha et al., 2003) or 10 g/day (Harvatine & Bauman, 2006), depressed milk fat yield, with, for the latter study, a joint decrease in the expression of genes involved in mammary uptake (*LPL*), *de novo* FA synthesis (*FASN*), and the regulation of lipid metabolism (*SREBP1*, *S14*, *INSIG-1*).

However, in the absence of duodenal infusion, the levels of *trans*-10, *cis*-12 CLA in the rumen, duodenal fluid, or milk always remained very low compared to the levels used in infusion studies (see above) or reached by the *trans*-10 C18:1 isomer (ratio between *trans*-10, *cis*-12 CLA, and *trans*-10 C18:1 of ~0.01; Bauman & Griinari, 2003; Loor et al., 2004a, b, 2005a, d; Piperova et al., 2000). In addition, in cows fed with marine oil for which an MFD was observed, little or only traces of milk *trans*-10, *cis*-12 CLA were detected, whereas substantial increases in *trans*-10 C18:1 were observed (Loor et al., 2005b; Offer et al., 2001). Furthermore, species-specific responses to *trans*-10, *cis*-12 CLA duodenal infusion have been noted, with no effect on milk fat secretion in goats (Andrade & Schmidely, 2005), contrary to what is observed in cows (see above), whereas in lactating sheep, lipid-encapsulated CLA supplement containing *trans*-10, *cis*-12 CLA significantly reduced milk fat synthesis (Lock et al., 2006).

Due to the lack of pure material, it was not possible until recently to investigate a direct effect of *trans*-10 C18:1 on milk fat synthesis, whereas the potent inhibitory effect of *trans*-10, *cis*-12 CLA was clearly established by postruminal infusion trials in dairy cows (Bauman & Griinari, 2003). However, a recent study (Lock et al., 2007) using chemically synthesized *trans*-10 C18:1 infused postruminally over 4 days at 42.6 g/day/cow showed that despite the fact that this isomer was absorbed, taken up by the mammary gland, and transferred to milk fat, it had no effect on milk fat synthesis. As suggested by the authors (Lock et al., 2007), it is likely that the formation of *trans*-10 C18:1 and of *trans*-10, *cis*-12-CLA due to alterations in the rumen environment is accompanied by the formation of other biohydrogenation intermediates that could as well inhibit (or co-inhibit with *trans*-10, *cis*-12-CLA) milk fat synthesis. Thus, from nutritional studies, several other rumen-derived FA were proposed recently as potential inhibitors of cow milk fat synthesis due to high negative correlations between their milk fat concentrations and milk fat content and secretion. These proposed FA were several *cis*- or *trans*-C18:1 isomers, and *cis*-9, *trans*-13 C18:2, *cis*-9, *trans*-12 C18:2, *trans*-11, *cis*-13 CLA, *trans*-11, *cis*-15 C18:2 (Loor et al., 2005a), and *trans*-9, *cis*-11 CLA (Roy et al., 2006a; Shingfield et al., 2005, 2006). To confirm the potential role of these FA as well as of other minor CLA isomers found in milk, when available, few of them were postruminally infused. Whereas *trans*-8, *cis*-10 CLA, *cis*-11, *trans*-13 CLA, and *trans*-10, *trans*-12 CLA did not

inhibit milk fat yield (Perfield et al., 2004, 2006; Sæbo et al., 2005), it was demonstrated that *cis*-10, *trans*-12 CLA (Sæbo et al., 2005) and *trans*-9, *cis*-11 CLA (Perfield et al., 2005) did reduce milk fat synthesis. However, the efficiency of the *trans*-9, *cis*-11 CLA was much lower than that of *trans*-10, *cis*-12 CLA (Perfield et al., 2005). This last result is in agreement with feeding trials on cows with or without MFD, with a greater slope of the equation between milk fat yield and *trans*-10, *cis*-12 CLA than *trans*-9, *cis*-11 CLA (Roy et al., 2006a).

Again, some of these isomers (*trans*-7, *cis*-9 CLA, *trans*-11, *cis*-13 CLA, *cis*-9, *trans*-13 C18:2, *trans*-11, *cis*-15 C18:2, *cis*-9, *trans*-13 C18:2) increased in goats supplemented with dietary PUFA, whereas no MFD was observed (Chilliard & Ferlay, 2004; Chilliard et al., 2003c; Ferlay et al., 2003).

In Vitro Studies on the Effect of Fatty Acids on Lipogenesis in Mammary Epithelial Cells

Studies several years ago on dispersed bovine MEC (Hansen et al., 1986; Hansen & Knudsen, 1987) demonstrated that the addition of C18:0, *cis*-9 C18:1, or C18:2 (*n*-6) inhibited *de novo* synthesis of FA with 16 carbons or less, except C4. More recently, looking at the effects of specific FA on the bovine mammary cell line (MAC-T cells), Jayan and Herbein (2000) showed that, compared to stearic acid, *trans*-11 C18:1 and, to a lesser extent, *cis*-9 C18:1 reduced ACC and FAS activities.

Furthermore, 35 years ago, Bickerstaffe and Annison (1970) observed negative effects of oleic, linoleic, and linolenic acids on goat mammary SCD activity measured *in vitro*, which have been partly confirmed by our *in vivo* studies on goats (see earlier section). The addition of *trans*-11 C18:1 increased *SCD* mRNA abundance in bovine MEC (Matitashvili & Bauman, 2000) and SCD activity in the bovine MAC-T cell line (Jayan & Herbein, 2000). Recently, Keating et al. (2006) demonstrated that treatment of bovine MAC-T cells with *trans*-10, *cis*-12 CLA (and, to a lesser extent, *cis*-9, *trans*-11 CLA) caused a significant reduction in *SCD* transcriptional activity, with this effect mediated through the stearoyl-CoA desaturase transcriptional enhancer element region (STE; see earlier section). The same study also showed that bovine *SCD* promoter was upregulated by insulin and downregulated by oleic acid, whereas linoleic, linolenic, stearic, and vaccenic acids had no effect. However, the *in vitro* effects of other specific *trans*-C18:1 and C18:2 isomers on mammary *SCD* gene expression are still unknown.

Elsewhere, in bovine mammary epithelial cell (BME-UV) cultures, the addition of *trans*-10, *cis*-12 CLA inhibited the stimulatory effect of prolactin on the cytosolic $NADP^+$-dependent isocitrate dehydrogenase (*IDH1*) gene expression, involved in the generation of NADPH required for *de novo* fatty acid synthesis, whereas the *cis*-9, *trans*-11 CLA isomer had no effect (Liu et al., 2006).

Further research is necessary in ruminants to identify the more important inhibitors of fat synthesis either *in vivo* (i.e., postruminal infusion of exogenous

FA) or *in vitro*, which is hampered by the lack of pure *trans*-C18:1 and C18:2 isomers and by the difficulty in obtaining an *in vitro* functional model for lipid synthesis and secretion (Barber et al., 1997).

Signaling Pathways Mediating Nutritional Regulation of Gene Expression

Whereas the signaling mechanisms involved in the regulation of lipogenic gene expression in rodent liver and adipose tissue have been comprehensively described (Clarke, 2001), little is known about these mechanisms in ruminants, particularly in the mammary gland. However, it was suggested that several genes involved in milk fat synthesis in the bovine mammary gland may share a common regulatory mechanism because of their coordinated downregulation observed in response to a postruminal infusion of *trans*-10, *cis*-12 CLA (Baumgard et al., 2002) and to an MFD diet (Peterson et al., 2003). Clarke (2001) reviewed rodent data and proposed that PUFA control the main metabolic pathways of lipid metabolism by governing the DNA-binding activity and nuclear abundance of selected transcription factors regulating the expression of key genes. The major transcription factors involved are sterol regulatory binding protein-1 (SREBP-1) and peroxisome proliferator-activated receptors (PPARs), with the FA or cholesterol acting by binding to the nuclear receptors PPAR (and LXR, HNF-4α), whereas the FA induce changes in the nuclear abundance of SREBP. As only few data are available in ruminants on these transcription factors, the following sections describe the state of knowledge on transcription factors mediating nutritional regulation of gene expression in rodents as well.

SREBP-1

SREBPs are basic-helix-loop-helix-leucine zipper (bHLH-LZ) transcription factors that belong to a family of transcription factors that regulate lipid homeostasis by controlling the expression of several enzymes required for endogenous cholesterol, FA, triacylglycerol, and phospholipid synthesis. Three SREBP forms have been characterized in rodents—SREBP-1a, -1c, and -2—differing in their roles in lipid synthesis. The SREBP-1a form is mainly expressed in cultured cells and tissues with a high cell proliferation capacity. The SREBP-1c form is expressed in many organs (mainly adipose tissue, brain, muscle, etc.) (Shimomura et al., 1997). These two forms derive from a single gene through the use of alternate promoters that give rise to different first exons (Brown & Goldstein, 1997). SREBP-2 derives from a different gene and is involved in the transcription of cholesterogenic enzymes.

SREBPs are synthesized as ~1,150 amino acid inactive precursors bound to the membrane of the endoplasmic reticulum (ER) through a tight association with SREBP cleavage activating protein (SCAP) (Miller et al., 2001). Upon sterol deprivation, the SREBP-SCAP complex moves to the Golgi apparatus, where two functionally active distinct proteases, site-1 and site-2 proteases (S1P and S2P), sequentially cleave the precursor protein SREBP to release the NH_2-terminal active domain (Sakai et al., 1996). It has been shown that this sterol-dependent trafficking requires an intact sterol-sensing domain located in the SCAP protein (Brown & Goldstein, 1997), demonstrating the dual role of SCAP as escorter and sensor. Recently, the insulin-induced gene (*INSIG-1*) protein that binds SCAP and thus facilitates retention of the SCAP/SREBP complex in the ER was identified (Yang et al., 2002). Upon appropriate conditions (low sterol concentrations or possibly insulin action; Eberlé et al., 2004), the interaction between INSIG and SCAP decreases and allows the SCAP to escort SREBPs to the Golgi apparatus for the cleavage activation process.

Finally, the mature SREBP, i.e., the NH_2-terminal portion (domain containing the bHLH-LZ), is translocated to the nucleus, where it binds (as a homodimeric form) its target genes on sterol binding elements or on palindromic sequences called E-boxes within their promoter regions (Wang et al., 1993). These target genes are implicated in cholesterol, FA, and lipid synthesis, including LPL, ACC, FAS, and SCD (Shimomura et al., 1998).

In rodents, FA downregulate the nuclear abundance of SREBP-1 by two described mechanisms: either an inhibition of the proteolytic activation process of SREBP-1 protein (under cholesterol dependence) or an inhibition of the *SREBP-1* gene transcription. Recently, in the ovine lactating mammary gland, Barber et al. (2003) identified SREBP-1 as a major regulator of *de novo* lipid synthesis through the activation of ACCα PIII, achieved together with NF-Y, USF-1, and USF-2 transcription factors. In addition, SREBP-1 binding motifs were also identified in the proximal promoter of ACCα PII, which is upregulated during lactation, indicating that SREBP-1 could play an important role in the joint regulation of PII and PIII in mammary tissue (Barber et al., 2003). Elsewhere it was shown that the addition of *trans*-10, *cis*-12 CLA to the bovine MEC line (MAC-T) had no effect on *SREBP-1* mRNA or SREBP-1 precursor protein content but reduced the abundance of the activated nuclear fragment of the protein (Peterson et al., 2004). This was accompanied by a reduction in transcriptional activation of the lipogenic genes *ACACA*, *FASN,* and *SCD*. These findings suggest that the inhibitory effect of this CLA isomer on lipid synthesis could be due to an inhibition of the proteolytic activation of SREBP1. Similarly, the use of microarray tools to characterize mammary gene profiling in cows fed an MFD diet (composed of 70% forage, 25% concentrate, and 5% soybean oil) showed a downregulation of several genes associated with fatty acid metabolism (see previous section) and of eight transcription factors without modification of *SREBP1* gene expression (Loor et al., 2005c). In addition, Harvatine and Bauman (2006) demonstrated the existence of a joint decrease in the mammary expression of *SREBP1*, *Spot 14* (*S14*), *INSIG-1*, *FASN,* and *LPL*

that could explain MFD in lactating cows either fed a low-forage/high-oil diet or infused with *trans*-10, *cis*-12 CLA. Again, the decreased expression of SREBP-1 and proteins associated with SREBP-1 activation, together with SREBP-1 responsive lipogenic enzymes, provides strong support for the central role of SREBP-1 in the regulation of milk fat synthesis. Furthermore, this study outlined a possible involvement of S14 in the regulation of FA synthesis in the bovine mammary gland, as shown in rodent liver and adipose tissue (Cunningham et al., 1998).

PPARs

PPARs belong to a superfamily of hormone receptors with, as for all of the members of this family, a DNA-binding domain, a gene-activating domain, and a ligand-binding domain. They regulate the transcription of genes involved in different lipid metabolism pathways including the transport of plasma triglycerides, the cellular FA uptake, and the peroxisomal and mitochondrial β-oxidation (Schoonjans et al., 1996a). The activating ligands of PPARs are peroxisome proliferators, including chemical molecules as fibrates, thiazolidinedione as well as molecules as FA, including PUFA, and their metabolites. PPARs heterodimerize with the *cis*-9 retinoic acid receptor (RXR) to bind to specific response elements located in the promoter region of the target genes.

Three PPAR subtypes have been identified: PPARα, expressed mainly in liver as well as in heart, kidney, intestinal mucosa, and brown adipose tissue, involved in FA transport and β– and ω-oxidation; PPARβ abundantly and ubiquitously expressed but mainly found in heart, lung, and kidney; PPARγ, most abundant in adipose tissue, stimulating adipocyte differentiation and lipogenesis of the mature adipocyte (Schoonjans et al., 1996b). PPARγ is also expressed in a number of epithelial tissues (breast, prostate, and colon), in which it seems to favor less malignant phenotype cells in human cancer (Sarraf et al., 1999). The *PPARγ* gene generates two transcripts, designated *PPARγ1* and γ2, resulting from differential mRNA splicing and promoter usage (Yeldandi et al., 2000), and leading to two protein isoforms, with PPARγ2 having 30 additional amino acid residues at the N terminal extremity. Among the activating compounds of the *PPAR* genes, (*n*-3) and (*n*-6) PUFA and mainly their metabolites (eicosanoids and oxidized FA) are the major natural activators of PPARα (Clarke, 2001), while 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 is the activator of the PPARγ subtype (Rosen & Spiegelman, 2001). In addition, *in vitro* studies on mature adipocytes revealed that *trans*-10, *cis*-12 CLA downregulates *PPARγ* gene expression, that could be a mechanism by which this CLA isomer prevents lipid accumulation in adipocytes (Granlund et al., 2003).

The few data available in bovine show similarities with those from rodent species. Thus, in bovine subcutaneous adipose tissue, the observed joint upregulation of PPARγ and FAS and *ACACA* and *LPL* gene expression by

propionate infusion (Lee & Hossner, 2002) suggests an implication of PPARγ in the nutritional or insulin activation of lipogenesis. Elsewhere, bovine *PPARγ1* and *PPARγ2* cDNAs have been characterized (Sundvold et al., 1997) with expression of the two isoforms in adipose tissue, whereas only PPARγ2 was expressed in the mammary gland. Recently, in primary cultured bovine MEC, the expression of PPARγ2 in response to the addition of acetate and octanoate was increased while ACC activity decreased (Yonezawa et al., 2004a), conversely to previous observations in adipose tissue (Lee & Hossner, 2002). Elsewhere, an upregulation of mammary *PPARγ* gene expression was shown in dairy cows between –14 and +14 days relative to parturition, using a bovine cDNA microarray (Loor et al., 2004c).

Other Transcription Factors

The molecular mechanisms that control milk protein and lipogenic gene expression are not fully understood and probably involve undiscovered proteins within the mammary gland. Thus, in the nuclear extract of bovine mammary gland, the transcription factors Sp1 and NF-1 were identified (Wheeler et al., 1997), which are already known in rodents to act in conjunction with other proteins such as SREBP1, as well as six other proteins whose abundance was positively related with lactation or pregnancy status. Four of these proteins were identified as lactoferrin, annexin II, vimentin, and heavy-chain immunoglobulin. The presence of lactoferrin in the nuclear extracts is consistent with a study demonstrating that lactoferrin binds to DNA in a sequence-specific manner and activates transcription (He & Furmanski, 1995). Nevertheless, the function of lactoferrin as a transcription factor has not yet been confirmed.

Elsewhere, over the past few years, response elements to lactogenic hormones have been mapped within the promoters of milk protein genes; in some cases, the proteins that mediate the lactational signals are known. Signal transducer(s) and activator(s) of transcription (STAT) form a family of cytoplasmic proteins that are activated in response to a large number of cytokines, growth factors, and hormones (Hennighausen, 1997). The STAT proteins are activated via a cascade of phosphorylation events in which Janus protein tyrosine kinases (Jak2) are first phosphorylated. Then the activated Jak2 phosphorylate STAT proteins. In turn, STAT detach from the receptor complex, form homo- or heterodimers, and translocate from the cytoplasm to the nucleus, where they interact with specific promoter regions and regulate gene expression (Hennighausen, 1997). Until now, seven bovine *STAT* genes have been identified, *STAT1-6*, *5a,* and *5b*, the latter two of which have already been sequenced (Seyfert et al., 2000). STAT5 was originally identified as a "mammary gland factor" mediating the prolactin signal to establish galactopoiesis (Rosen et al., 1999). Recently, Mao et al. (2002) demonstrated that STAT5 binding (at position –797) contributes to the lactational stimulation of

promoter III (PIII) of the *ACACA* gene in the mammary gland. Hence, prolactin, acting through STAT5, contributes to the activation of ACC expression in milk-producing cells. Similarly, in BME-UV bovine mammary epithelial cell, Liu et al. (2006) reported that prolactin enhances *IDH1* mRNA and protein expression, but the molecular mechanisms of this regulation were not investigated. Elsewhere, in bovine mammary gland explant culture, Yang et al. (2000a) showed a rapid stimulation of STAT5 DNA binding activity by prolactin, growth hormone, and IGF1. In addition, the same authors demonstrated *in vivo* that STAT5 protein level and DNA binding activity are modulated by several physiological signals, including GH infusion and milking frequency (Yang et al., 2000b). Altogether these findings suggest that STAT5 might be important in determining the milk composition by coordinating FA and protein synthesis during lactation and that STAT5 transcription factor may represent part of the common route by which different extracellular signals (linked to hormonal status as well as to milking frequency) could converge and be transduced intracellularly to coordinate cell functions in the mammary gland. Recently, a study reported an association between *STAT1* variants and milk fat and protein yield and percentages in Holstein dairy cattle, implicating the *STAT1* gene in the regulation of milk protein and fat synthesis (Cobanoglu et al., 2006).

Despite the recent increased knowledge in ruminants on the characterization of transcription factors in the mammary gland, many questions still remain unanswered, in particular the role of STAT and PPARs in the regulation of lipid metabolism.

Conclusions

Over the last several years, the biochemical pathways of lipid synthesis in the mammary gland have been elucidated, and many of the enzymatic proteins and their cDNAs have been characterized. This has allowed the development of studies on the nutritional regulation of a few "candidate", genes involved in mammary FA uptake (*LPL*), *de novo* synthesis (*ACACA* and *FASN*), and desaturation (*SCD*). These studies showed that the responses of mammary "candidate" gene expression to nutritional factors do not always match milk FA secretion responses. In goats and cows, data suggest that the availability of substrates rather than the LPL activity is the limiting factor in the uptake of long-chain FA, except with extreme MFD diets fed to cows, in which both mammary LPL mRNA and activity decreased. In cows and goats, data converged to demonstrate that *ACACA* and *FASN* gene expressions are key factors of short- and medium-chain FA synthesis, even though they are not always repressed by the addition of PUFA to the diet, in goats at least. In this species, *ACACA* and *FASN* gene expressions are regulated by dietary factors at a transcriptional level at least, and *SCD* is regulated at a transcriptional and/or

posttranscriptional level, depending on the lipid supplements. Conversely, in cows, the level of *SCD* mRNA varied little with the nutritional factors studied so far, except for a decrease when "protected" fish oil was fed. A fine balance between the exogenous unsaturated FA and the SCD desaturation products must be maintained within the mammary gland in order to preserve the fluidity of cellular membranes and milk fat (Chilliard et al., 2000; Parodi, 1982). In addition to its role on milk nutritional quality via the synthesis in *cis*-9, *trans*-11 CLA and *cis*-9-monounsaturated fatty acids, the impact of SCD on membrane fluidity underlines the importance of this enzyme.

The regulatory systems governing the nutritional response of mammary gene expression, in particular the intracellular signaling systems involved in these regulations, need to be further investigated in the future. The basis of the effects of nutrients and particularly the identification of specific *trans*-FA controlling lipogenic gene expression are obvious targets. Milk *trans*-10, *cis*-12 CLA is sometimes correlated with milk fat depression in cows (but not in goats) and, when infused postruminally at high doses, acts as a potent inhibitor of the expression of all the lipogenic genes. Conversely, *trans*-10 C18:1 does not directly control milk fat synthesis in dairy cows, although it largely responds to dietary factors, with its concentration being negatively related to milk fat response in cows and, to a lesser extent, in goats. Nevertheless, marked differences are observed between the milk fat yield responses of these two species, with few differences in milk FA profile responses, in particular lower increases in *trans*-10 C18:1 in goats. Elsewhere, more information on the promoter of the lipogenic genes should be acquired, which would help to clarify the roles and mechanisms of the action of PUFA and/or *trans*-FA, in order to better understand the molecular mechanisms involved in dietary- and/or species-related responses. Few data on transcription factors are available, and a central role for SREBP-1 has been outlined as mediator of FA effects, and STAT5 for hormonal and physiological effects at least, whereas the roles of PPARs need to be determined. It is expected that the development of *in vitro* functional systems for lipid synthesis and secretion would allow future progress in the identification of the inhibitors and activators of fat synthesis and in understanding differences between ruminant species.

This chapter reviewed studies focusing on the nutritional regulation of the expression of a few candidate genes controlling lipid synthesis. Nevertheless, the expression of specific milk fat globule membrane proteins (Mather, 2000) such as butyrophilin, xanthine oxidoreductase, and CD36, which intervene in milk lipid secretion, is also likely to have consequences on milk fat yield and composition (Ogg et al., 2004). The recent development of tools for studying the mammary transcriptome (macro- and microarrays; e.g., Bernard et al., 2005a; Leroux et al., 2003; Loor et al., 2004c, 2005c; Suchyta et al., 2003) and proteome (Daniels et al., 2006) will allow us to study the effect of nutritional changes on the expression (mRNA and protein) of a large number of genes putatively involved in mammary gland function, including lipid synthesis and secretion, in relationship to milk FA profile (Ollier et al., 2007). Such tools will allow us to

identify new "candidate" genes or proteins whose expression is regulated by nutrition, and to understand their regulation pathways. In addition, further investigations on ruminal digestion and body metabolism of nutrients (absorption, partitioning between tissues) and mammary metabolic flows will also contribute to highlight the mechanisms underlying the *in vivo* responses to dietary factors and the differences among ruminant species.

Acknowledgment The authors thank S. Ollier and M. Bonnet for helpful discussions during the preparation of the manuscript and P. Béraud for secretarial assistance.

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