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Role of *trans* fatty acids in the nutritional regulation of mammary lipogenesis in ruminants

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Fat is an important constituent contributing to the organoleptic, processing and physical properties of ruminant milk. Understanding the regulation of milk fat synthesis is central to the development of nutritional strategies to enhance the nutritional value of milk, decrease milk energy secretion and improve the energy balance of lactating ruminants. Nutrition is the major environmental factor regulating the concentration and composition of fat in ruminant milk. Feeding low-fibre/high-starch diets and/or lipid supplements rich in polyunsaturated fatty acids induce milk fat depression (MFD) in the bovine, typically increase milk fat secretion in the caprine, whereas limited data in sheep suggest that the responses are more similar to the goat than the cow. Following the observation that reductions in milk fat synthesis during diet-induced MFD are associated with increases in the concentration of specific trans *fatty acids in milk, the biohydrogenation theory of MFD was proposed, which attributes the causal mechanism to altered ruminal lipid metabolism leading to increased formation of specific biohydrogenation intermediates that exert anti-lipogenic effects.* Trans*-10,* cis*-12 conjugated linoleic acid (CLA) is the only biohydrogenation intermediate to have been infused at the abomasum over a range of experimental doses (1.25 to 14.0 g/day) and shown unequivocally to inhibit milk fat synthesis in ruminants. However, increases in ruminal* trans*-10,* cis*-12 CLA formation do not explain entirely diet-induced MFD, suggesting that other biohydrogenation intermediates and/or other mechanisms may also be involved. Experiments involving abomasal infusions (g/day) in lactating cows have provided evidence that* cis*-10,* trans*-12 CLA (1.2),* trans*-9,* cis*-11 CLA (5.0) and* trans*-10 18:1 (92.1) may also exert anti-lipogenic effects. Use of molecular-based approaches have demonstrated that mammary abundance of transcripts encoding for key lipogenic genes are reduced during MFD in the bovine, changes that are accompanied by decrease in sterol response element binding protein 1 (SREBP1) and alterations in the expression of genes related to the SREBP1 pathway. Recent studies indicate that transcription of one or more adipogenic genes is increased in subcutaneous adipose tissue in cows during acute or chronic MFD. Feeding diets of similar composition do not induce MFD or substantially alter mammary lipogenic gene expression in the goat. The available data suggests that variation in mammary fatty acid secretion and lipogenic responses to changes in diet composition between ruminants reflect inherent interspecies differences in ruminal lipid metabolism and mammary specific regulation of cellular processes and key lipogenic enzymes involved in the synthesis of milk fat triacylglycerides.*

Keywords: milk fat, *trans* fatty acids, gene expression, lipogenesis, ruminants

Implications

Understanding the regulation of milk fat synthesis is central to the development of nutritional strategies to enhance the nutritional value of milk, decrease milk-energy secretion and improve the energy balance of lactating ruminants. Studies in lactating cows have provided evidence that decreases in milk fat synthesis are related to increases in ruminal outflow of specific *trans* fatty acids that exert anti-lipogenic effects. Diets that cause milk fat depression in cows increase milk fat synthesis in goats with indications that interspecies differences are related to the impact on ruminal lipid metabolism and mammary specific regulation of cellular processes and key lipogenic enzymes. Molecular-based techniques have provided an insight into the molecular mechanisms underlying the nutritional regulation of mammary lipogenesis in ruminants. It is anticipated that future studies will elucidate a direct cause and effect and facilitate the development of several innovations to optimize milk fat content and composition including genomic-based selection of ruminant livestock.

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Introduction

Changes in the demand for milk fat during recent decades have resulted in the restructuring of major global milk markets, leading to protein commanding a higher premium than fat within milk payment systems (Lock and Shingfield, 2004). Fat is the major energy constituent in milk and represents a significant proportion of the total energy requirements for lactation in ruminants. Producing milk with a fat content more in line with market requirements could be expected to improve the economic returns from milk production enterprises and decrease the impact on the environment. Lowering milk energy content offers a major competitive advantage within milk purchase agreements, where payments are based on volume rather than the output of milk constituents. Several studies have also provided evidence to indicate that decreases in milk fat content may improve energy balance and the efficiency of nutrient utilization in cows during early lactation (Moore *et al*., 2004; Shingfield *et al*., 2004; Kay *et al*., 2006) or periods of limited nutrient availability (Kay *et al*., 2007). However, realizing these benefits is dependent on developing on-farm strategies to accurately manipulate milk composition and milk fat secretion.

Feeding diets containing plant oils, fish oil or marine algae or rations rich in rapidly fermented carbohydrates results in the production of milk containing a low-fat content, a phenomena often referred to as milk fat depression (MFD). Decreases in milk fat output during diet-induced MFD typically occur within a few days, and in more severe cases milk fat yield can be lowered by more than 50%, with little or no change in the yields of milk, milk protein or lactose (AbuGhazaleh *et al*., 2004; Roy *et al*., 2006; Shingfield *et al*., 2006a). Secretion of all fatty acids is decreased during diet-induced MFD, but the reductions in the output of fatty acids synthesized *de novo* are disproportionately greater (Bauman and Griinari, 2003).

Fat is an important component contributing to the processing attributes and organoleptic properties of milk and dairy products from ruminants (Palmquist *et al*., 1993; Chilliard and Ferlay, 2004). During recent decades, increasing emphasis has been placed on the contribution of ruminant-derived foods on the development or prevention of chronic human diseases including cancer, cardiovascular disease and insulin resistance. Milk and dairy products are the main source of 12:0 and 14:0 in the human diet and also provide a major contribution to total 16:0 and *trans* fatty acids (TFA) consumption in industrialized countries (Givens and Shingfield, 2006). Production of ruminant milk with a fatty acid profile more in line with public health recommendations has the potential to improve long-term human health without requiring substantial changes in consumer eating habits. Numerous studies have demonstrated that the use of specific forage species, forage conservation methods and dietary lipid supplements can be used to alter milk fat composition in ruminants (Dewhurst *et al*., 2006; Chilliard *et al*., 2007; Glasser *et al*., 2008a). Furthermore, plant oils and oilseeds in the diet also decrease methanogenesis in ruminants (Machmüller *et al*., 2003; Martin *et al*., 2008; Beauchemin *et al*., 2009). Due to the interrelationship between diet composition, rumen metabolism and mammary lipogenesis, on-farm feeding regimens for decreasing greenhouse gas emissions or altering milk fatty acid composition can also be expected to influence milk fat content (Chilliard *et al*., 2009) depending on diet composition and ruminant species.

Understanding the role of diet on milk fat synthesis and the underlying mechanisms regulating mammary lipogenesis in ruminants is central to the formulation of diets and/or supplements for strategic changes in milk fat content and composition. Numerous reviews have considered the effect of nutrition on milk fat synthesis in ruminants (Bauman and Griinari, 2003; Chilliard *et al*., 2007; Shingfield and Griinari, 2007), whereas several appraisals have considered possible molecular mechanisms underlying diet-induced changes in mammary lipogenesis and fat secretion (Bauman *et al*., 2008; Bernard *et al*., 2008; Harvatine *et al*., 2009a). In the following sections, the role of TFA formed during ruminal metabolism of dietary unsaturated fatty acids, lipogenic precursor supply and products of intermediary metabolism on milk fat secretion are examined. Recent evidence on factors regulating mammary lipogenic enzyme activity and gene expression in ruminants is also considered within the context of highlighting areas for further investigation and providing an insight into the possible causes for variable milk fat secretion responses to changes in diet composition between ruminant species.

Background

Decreases in milk fat synthesis are common in cows fed diets containing: (i) high proportions of concentrates, (ii) fish oil and marine algal lipids or (iii) ionophores (Bauman and Griinari, 2001 and 2003). Even though certain attributes of a diet are important in the establishment of MFD, there is considerable interaction between the composition of the basal diet, amount and source of dietary lipid supplement and feeding frequency (Chilliard *et al*., 2007; Shingfield and Griinari, 2007).

A number of theories have been proposed to explain diet-induced MFD. The major theories have attributed the decreases in milk fat synthesis during diet-induced MFD to (i) reductions in the supply of acetate and 3-hydroxy-butyrate for *de novo* fatty acid synthesis in the mammary gland, (ii) elevated insulin secretion stimulating the preferential partitioning of fatty acids towards adipose tissue at the expense of the mammary gland or (iii) direct inhibition of mammary lipogenesis by TFA formed during the biohydrogenation of dietary unsaturated fatty acids in the rumen. Several reviews have provided a comprehensive treatise of the historical background of diet-induced MFD and a critical evaluation of the main theories proposed to explain the decreases in milk fat synthesis (Bauman and Griinari, 2001 and 2003; Griinari and Bauman, 2003).

Early studies reported that decreases in milk fat output for cows fed diets causing MFD were associated with an increase in milk *trans* 18:1 concentrations (refer to Bauman

and Griinari, 2001). However, post-ruminal infusions of *trans*-9 18:1 had no effect on milk fat synthesis in lactating cows (Rindsig and Schultz, 1974) indicating a much more complicated cause and effect. Use of more advanced gas-chromatography methods for the analysis of milk fat composition provided the first indications that MFD was related to 18:1 isomer profiles rather than absolute increases in milk *trans* 18:1 content *per se* (Griinari *et al*., 1998). Following reports that diet-induced MFD was associated with an increase in milk *trans*-10 18:1 content (Griinari *et al*., 1998; Piperova *et al*., 2000) and *trans*-10, *cis*-12 conjugated linoleic acid (CLA) inhibits milk fat synthesis (Baumgard *et al*., 2000), the biohydrogenation theory of MFD was proposed (Bauman and Griinari, 2001), which states that 'under certain dietary conditions the pathways of biohydrogenation are altered to produce unique fatty acid intermediates which are potent inhibitors of milk fat synthesis'. Of all the hypotheses developed to explain diet-induced MFD, the biohydrogenation theory appears to be the most robust and offers a more convincing explanation for MFD over a wider range of diets. However, increases in ruminal outflow of *trans*-10, *cis*-12 CLA, do not explain the decreases in milk fat synthesis in all cases of diet-induced MFD, with the implication that additional biohydrogenation intermediates and/or mechanisms must be involved (Shingfield and Griinari, 2007).

Ruminal lipid metabolism

Ruminant diets typically contain between 20 and 40 g lipid/kg dry matter (DM). Even though the diet contains a high proportion of unsaturated fatty acids, ruminant meat and milk contain much higher levels of saturated fatty acids due in part, to extensive biohydrogenation of dietary unsaturated fatty acids in the rumen. Following ingestion, ester linkages in triacylglycerides (TAG), phospholipids and glycolipids are hydrolyzed by the action of bacterial lipases and the non-esterified fatty acids (NEFA) released into the rumen are adsorbed onto feed particles and biohydrogenated or incorporated directly into bacterial lipids. Numerous *in vitro* and *in vivo* studies have allowed the major pathways of ruminal biohydrogenation to be elucidated (Harfoot and Hazlewood, 1988; Palmquist *et al*., 2005; Jenkins *et al*., 2008). Metabolism of linoleic acid (LA; *cis*-9, *cis*-12 18:2) and linolenic acid (LNA; *cis*-9, *cis*-12, *cis*-15 18:3) is considered to involve at least two distinct populations of ruminal bacteria that under normal conditions is thought to proceed via isomerization of the *cis*-12 double bond resulting in the formation of a conjugated 18:2 or 18:3 fatty acid, respectively. Conjugated products are transient and are sequentially reduced to 18:0 as the final end product via *trans*-11 18:1. The final reduction step is considered rate limiting and therefore *trans* 18:1 intermediates can accumulate and flow out of the rumen. On most diets, biohydrogenation of LA and LNA varies between 70% and 95% and 85% and 100%, respectively (Doreau and Ferlay, 1994; Glasser *et al*., 2008c), indicating that with the exception of diets containing marine oils, 18:0 is the major fatty acid leaving the rumen.

Fewer studies have examined the fate of oleic acid (OA; *cis*-9 18:1) in the rumen. Incubations with mixed or pure cultures of rumen bacteria demonstrated that OA can be biohydrogenated to 18:0 (Harfoot and Hazlewood, 1988), whereas more recent studies indicated that metabolism of OA *in vitro* results in the formation of oxygenated fatty acids (10-OH 18:0 and 10-O 18:0) and numerous *trans*-18:1 intermediates with double bonds at positions Δ6-Δ16 (Mosley *et al*., 2002; Jenkins *et al*., 2006; McKain *et al*., 2010; Figure 1). Direct comparisons indicate that the extent of biohydrogenation of OA is also lower compared with LA and LNA and typically varies between 58% and 87% (Loor *et al*., 2004 and 2005d; Doreau *et al*., 2009).

Ruminal biohydrogenation of dietary unsaturated fatty acids in lactating cows results in the formation of a wide and diverse range of fatty acid intermediates that following digestion and absorption can be incorporated into milk fat (Piperova *et al*., 2002; Shingfield *et al*., 2003; Loor *et al*., 2004). The occurrence of numerous isomers of *trans* 18:1,

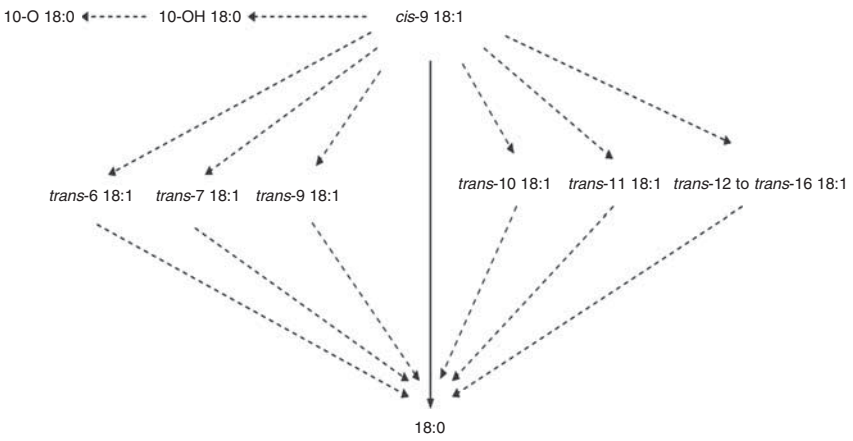

Figure 1 Putative pathways describing *cis*-9 18:1 metabolism in the rumen (adapted from Mosley *et al*., 2002; Jenkins *et al*., 2006; McKain *et al*., 2010). Arrows with solid lines highlight the major biohydrogenation pathway, whereas arrows with dashed lines describe the formation of minor fatty acid metabolites.

18:2 and 18:3 fatty acids containing one or more *trans* double bonds indicate that the metabolic pathways often used to describe ruminal biohydrogenation are an over simplification of the actual intermediates formed in the rumen. Characterizing the biohydrogenation process in more detail represents a major challenge, owing to the limited number of studies reporting sufficiently comprehensive determinations of ruminal fatty acid outflow and because changes in the production of intermediates to oils rather than pure fatty acids in the diet have been examined. Consequently, it is often difficult or impossible to elucidate unequivocally the metabolic origins of a specific minor biohydrogenation intermediate. Nevertheless, recent *in vitro* and *in vivo* studies have provided additional insight into possible biochemical pathways accounting for the formation of specific intermediates during the metabolism of LA (Figure 2) and LNA (Figure 3).

Fish oil or marine algal oil rich in long chain polyunsaturated fatty acids (PUFA) in the diet are known to inhibit the complete biohydrogenation of C18 unsaturated fatty acids resulting in an increase in ruminal *trans* 18:1 and *trans* 18:2 outflow (Shingfield *et al*., 2003; Kim *et al*., 2008; Lee *et al*., 2008). Incubations with rumen micro-organisms have shown that eicosapentaenoic acid (EPA; *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 20:5) and docosahexaenoic acid (DHA; *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 22:6), cause *trans* 18:1

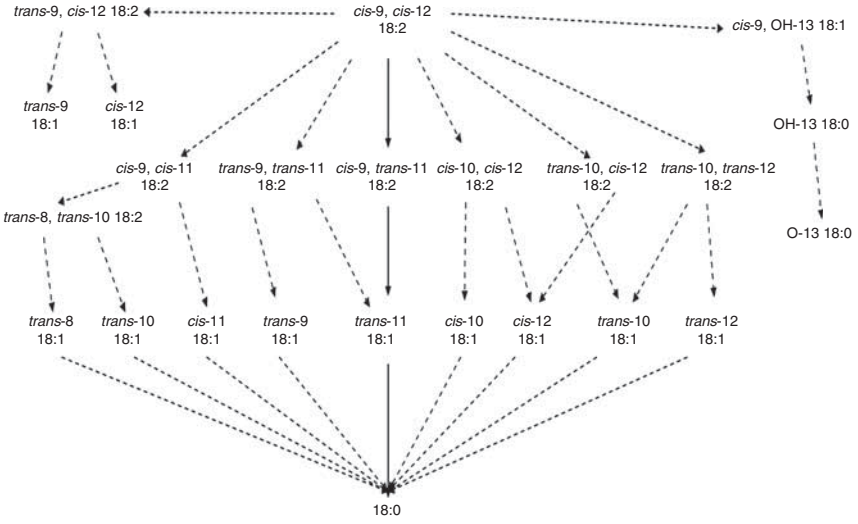

Figure 2 Putative pathways describing *cis*-9, *cis*-12 18:2 metabolism in the rumen (adapted from Harfoot and Hazlewood, 1988; Hudson *et al*., 1998; Wallace *et al*., 2007; Shingfield *et al*., 2008a). Arrows with solid lines highlight the major biohydrogenation pathway, whereas arrows with dashed lines describe the formation of minor fatty acid metabolites.

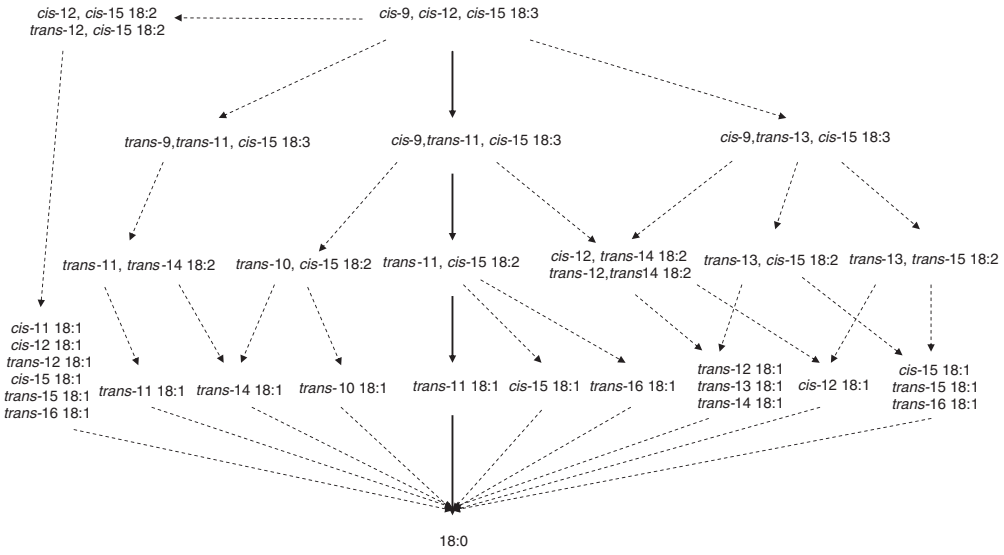

Figure 3 Putative pathways describing *cis*-9, *cis*-12, *cis*-15 18:3 metabolism in the rumen (adapted Harfoot and Hazlewood, 1988; Destaillats *et al*., 2005; Wąsowska *et al*., 2006 and Shingfield *et al*., 2008a). Arrows with solid lines highlight the major biohydrogenation pathway, whereas arrows with dashed lines describe the formation of minor fatty acid metabolites.

to accumulate (AbuGhazaleh and Jenkins, 2004), but it is possible that other 20 and 22 carbon unsaturated fatty acids also contribute to the effects of fish oil on ruminal lipid metabolism. Even though numerous studies have demonstrated that EPA and DHA are extensively metabolized in the rumen, the mechanisms responsible and intermediates formed are not known. Due to a lack of substantive data there is a need to characterize the biochemical pathways of ruminal EPA and DHA metabolism and the biohydrogenation intermediates formed to provide further insight into the causes of MFD on diets containing marine lipids.

Mammary lipogenesis

Bovine and caprine milk typically contains between 30 and 50 g fat/kg depending on diet, stage of lactation and genotype (Chilliard *et al*., 2003; Givens and Shingfield, 2006). The same nutritional, physiological and genetic factors also contribute to variations (40 to 110 g/kg) in ovine milk fat content (Pulina *et al*., 2006). Milk fat globules are essentially comprised of TAG (96% to 98% of total milk lipids) and small amounts of 1,2-diacylyglycerides and monoacylglycerides (0.02%), free fatty acids (0.22%) and retinol esters (Jensen, 2002). Milk TAG are thought to contain more than 400 individual fatty acids, but quantitatively saturated fatty acids of chain lengths from 4 to 18 carbon atoms, *cis*-9 16:1, OA, *trans* 18:1 and LA are the most abundant (Jensen, 2002). Fatty acids incorporated into milk TAG are derived from two sources, uptake of preformed fatty acids from peripheral circulation and fatty acid synthesis in mammary secretory cells (Figure 4). Depending on breed, stage of lactation and diet, fatty acid synthesis *de novo* in the bovine contributes to proportionately 0.60 on a molar basis or 0.40 by weight to total fatty acid secretion in milk (Bauman and Davis, 1974).

Mammary epithelial cells synthesize short- and medium-chain fatty acids using acetate and 3-hydroxy-butyrate as substrates. Fatty acid synthesis *de novo* accounts for all 4:0 to 12:0, most of the 14:0 (ca. 95%) and about 50% of 16:0 secreted in milk, whereas all 18 carbon and longer chain fatty acids are thought to be derived from circulating plasma lipids (Chilliard *et al*., 2000). *De novo* fatty acid synthesis has an absolute requirement for acetyl-CoA, the presence of two key enzymes, acetyl-CoA carboxylase (ACC, E.C. 6.4.1.2) and fatty acid synthetase (FAS, E.C. 2.3.1.85) and a supply of NADPH reducing equivalents (Barber *et al*., 1997). Both 3-hydroxy-butyrate and acetate contribute equally to the initial four carbon unit. Acetate is converted to acetyl CoA in the cytosol and used to extend the chain length of synthesized fatty acids via the malonyl-CoA pathway, whereas 3-hydroxy-butyrate is incorporated directly following activation to butyryl CoA (Figure 4). Conversion of acetate to malonyl-CoA, catalyzed by ACC, is considered to be the rate-limiting step (Bauman and Davis, 1974). Recent studies have reported that expression of acyl-CoA synthetase short-chain family member *ACSS2* gene increases in mammary tissue of cows during lactation, and that the changes in transcript

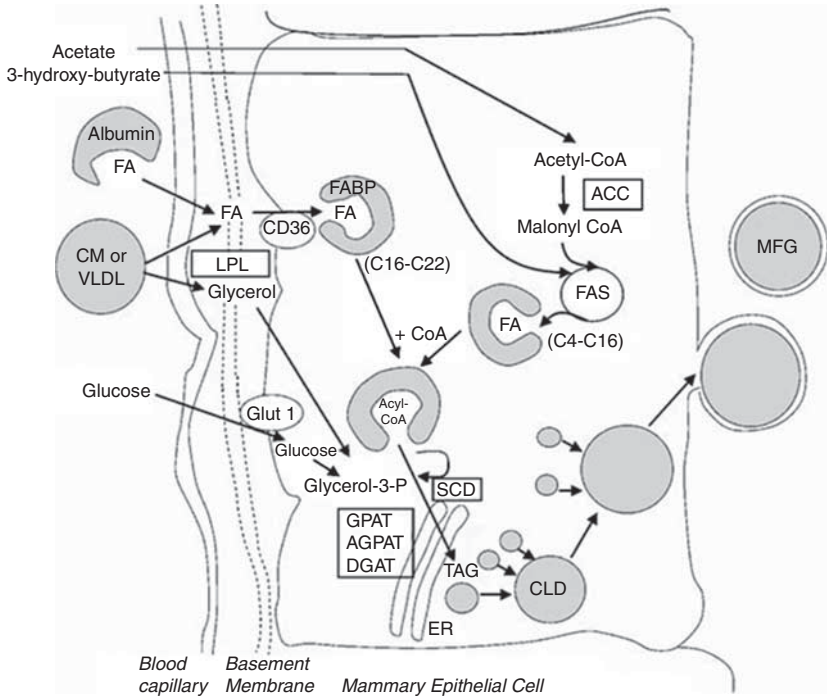

Figure 4 Synthesis of milk fat in the bovine mammary epithelial cell (adapted from Bernard *et al*., 2008). Abbreviations: ACC, acetyl-CoA carboxylase; AGPAT, 1-acyl glycerol 3-phosphate acyl transferase; CD36, cluster of differentiation 36; CLD, cytoplasmic lipid droplet; CoA, coenzyme A; CM, chylomicron; DGAT, diacylglycerol acyltransferase 1; ER, endoplasmic reticulum; FA, fatty acid; FABP, fatty acid binding protein; FAS, fatty acid synthase; Glut 1, glucose transporter 1; GPAT, glycerol-3 phosphate acyl transferase; LPL, lipoprotein lipase; MFG, milk fat globule; SCD, stearoyl-CoA desaturase; TAG, triacylglyceride; VLDL, very low density-lipoprotein.

abundance correspond with increases in mammary acetyl CoA synthesis suggesting that the protein encoded by this gene supplies activated acetate for *de novo* synthesis in the bovine (Bionaz and Loor, 2008b). Long chain fatty acids containing 16 or more carbon atoms inhibit mammary fatty acid synthesis in bovine or caprine mammary epithelial cells *in vitro* with the effects being more potent during incubations with fatty acids containing a longer carbon chain and/or higher degree of unsaturation (Barber *et al*., 1997). The inhibitory effects on ACC enzyme activity offer an explanation for the decreases in the proportions of 8 to 14 carbon fatty acids in milk following an increase in the supply of long chain fatty acids at the mammary gland derived either from the diet or mobilization of body fat (Chilliard *et al*., 2000). Secretion of 16:0 and all longer fatty acids in milk are derived from the TAG fractions of circulating very low-density lipoproteins (VLDL) and chylomicrons or plasma albumin bound NEFA. Fatty acids are imported into the mammary secretory cell following the action of mammary lipoprotein lipase (LPL, E.C. 3.1.1.34). Both VLDL and chylomicrons are anchored to mammary endothelium by LPL that acts on TAG contained within the lipoprotein core to release NEFA. Studies using rodent models suggest that the VLDL receptor (VLDLR) is an essential component of mammary LPL activity (Tacken *et al*., 2001). Expression of *VLDLR* has been shown to be upregulated in the bovine mammary gland throughout lactation, particularly during the first month postpartum (Bionaz and Loor, 2008b).

In vitro studies with cultured mammalian cell lines and use of fatty acid transport protein transgenic and knock out mouse models have allowed a number of candidate proteins involved in the uptake of long chain fatty acids by various tissues to be elucidated including acyl-CoA binding proteins (ACBP), fatty acid binding proteins (FABP), fatty acid transport proteins (FATP; gene name: solute carrier family 27, SLC27), fatty acid translocase (FAT/CD36) and long chain acyl-CoA synthetases (ACSL) (Doege and Stahl, 2005). FABP and ACBP are the main intracellular fatty acid transporters in non-ruminant cells enabling the selective targeting of long-chain fatty acids for metabolism in specific organelles (Doege and Stahl, 2005). Long-chain fatty acids are esterified to CoA esters in the inner surface of the plasma membrane before entering intracellular metabolic pathways. Activation of fatty acids occurs primarily via an ATP-dependent mechanism involving the action of ACSL isoforms (Watkins *et al*., 2007). Measurements in lactating cows have shown that several *ACSL* (*ACSL1, 3, 4* and *5*) and *FABP* (*FABP 1, 3, 4, 5* and *6*) isoforms are transcribed in mammary tissue (Bionaz and Loor, 2008a) and demonstrated that the expression of *ACSL1, FABP3*, *FABP4* and *FABP5* increases several-fold during lactation, whereas changes in the relative abundance of *ACBP* mRNA are much lower suggesting a minor role in mammary lipogenesis in the bovine (Bionaz and Loor 2008b). Passive diffusion is thought to play only a minor role in the transport of fatty acids across cell membranes compared with protein-mediated uptake, which in non-ruminants involves CD36 and SLC27A (Doege and Stahl, 2005). Studies in cows have revealed the occurrence of transcripts encoding for *CD36* (Bionaz and Loor, 2008b) and several *SLC27A* isoforms (1, 2, 3, 5 and 6; Bionaz and Loor, 2008a) in mammary tissue and demonstrated that *CD36* and *SLC27A6* mRNA abundance increases during the first months of lactation (Bionaz and Loor, 2008b). Even though the mechanisms responsible for the transport of fatty acids across the capillary endothelium and interstitial space are not well characterized (Barber *et al*., 1997; Bernard *et al*., 2008), it has been suggested that *SLC27A6*, *ACSL1* and *FABP3* are involved in the coordinate regulation of channelling fatty acids towards milk fat TAG synthesis in the bovine mammary gland (Bionaz and Loor, 2008a).

Long-chain fatty acids entering the mammary secretory cell can be desaturated but there is no evidence that preformed fatty acids are elongated (Chilliard *et al*., 2000). Mammary epithelial cells contain the stearoyl-CoA desaturase (SCD) complex (E.C. 1.14.99.5), often referred to as Δ-9 desaturase, an enzyme that catalyses the oxidation of fatty acyl CoA esters resulting in the introduction of a *cis* double bond between carbon atoms 9 and 10. The SCD catalyzed reaction involves cytochrome b_5, NAD(P)-cytochrome b_5 reductase and molecular oxygen and the CoA ester of fatty acids as a substrate (Palmquist *et al*., 2005). Activity of SCD in the ruminant mammary gland is thought to occur as a mechanism to maintain and regulate the fluidity of milk to ensure efficient ejection from the mammary glands (Timmen and Patton, 1988). Stearoyl and palmitoyl-CoA are the preferred substrates for SCD with 18:0 to OA being the predominant precursor:product of SCD in the bovine mammary epithelial cell (bMEC) (Palmquist *et al*., 2005; Bernard *et al*., 2008). Between 49% and 60% of 18:0 available for mammary TAG synthesis is desaturated with the extent of endogenous conversion accounting for ca. 60% of the OA secreted in milk (Enjalbert *et al*., 1998; Mosley and McGuire, 2007; Glasser *et al*., 2008b). Activity of SCD also contributes to approximately 90% of *cis*-9 14:1 and 50% to 56% of *cis*-9 16:1 in bovine milk fat (Mosley and McGuire, 2007). A number of other saturated acyl CoA also serve as substrates for SCD including 10:0, 12:0, 14:0, 15:0 and 17:0 (Bauman and Davis, 1974; Shingfield *et al*., 2008c). Endogenous synthesis via the action of SCD on *trans*-11 18:1 is responsible for 64% to 97% of *cis*-9, *trans*-11 CLA (Palmquist *et al*., 2005; Shingfield *et al*., 2007; Glasser *et al*., 2008b) and virtually all the *trans*-7, *cis*-9 CLA secreted in bovine milk (Palmquist *et al*., 2005). Experiments with ^{13}C-labelled *trans*-11 18:1 have estimated that 25% or 32% is desaturated to *cis*-9, *trans*-11 CLA in the bovine (Mosley *et al*., 2006) and caprine (Bernard *et al*., 2010) mammary gland, respectively.

De novo fatty acid synthesis, uptake of long chain fatty acids and the formation of SCD products contribute to fatty acid pool available for TAG synthesis. Esterification of fatty acids to glycerol proceeds via the glycerol-3-phosphate pathway. Synthesis of TAG in the mammalian mammary epithelial cell is initiated by acylation at the *sn*-1 position catalyzed by glycerol-3 phosphate acyl transferase (GPAT, E.C. 2.3.1.15). Esterification of fatty acids to *sn*-2 is catalyzed

by 1-acylglycerol 3-phosphate acyl transferase (AGPAT, E.C. 2.3.1.51), whereas diacylglycerol acyltransferase 1 (DGAT1, EC 2.3.1.20) catalyzes the transfer of an acyl moiety from acyl-CoA to *sn*-1,2 diacylglycerol as the final step in TAG synthesis. Eight isoforms of *AGPAT* are expressed in bovine mammary tissue, with *AGPAT6*, *AGPAT1* and *AGPAT3* being the most abundant accounting for 60%, 18% and 10% of total *AGPAT* mRNA, respectively (Bionaz and Loor, 2008a). Expression of *AGPAT6* is the most affected by stage of lactation being some 15-fold higher in mammary tissue of cows on day 60 of lactation compared with day 15 *pre partum* (Bionaz and Loor, 2008a).

Despite the sequential addition to the glycerol backbone, fatty acids are not randomly distributed during the synthesis of TAG. Medium and long chain saturated fatty acids (10:0 to 18:0) account for a between 56% and 62% of fatty acids esterified at *sn*-1 and *sn*-2, with the distribution of 16:0 being equal, whereas 18:0 is preferentially esterified at *sn*-1 and 8:0, 10:0, 12:0 and 14:0 at *sn*-2 (Jensen, 2002). Relatively high molar proportions (44%) of short chain saturated fatty acids (4:0, 6:0 and 8:0) and OA (27%) are located at *sn*-3 in milk fat TAG (Jensen, 2002). The esterification of fatty acids with a relatively low melting point at *sn*-3 is thought to be important in the regulation and maintenance of-milk fat fluidity (Hawke and Taylor, 1995).

Role of substrate supply on mammary lipogenesis

Production of volatile fatty acids (VFA) in the rumen is the main form in which energy yielding substrates enter the blood in ruminant animals. Most of the propionate and butyrate absorbed across the rumen wall is metabolized in the liver and only acetate enters peripheral blood in appreciable amounts (Seal and Reynolds, 1993). Owing to the importance as a carbon source for *de novo* fatty acid synthesis in the mammary gland early theories attributed diet-induced MFD to a decrease in acetate supply. Diets causing MFD often alter rumen fermentation characteristics increasing the ratio of glucogenic : lipogenic precursors that are not always accompanied by a decrease in acetate yield, whereas the extent of decreases in molar acetate proportions are not in direct proportion to the magnitude of MFD (Bauman and Griinari, 2001). Continual intraruminal infusions of acetate and butyrate have been reported to enhance milk fat secretion, whereas propionate tends to decrease milk fat synthesis in lactating cows (Rulquin *et al*., 2007). However, the findings from these studies cannot be considered as direct evidence that MFD is caused by a shortage of short chain fatty acid precursors, but simply indicate that in the absence of other changes, increases in acetate and butyrate supply stimulate mammary lipogenesis. Even though diets causing MFD often induce changes in molar VFA proportions, such changes do not occur in isolation and are also accompanied by alterations in ruminal outflow of biohydrogenation intermediates and end products. For example, increases in the proportion of concentrates in the diet from 400 to 910 g/kg DM in cows fed grass hay were reported to lower milk fat content from 34.5 to 22 g/kg, but MFD under these circumstances was associated with numerical, but non-significant 13% and 27% decreases in net production rates of acetate and butyrate in the rumen (Sutton *et al*., 2003). Due to the interrelationship between diet induced changes in rumen fermentation characteristics and ruminal biohydrogenation the contribution of reduced supply of acetate and butyrate to the decrease in milk fat synthesis during MFD remains uncertain. Recent investigations also indicate that the short chain fatty acids acetate, propionate and butyrate through their binding to and activation of two G-protein-coupled cell surface receptors (bGPR-41 and bGPR-43) are involved in second messenger signalling pathways in bMEC but do not alter cell proliferation (Yonezawa *et al*., 2009). These findings indicate that the supply of VFA may also play an important regulatory role on mammary metabolism further highlighting the challenges in discriminating between the effects of decreases in substrate supply and increases in ruminal TFA outflow in the etiology of diet induced MFD.

In addition to short chain fatty acids, medium and long chain fatty acids contribute to the pool of fatty acids available for incorporation into milk fat TAG in mammary secretory cells. Abomasal infusions of pure fatty acid substrates, plant oils, mixtures of free fatty acids and fish oil have been used to investigate the role of increases in long chain fatty acid supply on mammary lipogenesis. Often lipid in the diet or oils and fatty acids administered at the rumen or abomasum elicit an increase in milk fat synthesis in the lactating cow, but not in all cases, due to negative effects on DM intake and milk yield (Table 1). However, infusion of partially hydrogenated vegetable oils at the abomasum have been shown to decrease milk fat secretion in lactating cows in the absence of changes in milk yield (Gaynor *et al*., 1994; Romo *et al*., 1996).

Role of intermediary metabolism on mammary lipogenesis

It has long been recognized that MFD in the bovine alters nutrient partitioning in favour of non-mammary tissues, adipose in particular (Bauman and Griinari, 2003; Griinari and Bauman, 2006). A higher lipogenic activity in adipose coupled with a simultaneous increase in circulating plasma glucose and insulin concentrations led to the development of the 'glucogenic-insulin' theory of MFD (refer to Bauman and Griinari, 2003). However, analysis of available experimental data indicated that this theory could only explain a small proportion of the overall decrease in milk fat synthesis during diet induced MFD (Griinari and Bauman, 2006). Furthermore, it was argued that the energy spared for milk fat synthesis during short-term MFD would be partitioned toward fat stores, rather than the changes in adipose tissue metabolism induced by a propionate-glucose-insulin cascade being the cause of a shortage of precursors for mammary lipogenesis. Recent studies have shown that decreases in milk energy secretion during acute (Harvatine *et al*., 2009b)

Table 1 *Effect of administration of lipid supplements at the rumen, abomasum or duodenum on mammary lipogenesis in lactating cows*

Infusate	Site	Amount (g/day)	Duration	DMI (kg/day)	Milk (kg/day)	Milk fat (g/day)	Composition (g/kg)			Reference
							Fat	Protein	Lactose	
Control	Rumen	0	21	24.4	28.8	991	34.4	29.3	NR	Hristov *et al.* (2004)
12:0		240	21	23.1	29.6	1000	33.8	27.9	NR	
Control	Diet	0	10	15.2	14.9	626	42	35	NR	Odongo *et al.* (2007)
14:0		710	10	14.2	13.4	549	41	36	NR	
Control	Duodenum	0	10	NR	23.3	958	41.1	NR	NR	Enjalbert *et al.* (1998)
16:0		490	10	NR	25.1	1252	49.9	NR	NR	
18:0/16:0		460/31	10	NR	24.5	1115	45.5	NR	NR	
OA/LA/16:0		400/37/31	10	NR	21.3	1063	49.9	NR	NR	
Control	Duodenum	0	10+	16.5	24.1	995	41.3	30.9	46.9	Gagliostro and Chilliard (1991)
Rapeseed oil		1100	10+	13.9	22.9	971	42.4	28.2	47.1	
Control	Duodenum	0	21	18.4	22.5	976	43.4	31.1	47.7	Ottou *et al.* (1995)
Rapeseed oil		650	21	17.9	23.7	1000	42.3	29.3	48.6	
Control	Abomasum	0	14	18.0	25.0	923	36.9	NR	NR	DePeters *et al.* (2001)
Rapeseed oil		330	14	17.7	26.6	1019	38.3	NR	NR	
Control	Abomasum[1]	0	7	23.1	41.3	1491	36.1	29.3	48.0	Benson *et al.* (2001)
Rapeseed oil/		400	7	21.4	38.6	1390	36.0	29.3	48.6	
sunflower oil	Abomasum[2]	0	7	22.4	35.2	1309	37.2	33.2	47.9	
(50:50)		400	7	21.5	35.0	1428	40.8	34.2	48.1	
Control	Abomasum	0	5	19.8	21.8	850	39	34	44	Litherland *et al.* (2005)
Soya bean oil		200	5	18.7	20.9	857	41	34	46	
Soya bean oil		400	5	17.2	18.7	842	45	32	45	
Soya bean oil		600	5	15.4	18.0	810	45	33	45	
Control	Abomasum	0	21	24.2	47.0	1605	34.5	30.8	49.3	Gaynor *et al.* (1994)
SFO[3] + CB		750	21	22.8	46.3	1512	32.7	29.7	49.3	
PHVO[4]		750	21	23.5	47.0	1211	25.9	30.6	48.5	
Control	Abomasum	0	21	22.9	31.7	1249	39.4	34.7	48.2	Romo *et al.* (1996)
SFO[3] + CB		630	21	21.4	34.5	1421	41.2	32.3	48.5	
PHVO[5]		630	21	20.4	33.9	1068	31.5	30.9	47.6	
Control	Abomasum	0	14	23.7	31.8	1178	37.4	33.0	46.3	Kadegowda *et al.* (2008a)
Fat blend[6]		245	14	25.7	33.1	1279	37.9	32.8	47.0	
Butter fat		400	14	24.2	33.7	1421	42.6	32.7	45.9	
Control	Abomasum		5	22.0	30.1	1211	40.3	33.5	48.6	Shingfield *et al.* (2009b)
Methyl esters[7]		247	5	20.6	30.7	975	31.7	33.7	49.3	
Control	Abomasum	0	9	22.9	33.1	1089	32.6	32.9	49.5	Christensen *et al.* (1994)
Saturated FFA		450	9	21.3	32.8	1010	33.7	30.8	49.7	
Rapeseed FFA		450	9	20.9	30.6	970	36.2	31.7	50.7	
Soya bean FFA		450	9	20.4	30.2	915	33.3	30.3	51.2	
Sunflower FFA		450	9	19.3	29.4	876	32.2	29.8	48.5	
Control	Abomasum	0	21	22.8	34.3	1067	31.1	30.5	NR	Bremmer *et al.* (1998)
Saturated FFA		450	21	21.6	34.7	1121	32.3	29.9	NR	
Palm FFA		450	21	20.3	33.4	878	26.3	30.4	NR	
Soya bean FFA		450	21	19.1	34.6	1176	34.0	30.5	NR	
Control	Abomasum	0	5	20.4	22.3	914	41	33	45	Litherland *et al.* (2005)
Soya bean FFA		200	5	16.6	21.4	813	38	34	45	
Soya bean FFA		400	5	14.9	17.9	752	42	34	43	
Soya bean FFA		600	5	9.8	13.0	585	45	35	39	
Control	Abomasum	0	7	22.0	27.1	892	32.9	31.4	55.9	Drackley *et al.* (2007)
Sunflower FFA[8]		250	7	21.7	26.4	921	34.9	31.7	56.6	
Sunflower FFA[8]		500	7	18.6	23.8	883	37.1	31.2	55.1	
Sunflower FFA[8]		750	7	13.1	14.0	624	44.6	32.8	51.6	
Sunflower FFA[8]		1000	7	5.8	6.8	403	59.3	35.2	47.5	
Control	Abomasum	0	21	NR	21.9	790	36.3	NR	NR	Pennington and Davis (1975)
Fish oil		225	21	NR	23.0	750	32.8	NR	NR	
Control	Duodenum	0	25	19.8	22.2	783	35.4	31.7	47.2	Loor *et al.* (2005a)
Fish oil		270	25	18.0	23.3	737	32.2	30.4	49.2	

DMI = dry matter intake; NR = not reported; CB = cocoa butter; FFA = free fatty acid; PHVO = partically hydrogenated vegetable oil; OA = oleic acid (*cis*-9 18:1); LA = linoleic acid (*cis*-9, *cis*-12 18:2); SFO = sunflower oil.
[1]Infusions in cows of mean 55 days in lactation.
[2]Infusions in cows of mean 111 days in lactation.
[3]Sunflower oil rich in OA.
[4]Mixture (93:7, on a weight basis) of shortening rich in *trans* fatty acids and corn oil.
[5]Mixture (90:10, on a weight basis) of partially hydrogenated soyabean oil and safflower oil.
[6]Blend of fat comprised of (g/kg): cocoa butter (590), olive oil (360) and palm oil (50).
[7]Mixture of methyl esters containing (g/kg): *cis*-9 18:1 (94.5), *cis*-12 18:1 (33.5), *trans*-10 18:1 (373), *trans*-11 18:1 (374) and *trans*-12 18:1 (26.6) as major components.
[8]Prepared from OA enriched sunflower oil.

or chronic (Thering *et al*., 2009) MFD in cows is associated with increased transcription of one or more genes involved in adipogenesis in subcutaneous adipose tissue consistent with the increased utilization of acetate, glucose and long chain fatty acids for lipogenesis and esterification in non-mammary tissues. It has also been suggested that specific TFA biohydrogenation intermediates may inhibit adipose tissue leptin expression or prevent increased secretion of this hormone in response to starch or LA intake in lactating cows and goats (Bonnet *et al*., 2009), but the role of lowered circulating levels of leptin in the regulation of lipogenesis in mammary or adipose tissue remains unclear. Nevertheless, available data do not exclude the possibility that an increase in propionate and glucose supply combined with a decrease in acetate and butyrate availability may contribute to the decreases in milk fat synthesis during diet-induced MFD (Rulquin *et al*., 2007).

Role of TFA on mammary lipogenesis in ruminants

Following the identification of exogenous *trans*-10, *cis*-12 CLA as a potent inhibitor of mammary fatty acid synthesis in the lactating cow (Baumgard *et al*., 2000). A number of studies have been conducted to characterize the anti-lipogenic effects of *trans*-10, *cis*-12 CLA in lactating ruminants. Abomasal infusions of relatively pure preparations supplying between 1.25 and 14.0 g/day over 4- or 5-day periods have established that *trans*-10, *cis*-12 CLA decreases milk fat synthesis in a dose-dependent curvi-linear manner in the lactating cow (de Veth *et al*., 2004; Shingfield and Griinari, 2007). Administration of 1.25 to 5.0 g of *trans*-10, *cis*-12 CLA/day typically results in similar decreases in the output of preformed fatty acids and fatty acids synthesized *de novo*, often in the absence of changes in milk fat SCD product and precursor concentration ratios. However, infusion of *trans*-10, *cis*-12 CLA/day in higher amounts (7.0 to 14.0 g/day) causes a disproportionately larger reduction in the secretion of fatty acids synthesized *de novo*, effects that are also accompanied by significant decreases in milk fat SCD desaturase indices. Observations from several studies indicate that anti-lipogenic potential of *trans*-10, *cis*-12 CLA supplied as calcium salts of a mixture of CLA isomers in the diet is lower immediately in cows *post partum* compared with established lactation (Bernal-Santos *et al*., 2003; Moore *et al*., 2004; Gervais *et al*., 2005). Lowered inhibition of mammary lipogenesis does not appear to be related to variations in mammary supply and incorporation of *trans*-10, *cis*-12 CLA in milk fat (Bernal-Santos *et al*., 2003; Moore *et al*., 2004; Castañeda-Gutiérrez *et al*., 2005); or due to specific changes in plasma glucose, insulin, leptin or NEFA concentrations (Castañeda-Gutiérrez *et al*., 2005; Kay *et al*., 2006). It has been suggested that the coordinated reduction in the expression of genes encoding for key lipogenic enzymes in mammary tissue as a direct response to *trans*-10, *cis*-12 CLA may be prevented due to the attenuation of cellular signalling systems at the onset of lactation (Bernal-Santos *et al*., 2003; Moore *et al*., 2004).

In contrast, intravenous administration of 0.2 g *trans*-10, *cis*-12 CLA/day over a 48 h interval (Schmidely and Morand-Fehr, 2004) or 10 h duodenal infusions supplying 2.0 g/day of *trans*-10, *cis*-12 CLA over a 72 h period (Andrade and Schmidely, 2006) have been shown to have relatively minor or no effect on mammary lipogenesis in goats. Further studies have shown that lipid encapsulated (Lock *et al*., 2008) or calcium salts (Shingfield *et al*., 2009a) of a mixture of CLA isomers containing *trans*-10, *cis*-12 CLA in the diet lowers milk fat secretion from 8.0% to 49.3% depending on the level of supplementation in the goat. Indirect comparisons of the relationship between the relative decreases in milk fat output and milk fat *trans*-10, *cis*-12 CLA content in mid-to-late lactation cows and goats fed diets supplemented with calcium salts of a mixture of CLA isomers suggest that the sensitivity of mammary lipogenesis to the inhibitory effects of *trans*-10, *cis*-12 CLA is several-fold lower in the caprine than bovine (Shingfield *et al*., 2009a; Figure 5). No studies thus far have examined the effects of post-ruminal infusions of *trans*-10, *cis*-12 CLA in the ovine. However, lipid encapsulated supplements comprised of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA in the diet have been shown to decrease milk fat synthesis in the sheep (Lock *et al*., 2006; Sinclair *et al*., 2007).

Relatively few additional TFA have been synthesized to a purity $>$90% and tested for anti-lipogenic activity in lactating cows (Table 2). Due to the lack of pure materials, the effects of lipid supplements containing several positional or geometric isomers on milk fat synthesis have also been evaluated. This approach is valuable in eliminating the involvement of one or more specific biohydrogenation intermediates in the regulation of mammary lipogenesis, but is less informative when infusions cause a reduction in milk fat synthesis. Experiments involving the infusion of mixture of CLA isomers at the abomasum have provided evidence that *trans*-9, *cis*-11 CLA (Perfield *et al*., 2007) and *cis*-10, *trans*-12 CLA (Sæbø *et al*., 2005b) inhibit milk fat synthesis in the lactating cow, but further studies are required to confirm these findings and characterize the changes in milk fat over a range of doses. Investigations with lactating ruminants, experiments with animal models and cultured adipocytes have provided an insight into the structural components associated with anti-lipogenic activity. Available data indicate that for a fatty acid to exhibit anti-lipogenic activity several structural features are required: (i) free carboxyl group on the first carbon atom; (ii) *cis-trans* or *trans-cis* conjugated double bond system; and (iii) *trans* or *cis* double bond on carbon 10 relative to the carboxyl group (Park *et al*., 2004; Shingfield and Griinari, 2007). There are also data indicating that monoenoic fatty acids with a *cis* or *trans* double bond on carbon 10 may alter LPL activity and lipolysis of TAG in fully differentiated 3T3-L1 adipocytes (Park *et al*., 2004). A recent experiment also reported that *trans*-10 18:1 decreases the expression of *ACACA, ACSS2, FABP3*, *FASN, LPL* and *SCD* ($-$41.4%, $-$16.1%, $-$92.1%, $-$60.1%, $-$251.9% and $-$99.7%, respectively, relative to the control) in bMEC (Kadegowda *et al*., 2009). Studies in

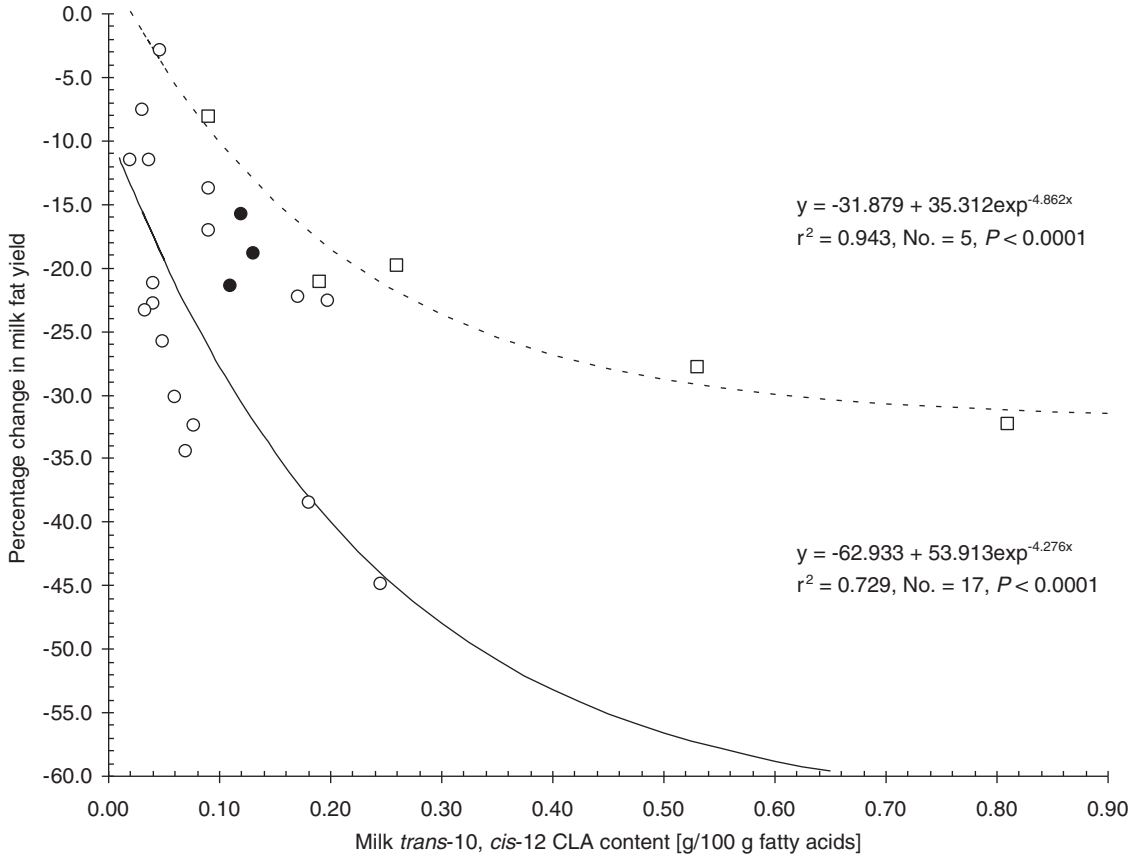

Figure 5 Relationship between the percentage decrease in milk fat yield with milk fat *trans*-10, *cis*-12 conjugated linoleic acid (CLA) content in response to rumen protected CLA supplements in lactating cows (○), goats (□) and sheep (●). The data in lactating cows derived from 11 studies evaluating responses to calcium salts of CLA during early or established lactation (Appendix 1). Measurements in lactating goats determined in two experiments examining the effects of calcium salts of CLA (Shingfield *et al*., 2009a) or lipid encapsulated CLA in the diet (Lock *et al*., 2008). Values for lactating sheep are derived from two studies investigating the impact of lipid encapsulated CLA supplements (Lock *et al*., 2006; Sinclair *et al*., 2007). Fitted lines indicates the relationship between milk fat *trans*-10, *cis*-12 CLA content and relative changes in milk fat yield in cows (solid line) and goats (dotted line) based on comparisons between treatment groups and corresponding controls from reports in the literature.

3T3 L1 adipocytes (Granlund *et al*., 2005) and lactating cows (Sæbø *et al*., 2005a) have also demonstrated that the inhibitory effects associated with a *trans*-10, *cis*-12 conjugated bond system are attenuated or completely abolished when double bonds or methyl groups are introduced between carbon atoms 1 and 10 of the fatty acid moiety (Figure 6).

Role of ruminal biohydrogenation on mammary lipogenesis in the bovine

Trans-10, *cis*-12 CLA formed during the isomerization of LA (Wallace *et al*., 2007) is the only biohydrogenation intermediate to have been infused at the abomasum over a range of doses and shown unequivocally to inhibit milk fat synthesis in the bovine. Often increases in ruminal *trans*-10, *cis*-12 CLA production have been considered as the cause of MFD, but direct measurements indicate that ruminal outflow of *trans*-10, *cis*-12 CLA in lactating cows is less than 1.5 g/day (Shingfield and Griinari, 2007; Table 3). Considerations of the enrichment of *trans*-10, *cis*-12 CLA in bovine milk (0 to 0.12 g/100 g fatty acids) on diets causing MFD also indicate that increases in the supply of *trans*-10, *cis*-12 CLA at the mammary gland contribute to between 0% and 52% of the observed reductions in milk fat secretion (Shingfield and Griinari, 2007). Diets causing MFD are known to alter ruminal lipid metabolism resulting in a shift towards the formation of *trans*-10 18:1 at the expense of *trans*-11 18:1 (Bauman and Griinari, 2001; Loor *et al*., 2005b; Shingfield and Griinari, 2007). Over a wide range of diets causing MFD, increases in milk fat *trans*-10 18:1 concentrations have been consistently reported (Figure 7a and b). However, abomasal infusion of 42.6 g/day of relatively pure (95%) *trans*-10 18:1 in free fatty acid form in lactating cows over a 4-day period was shown to enrich the concentration of this TFA in milk from 0.47 to 1.11 g/100 g fatty acids but have no effect on milk fat secretion with the implication that *trans*-10 18:1 does not exert anti-lipogenic effects (Lock *et al*., 2007). Owing to the relatively low recovery in milk fat it has been postulated that the associated increases in milk fat *trans*-10 18:1 content to post-ruminal infusions were too small to allow a reliable detection of the effects on mammary lipogenesis (Kadegowda *et al*., 2008b). Furthermore, under certain conditions, ruminal outflow of *trans*-10 18:1 in cows

Table 2 *Summary of* trans *fatty acids evaluated for anti-lipogenic activity and effects on milk fat desaturase index*[1] *in lactating cows*

Isomer investigated	Dose tested (g/day)	Purity (%)	Lipogenic (activity)	Desaturase (index)	Reference
trans-9 18:1	25.0	99.0	No change	ND	Rindsig and Schultz (1974)
trans-9 18:1	41.7	83.0	No change	No change	Tyburczy *et al.* (2008)
trans-10 18:1	42.6	94.7	No change	No change	Lock *et al.* (2007)
trans-10 18:1	92.1	37.3	Tentative[2]	No change	Shingfield *et al.* (2009b)
trans-11 18:1	12.5	50.0	No change	No change	Griinari *et al.* (2000)
trans-11 18:1	7.4–29.4	29.5	No change	No change	Shingfield *et al.* (2007)
trans-11 18:1	41.4	83.0	No change[3]	No change[3]	Tyburczy *et al.* (2008)
trans-12 18:1	12.5	50.0	No change	No change	Griinari *et al.* (2000)
trans-12 18:1	7.1–28.5	28.6	No change	No change	Shingfield *et al.* (2007)
cis-9, *trans*-11 CLA	10.1	73.9	No change	No change	Baumgard *et al.* (2000)
cis-9, *trans*-11 CLA	3.0–11.8	88.8	No change	No change	Shingfield *et al.* (2007)
cis-10, *trans*-12 CLA	1.2	23.2	No change[4]	ND	Sæbø *et al.* (2005b)
cis-11, *trans*-13 CLA	3.8	39.0	No change	No change	Perfield *et al.* (2004)
trans-8, *cis*-10 CLA	3.5	32.0	No change[4]	ND	Perfield *et al.* (2004)
trans-9, *cis*-11 CLA	5.0	32.0	Tentative[4]	ND	Perfield *et al.* (2007)
trans-10, *cis*-12 CLA	10.3	89.5	Inhibitory	Decreased	Baumgard *et al.* (2000)
trans-10, *cis*-12 CLA	3.5–14.0	94.5	Inhibitory	Decreased	Baumgard *et al.* (2001)
trans-9, *trans*-11 CLA	5.0	>95.0	No change	Decreased	Perfield *et al.* (2007)
trans-10, *trans*-12 CLA	4.6	95.7	No change	Decreased	Sæbø *et al.* (2005b)
trans-10, *trans*-12 CLA	5.0	>94.0	No change	Decreased	Perfield *et al.* (2006)
cis-6, *trans*-8, *cis*-12 CLNA	4.0	21.8	No change[4]	No change[4]	Sæbø *et al.* (2005a)
cis-6, *trans*-10, *cis*-12 CLNA	2.6	14.6	No change[4]	No change[4]	Sæbø *et al.* (2005a)
cis-9, *trans*-11, *cis*-15 CLNA	5.0	20.3	No change[4]	No change[4]	Gervais and Chouinard (2008)
cis-9, *trans*-13, *cis*-15 CLNA	5.0	20.3	No change[4]	No change[4]	Gervais and Chouinard (2008)

CLA = conjugated linoleic acid; CLNA = conjugated linolenic acid; ND = not determined.
Observed inhibitory effects on lipogenesis are defined as tentative for isomers that have been administered as part of a mixture of fatty acids at the abomasum preventing a direct cause and effect to be established.
[1]Defined as changes in milk fat concentration ratios of product/substrate for stearoyl-CoA desaturase.
[2]Relative abundance of constituent methyl esters implicated *trans*-10 18:1 responsible for the observed reduction in milk fat synthesis.
[3]No change relative to treatments supplying 45.5 g/day of *cis*-9 18:1.
[4]Based on comparisons with infusion treatments supplying the same amount of *trans*-10, *cis*-12 CLA as the test fatty acid preparations.

has been reported to be 90 g/day or higher (Shingfield and Griinari, 2007), whereas *trans*-10 18:1 concentrations in bovine milk during MFD approach or exceed 10 g/100 g fatty acids (Figure 7a and b). A recent experiment demonstrated that post-ruminal infusions of a mixture of 18:1 fatty acid methyl esters providing 92.1 g/day of *trans*-10 18:1 in lactating cows over a 5-day period resulted in 21.3% and 19.5% reductions in milk fat content and output, respectively (Shingfield *et al.*, 2009b). Even though no direct cause and effect could be established, the lack of MFD associated with other constituent methyl esters implicated *trans*-10 18:1 as the most probable candidate responsible for the inhibitory effects on milk fat synthesis. Further studies are required using relatively pure preparations at equal or in higher amounts to allow unambiguous conclusions on the role of *trans*-10 18:1 on mammary lipogenesis in ruminants.

Few biohydrogenation intermediates of known (*trans*-10, *cis*-12 CLA) or putative (*trans*-9, *cis*-11 CLA, *cis*-10, *trans*-12 CLA) anti-lipogenic activity have been identified. Collective increases in ruminal outflow of these fatty acid metabolites are unable to explain all of the reduction in milk fat synthesis over a range of diets causing MFD in lactating cows (Shingfield and Griinari, 2007; Table 3). A number of studies have attempted to identify additional biohydrogenation intermediates involved in the regulation of milk fat synthesis based on the evaluation of changes in specific fatty acids in milk and changes in milk fat secretion. Over a range of diets causing MFD a close inverse relationship has been identified between milk *trans*-9, *cis*-11 CLA concentrations and milk fat secretion (Shingfield *et al.*, 2005; Roy *et al.*, 2006; Shingfield *et al.*, 2006a). In all cases, enrichment of *trans*-9, *cis*-11 CLA in milk fat has been shown to be highly correlated with increases in *trans*-10 18:1 concentrations. There are no reports on ruminal *cis*-10, *trans*-12 CLA outflow or enrichment of this CLA isomer in milk fat in cows fed diets causing MFD.

Principle component analysis of the relationship between milk fatty acid composition and milk fat yield confirmed that MFD in lactating cows is associated with increases in milk *trans*-10 18:1 and *trans*-10, *cis*-12 CLA concentrations, but also highlighted that the changes in *trans*-7 18:1 and *trans*-7, *cis*-9 CLA content were consistent with a possible role in the regulation of mammary lipogenesis (Kadegowda *et al.*, 2008b). However, there are no data on the anti-lipogenic activity of these additional biohydrogenation intermediates, but the available evidence indicates that a *cis*-*trans* or *trans*-*cis* conjugated double bond system with a *trans* or *cis* double bond on carbon 10 relative to the carboxyl group are prerequisites of a fatty acid for anti-lipogenic activity (refer to

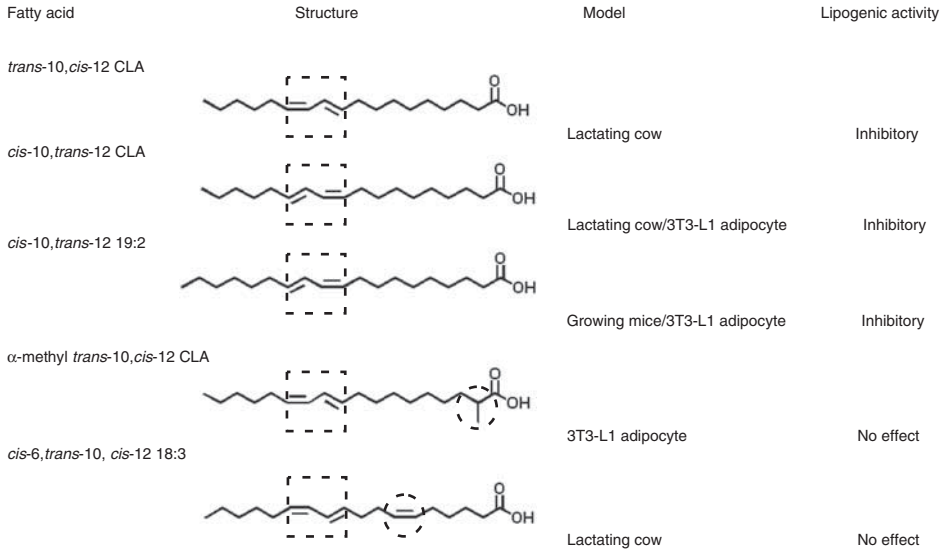

Figure 6 Structural features of *trans* fatty acids known to be associated with anti-lipogenic activity in a range of biological models. Numerous studies have demonstrated that *trans*-10, *cis*-12 conjugated linoleic acid (CLA) exerts anti-lipogenic activity in numerous mammalian species (Bauman and Griinari, 2003), whereas experiments in lactating cows (Sæbø *et al*., 2005b) and 3T3-L1 adipocytes (Griinari *et al*., 2005) have provided evidence that *cis*-10, *trans*-12 CLA inhibits lipogenesis. Studies in growing mice also suggest that a *cis*, *trans* or *trans*, *cis* conjugated bond system with the first double bond being located 10 carbon atoms from the carboxyl group of the fatty acid moiety are prerequisites for anti-lipogenic activity that account for the reduction of body fat in response to a mixture of conjugated nonadecadienoic acids in the diet (Park and Pariza, 2001). Observations in lactating cows (Sæbø *et al*., 2005a) or 3T3 L1 adipocytes (Granlund *et al*., 2005) have also demonstrated that the introduction of a *cis* double bond or a methyl group between the *trans*-10, *cis*-12 conjugated double bond system and the carboxyl group reduces or abolishes the anti-lipogenic effects of *trans*-10, *cis*-12 CLA.

Figure 6) that would not support a role for *trans*-7 18:1 or *trans*-7, *cis*-9 CLA as milk fat inhibitors.

The biohydrogenation theory attributes the causal mechanism underlying diet-induced MFD to increased formation of specific biohydrogenation intermediates that directly inhibit milk fat synthesis. However, it is possible that alterations in ruminal lipid metabolism during diet-induced MFD and associated changes in the relative abundance of preformed fatty acids available for milk fat TAG synthesis may also have a direct or indirect effect on the regulation of mammary lipogenesis (Shingfield and Griinari, 2007). It is well established that endogenous synthesis of OA via the action of SCD on 18:0 in the mammary gland is an important point of regulation in milk TAG synthesis and maintenance of milk fat fluidity. In addition to fatty acid chain length, degree of unsaturation and the presence of a *cis* or *trans* double bond, the maintenance of milk fat globule fluidity also requires that most fatty acids are esterified at specific positions in milk fat TAG to ensure that milk fat has a melting point at or below 39°C (Timmen and Patton, 1988). Under certain conditions, decreases in the availability of 18:0 for endogenous OA synthesis combined with an increase in *trans* 18:1 at the mammary gland have been proposed as a possible mechanism to explain part of the decrease in milk fat secretion during diet-induced MFD (Loor and Herbein, 2003; Loor *et al*., 2005a; Gama *et al*., 2008). In a recent experiment, decreases in milk fat secretion to fish oil in the diet were reported to be associated with increases in mean milk fat melting point (Gama *et al*., 2008) that was considered to be an important component of MFD due to compromized exportation of TAG from the mammary epithelial cell. Studies in lactating cows have demonstrated that *trans*-7 18:1, *trans*-11 18:1 and *trans*-12 18:1 serve as substrates for SCD in the bovine based on concomitant increases in the concentration of *cis*-9 containing 18:2 products in milk (Palmquist *et al*., 2005; Shingfield *et al*., 2007 and 2008c). Therefore for an increase in the mean melting point of long chain fatty acid precursors to occur, decreases in ruminal 18:0 outflow must be accompanied by collective increases in TFA that cannot be desaturated by SCD in the ruminant mammary gland. Studies involving incubations with rat liver microsomes demonstrated that isomers of *trans* 18:1 with double bonds at Δ4 to 7 and Δ11 to 15 but not Δ8 to 10 can serve as substrates for SCD *in vitro* with the extent of conversion being dependent on double-bond position (Mahfouz *et al*., 1980; Pollard *et al*., 1980) with the implication that *trans*-8 18:1, *trans*-9 18:1 and *trans*-10 18:1 are not desaturated in the ruminant mammary gland. Although post-ruminal infusions of *trans*-9 18:1 (Rindsig and Schultz, 1974; Tyburczy *et al*., 2008) have been shown to have no effect on lipogenesis in the lactating cow, under the specified conditions of these experiments the supply of 18:0 at the mammary gland remained unchanged, and therefore these observations do not provide a rigorous test of the possible role of lowered milk fat fluidity in the etiology of diet-induced MFD. Short-term abomasal infusions of sterculic oil (Griinari *et al*., 2000; Corl *et al*., 2001; Kay *et al*., 2004), *trans*-9, *trans*-11 CLA (Perfield *et al*., 2007) and *trans*-10, *trans*-12 CLA (Sæbø *et al*., 2005b; Perfield *et al*., 2006) as well as administration of Co-EDTA in the rumen (Shingfield *et al*., 2006b and 2008b) have reported that acute decreases in the ratio of product to substrate concentrations for SCD in milk fat are not associated with MFD. Furthermore, it is

Table 3 *Changes in the flow of* trans *fatty acid biohydrogenation intermediates at the omasum or duodenum of lactating cows in response to diets causing a reduction in milk fat synthesis*

Sampling site	Duodenum[1]			Duodenum[2]			Omasum[3]	
Forage/concentrate ratio	60 : 40	25 : 75	65 : 35	65 : 35	35 : 65	35 : 65	60 : 40	60 : 40
Oil (g/kg DM)	–	–	–	LO (30)	–	LO (30)	–	FO (16)
Milk fat								
Content (g/kg)	40.9	34.2	33.0	34.5	24.0	22.2	46.0	42.8
Yield (g/day)	1144	1074	799	956	691	582	788	602
Flow (g/day)								
trans-4 18:1	–	–	0.37	1.06	0.88	1.87	0.45	0.65
trans-5 18:1	–	–	1.29	3.12	1.81	3.36	0.37	0.56
trans-6, -7, -8 18:1	1.02	3.72	1.83	6.75	5.98	16.2	1.67	6.65
trans-9 18:1	2.41	4.55	1.38	3.89	2.96	13.1	1.07	6.21
trans-10 18:1	5.73	29.1	1.46	6.61	20.2	50.6	1.71	14.3
trans-11 18:1	20.8	33.6	21.4	61.7	26.0	139	17.0	121
trans-12 18:1	5.68	9.52	1.93	8.32	3.78	9.57	2.21	9.41
trans-13, -14 18:1	14.1	22.9	4.17	29.6	10.3	42.9	6.46	14.0
trans-15 18:1	5.74	8.53	1.95	12.1	4.84	16.8	3.15	5.70
trans-16 18:1	5.45	7.98	2.34	11.5	3.98	10.7	3.99	3.05
Σ *trans* 18:1	60.9	120	37.1	145	80.7	304	38.2	182
cis-9, *trans*-11 CLA	0.330	0.529	0.31	0.52	0.31	0.86	2.86	2.08
cis-10, *trans*-12 CLA			0.16	0.08	0.08	0.12		
cis-11, *trans*-13 CLA	0.021	0.035	0.14	0.11	0.06	0.12	0.013	0.011
cis-12, *trans*-14 CLA	–	–	–	–	–	–	0.052	0.002
trans-7, *cis*-9 CLA	0.005	0.005	–	–	–	–	–	–
trans-8, *cis*-10 CLA	0.009	0.022	0.26	0.15	0.15	0.22	–	–
trans-9, *cis*-11 CLA	–	–	0.28	0.14	0.14	0.19	–	–
trans-10, *cis*-12 CLA	0.086	0.256	0.08	0.07	0.07	0.10	0.095	0.021
trans-11, *cis*-13 CLA	0.009	0.013	0.26	0.59	0.12	0.48	0.460	0.197
trans-7, *trans*-9 CLA	0.014	0.021	–	–	–	–	0.000	0.046
trans-8, *trans*-10 CLA	0.038	0.096	–	–	–	–	0.009	0.099
trans-9, *trans*-11 CLA	0.200	0.391	–	–	–	–	0.224	0.552
trans-10, *trans*-12 CLA	0.109	0.234	–	–	–	–	0.047	0.057
trans-11, *trans*-13 CLA	0.156	0.154	0.33	1.34	0.22	1.37	0.403	0.089
trans-12, *trans*-14 CLA	0.088	0.082	–	–	–	–	0.193	0.078
Σ CLA	1.07	1.84	2.21	3.57	1.70	4.71	4.36	3.50
cis-9, *trans*-12 18:2	–	–	0.39	1.51	0.69	1.99	–	–
trans-9, *cis*-12 18:2	–	–	0.04	0.56	0.38	3.93	0.18	1.60
trans-11, *cis*-15 18:2	–	–	0.99	9.47	2.11	64.1	2.81	18.76
trans-9, *trans*-12 18:2	–	–	0.37	0.16	0.37	4.06	0.03	0.93

DM = dry matter; CLA = conjugated linoleic acid; FO = fish oil; LO = linseed oil.
[1]Data derived from Kalscheur *et al.* (1997) and Piperova *et al.* (2002).
[2]Data derived from Loor *et al.* (2004 and 2005b).
[3]Data derived from Shingfield *et al.* (2003).

not clear whether the implied inhibition of SCD activity is truly analogous to changes in ruminal biohydrogenation leading to simultaneous increases in the supply of TFA that are not desaturated and decreases in 18:0 availability at the mammary gland. It is conceivable that compensatory mechanisms may operate allowing the mammary gland to accommodate short-term reductions in SCD activity, and that these differ from the adaptations to chronic increases in the average melting point of milk fat precursors. Even though increases in *trans* 18:1 and decreases in 18:0 leaving the rumen are characteristic responses to fish oil in the diet, it remains possible that specific intermediates formed during the metabolism of constituent PUFA may also contribute to MFD in cows fed diets containing marine lipids (Shingfield and Griinari, 2007).

Recent investigations based on two different experimental approaches have also provided evidence to suggest that the availability of short and medium chain fatty acids may be an important regulatory component of mammary lipogenesis in the bovine. Comparisons of the flow of fatty acids at the duodenum and secretion of fatty acids in milk in cows fed diets causing MFD indicated that the secretion of 18 carbon fatty acids in milk was independent of the absorption of 18 carbon fatty acids in the small intestine and varied according to mammary output of 4:0 to 16:0 (Glasser *et al.*, 2007). A meta-analysis based on data derived from 39 experiments also inferred an upper limit in the concentration of 18 carbon fatty acids that could be incorporated into milk fat (ca. 52%) and that the effects of lipid supplements on the secretion of

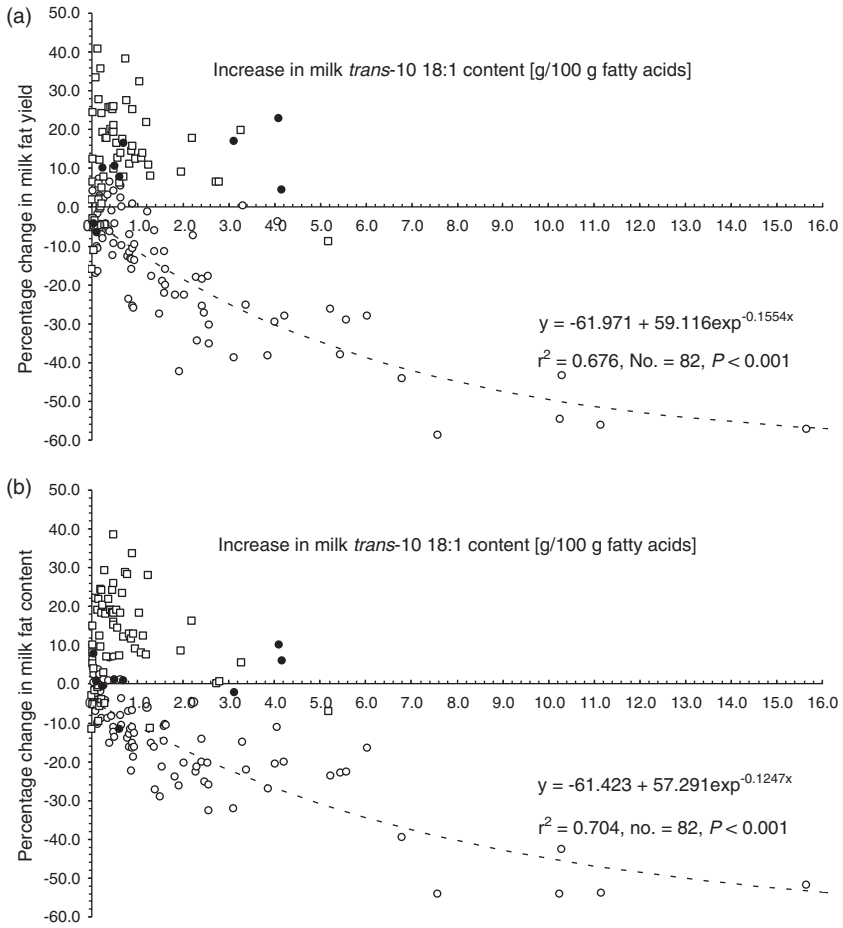

Figure 7 Association between increases in milk fat *trans*-10 18:1 content and corresponding changes in milk fat yield (a) or milk fat content (b) in lactating cows (○), goats (□) and sheep (●). Each point represents comparisons between treatment groups and corresponding controls calculated from studies reported in the literature (Appendix 2) in cows ($n = 82$), goats ($n = 63$) and sheep ($n = 9$). Fitted lines indicate the relationship between an increase in milk fat *trans*-10 18:1 content and relative changes in milk fat yield or milk fat content determined in lactating cows. No obvious associations between these parameters were observed for data derived from studies in lactating goats and sheep.

fatty acids synthesized *de novo* was an important component of the overall lipogenic response (Glasser *et al*., 2008b). Abomasal infusion of milk fat or a mixture of plant oils supplying the same amount of 18 carbon fatty acids but different amounts of 4:0 to 16:0 were recently reported to elicit different milk fat responses in lactating cows (Kadegowda *et al*., 2008a; Table 1). Administration of butter fat increased milk fat content and secretion (14% and 21%, respectively), whereas the infusion of the same amount of 18 carbon fatty acids but only 50% of 16:0 and no additional amounts of 4:0 to 14:0 had no effect. Overall, it appears that several adaptive mechanisms may operate allowing the mammary gland to accommodate for the direct and indirect effects of changes in milk fat precursor supply, with some indications that once exceeded may contribute to lowered rates of milk fat synthesis during diet-induced MFD.

Role of ruminal biohydrogenation on mammary lipogenesis in small ruminants

In contrast to the bovine, the occurrence of MFD is not common in small ruminants, even when goats (Chilliard *et al*., 2003 and 2007; Gagliostro *et al*., 2006) or sheep (Pulina *et al*., 2006; Reynolds *et al*., 2006) are fed starch rich diets containing plant oils or marine oils. Owing to a lack of data on the flow of fatty acids at the duodenum of lactating small ruminants, understanding the reasons underpinning the differences in lipogenic response between small ruminants and the bovine has to be based on an evaluation of diet-induced alterations in the concentration of specific fatty acids in milk and the associated changes in milk fat secretion. Comparisons reported several years ago indicated marked differences in the relationship between changes in milk fat secretion and increases in milk fat *trans*-10 18:1 content in response to lipid supplements between goats and cows (Bernard *et al*., 2006). Within the range of milk *trans*-10 18:1 concentrations observed in goats (0 to 5.2 g/100 g fatty acids) almost all milk fat yield responses are positive, whereas the converse is true in cows (Figure 7a). Recent experiments in lactating sheep, resulting in a wide range in milk *trans*-10 18:1 concentrations (0 to 4.2 g/100 g fatty acids) also highlight differences in lipogenic responses to changes in diet composition between sheep and cows (Figure 7a and b).

Comparisons across ruminant species reveal that increases in milk *trans*-10 18:1 concentrations are some three-fold higher in cows compared with goats and sheep and highlight that the secretion of *trans*-10 18:1 in the milk of small ruminants is not accompanied by substantial decreases in milk fat synthesis as reported for cows (Figure 7). These observations suggest that ruminal biohydrogenation pathways in sheep and goats are less susceptible to diet-induced changes causing a shift from *trans*-11 18:1 to *trans*-10 18:1 compared with the cow and that mammary cells in the caprine and ovine are less sensitive to the anti-lipogenic activity of specific TFA biohydrogenation intermediates relative to the bovine.

Detailed analysis of *trans* 18:1 and CLA isomers in ovine (Gómez-Cortés *et al*., 2008; Hervás *et al*., 2008) and caprine (Bernard *et al*., 2009c) milk produced on starch rich diets containing plant oils enriched in LA provide further insight into possible differences between ruminant species when the changes in milk fatty acid composition are compared with data from studies in cows fed similar diets that result in MFD (Table 4). Relative to the bovine, reductions in the secretion of 4:0 to 16:0 are lower and the output of all 18 carbon fatty acids, including 18:0 and OA are increased in the caprine and ovine. Furthermore, responses in goats and sheep were characterized by: (i) no significant change or a decrease in milk fat *cis*-9 14:1/14:0 and *cis*-9 18:1/18:0 concentration ratios compared with an increase in these desaturase indices in cows; (ii) minor increases in the *trans*-10 18:1/*trans*-11 18:1 ratio relative to several-fold increases in the cow; and (iii) an increase in the *trans*-10, *cis*-12 CLA/*trans*-10 18:1 ratio, whereas this ratio was unchanged or decreased in cows. Relative to sheep, lipogenic responses to plant oil supplements rich in LA in goats are characterized by greater decreases in 16:0 secretion in milk, higher increases in milk 18:0 output, larger reductions in milk fat desaturase ratios, lower concentrations of *trans*-9, *cis*-11 CLA and a trend towards higher milk fat *trans*-9, *trans*-11 CLA enrichment. Indirect comparisons of changes in milk fat composition to diets of similar composition (Table 4) combined with comparisons of changes in milk fat secretion to rumen protected sources of *trans*-10, *cis*-12 CLA (Figure 5) and the relationship between increases in *trans*-10 18:1 and changes in milk fat synthesis (Figure 7a) suggest that small ruminants differ from cows with respect to both ruminal lipid metabolism and the regulation of mammary lipogenesis. Ruminal biohydrogenation pathways appear to be more stable and robust to alterations due to diet in small ruminants compared with the cow that may reflect differences between species related to eating behavior, rumination, buffering of rumen pH, rumen digestion kinetics and transit rates (Chilliard *et al*., 2003; Pulina *et al*., 2006; Bernard *et al*., 2009c) resulting in less exposure of the mammary gland to TFA that may inhibit milk fat synthesis. This is supported by a recent experiment demonstrating that supplements of fish oil and soya bean oil (30 and 109 g/day) to goats grazing fresh grass enhanced milk fat content (mean response 8 g/kg) and milk *trans*-11 18:1 concentrations (mean response 11.9 g/100 g fatty acids) in the absence of large changes (mean response 0.9 g/100 g fatty acids) in milk *trans*-10 18:1 content (Gagliostro *et al*., 2009), whereas fish oil and sunflower oil causes MFD in lactating cows fed maize silage based diets and increases in milk fat *trans*-10 18:1 and *trans*-9, *cis*-11 CLA concentrations (Shingfield *et al*., 2006a).

Nutritional regulation of ruminant mammary lipogenic gene expression

In order to synthesize milk components, the mammary gland requires efficient transcriptional, translational and secretory mechanisms involving the coordinated and concerted action of multiple genes. Studies on the nutritional regulation of molecular mechanisms underlying changes in milk fat secretion in ruminants have initially been based on candidate gene approaches that focus on a few of the major lipogenic genes and measurements of mammary transcript abundance based on northern-blot or reverse transcriptase-PCR and/or enzyme activity (Piperova *et al*., 2000; Delbecchi *et al*., 2001; Ahnadi *et al*., 2002). Milk fat synthesis involves several biochemical pathways involved in fatty acid uptake, *de novo* fatty acid synthesis, desaturation and esterification (Figure 4) and there is an increasing body of evidence that various fatty acids including TFA alter the expression of one or more key genes that encode for enzymes involved in mammary lipogenesis.

De novo *fatty acid synthesis*

Inclusion of plant oils and oilseeds decreases the secretion of medium chain fatty acids (10:0 to 16:0) synthesized *de novo* in the bovine and caprine (Chilliard *et al*., 2007). Indirect comparisons between species indicate that the reductions in milk 10:0 to 16:0 output in goats fed grass hay based diets supplemented with linseed oil (Bernard *et al*., 2009b) or maize-silage-based diets supplemented with sunflower-seed oil (Bernard *et al*., 2009a) of 27% and 32%, respectively, are lower than corresponding decreases of 46% and 69% reported in cows fed diets of similar composition (Roy *et al*., 2006). Feeding diets that cause MFD (Piperova *et al*., 2000; Ahnadi *et al*., 2002; Peterson *et al*., 2003) or administration of *trans*-10, *cis*-12 CLA at the abomasum (Baumgard *et al*., 2002) or intravenously (Harvatine and Bauman, 2006; Gervais *et al*., 2009) results in 30% to 59% decreases in the output of 4:0 to 16:0 in milk that are associated with a decrease in mammary *ACACA* and *FASN* mRNA abundance and/or activity of the transcribed proteins in cows. In goats, diets causing 18% to 32% decreases in the output of fatty acids synthesized *de novo* in milk do not result in significant changes in mammary *ACACA* or *FASN* expression or ACC and FAS activity (Bernard *et al*., 2005b, 2009a and 2009b). Mammary abundance of *ACACA* and *FASN* mRNA in the bovine and caprine is known to be positively related with the output of 4:0 to 16:0 in milk (Bernard *et al*., 2008) suggesting that variation in the expression of *ACACA* and *FASN* is an important component in the regulation of *de novo* fatty acid synthesis in both ruminant species. Furthermore,

Table 4 *Milk fatty acid secretion and composition responses to the inclusion of plant oils containing* cis-*9,* cis-*12 18:2 into starch-rich diets in the caprine, ovine and bovine*

Species	Caprine		Bovine		Ovine		Bovine		Ovine		Bovine	
Reference	Bernard *et al.* (2009c)		Roy *et al.* (2006)		Hervás *et al.* (2008)		Roy *et al.* (2006)		Gómez-Cortés *et al.* (2008)		Piperova *et al.* (2000)	
Main basal forage in the diet	Maize silage		Maize silage		Dehydrated lucerne hay		Maize silage		Dehydrated lucerne hay		Maize silage	
Oil supplement	None	Sunflower	None	Sunflower	None	Sunflower	None	Sunflower	None	Soya bean	None	Soybean
Oil inclusion rate (g/kg DM)	0	63	0	52	0	60	0	51	0	60	0	50
Duration (day)	28	28	18	18	28	28	18	18	28	28	21	21
Animals per group	14	14	6	6	12	12	6	6	12	12	6	6
Diet composition												
Concentrate (g/kg DM)	612	545	520	537	800	812	733	750	800	812	400	750
Neutral detergent fibre (g/kg DM)	310	295	285	266	251	257	206	180	226	222	312	175
Ether Extract (g/kg DM)	20	82	28	72	22	69	32	78	25	77	30	72
MFC and secretion												
MFC (g/kg or response)[1]	31.4	(+0.6%)	40.7	(−39.6%)	48	(+10.0%)	32.8	(−51.8%)	54	(+5.9%)	32.8	(−42.7%)
Milk fat output (g/day or response)	107	(+6.5%)	1380	(−44.2%)	79	(+22.8%)	960	(−57.3%)	90	(+4.4%)	945	(−43.3%)
14:0 (g/day or response)	12.0	(−28.5%)	156.0	(−67.5%)	7.5	(+1.3%)	114.6	(−75.4%)	10.4	(−28.3%)	105.8	(−56.0%)
16:0 (g/day or response)	29.8	(−33.0%)	416.1	(−67.3%)	18.5	(−5.3%)	257.3	(−71.6%)	23.5	(−16.7%)	270.7	(−60.6%)
18:0 (g/d or response)	4.9	(+96.7%)	111.1	(−11.9%)	4.6	(+62.8%)	51.7	(−53.6%)	4.1	(+63.3%)	85.5	(−48.5%)
cis-9 18:1 (g/day or response)	13.7	(+22.0%)	213.6	(−4.9%)	10.2	(+55.7%)	133.5	(−44.4%)	10.2	(+31.3%)	173.7	(−44.4%)
trans-10 18:1 (g/day or response)	0.44	(+682%)	5.54	(+837%)	0.62	(+622%)	26.51	(+169%)	1.60	(+233%)	2.29	(+1915%)
trans-11 18:1 (g/day or response)	1.17	(+674%)	16.35	(−36.7%)	1.64	(+374%)	9.32	(−44.2%)	1.75	(+211%)	4.77	(+78.1%)
Fatty acid (g/100 g fatty acids)												
4:0	2.38	2.56	3.35	2.31	4.12	4.37	3.28	1.84	3.51	4.27	3.80	3.20
6:0	2.47	2.22	2.67	1.21	3.71	2.45	2.7	1.03	3.27	2.28	2.30	1.40
8:0	2.74	2.19	1.51	0.54	3.45	2.00	1.62	0.48	2.90	1.98	1.60	0.90
10:0	10.58	6.91	3.46	1.2	10.24	4.92	4.26	1.16	9.64	5.15	3.50	2.40
12:0	5.72	3.12	3.94	1.65	5.10	2.80	5.14	1.81	5.09	2.95	4.20	3.20
14:0	12.07	8.10	12.12	7.06	10.18	8.40	12.79	7.36	12.35	8.48	12.00	9.30
cis-9 14:1	0.23	0.10	1.14	0.93	0.16	0.10	1.49	1.03	0.29	0.15	1.00	1.00
16:0	29.85	18.78	32.32	18.93	25.06	19.33	28.73	19.13	27.96	22.29	30.70	21.30
18:0	4.88	9.01	8.63	13.62	6.23	8.26	5.77	6.27	4.86	7.60	9.70	8.80
cis-9 18:1	13.7	15.69	16.59	28.29	13.8	17.5	14.9	19.41	12.18	15.31	19.70[2]	19.30[2]
c*is*-9, *cis*-12 18:2	2.41	3.01	2.19	2.34	2.87	3.76	2.96	4.59	2.70	3.46	3.10	6.30
cis-9, *cis*-12, *cis*-15 18:3	0.19	0.15	0.21	0.20	0.46	0.37	0.09	0.15	0.35	0.46	0.60	0.50
trans-6, -7, -8 18:1	0.14	0.56	0.23	0.95	0.35	0.90	0.27	1.43	0.46	0.83	0.05	1.08
trans-9 18:1	0.21	0.58	0.22	0.46	0.3	0.74	0.18	0.59	0.44	0.65	0.10	0.95
trans-10 18:1	0.44	3.23	0.43	7.22	0.84	4.94	2.96	18.62	1.90	6.06	0.26	9.24
trans-11 18:1	1.17	8.50	1.27	1.44	2.23	8.61	1.04	1.36	2.08	6.20	0.54	1.70
trans-12 18:1	0.16	0.67	0.34	0.86	0.48	0.76	0.33	0.74	0.42	0.68	0.23	0.67
trans-13 and *trans*-14 18:1	0.29	0.87	0.30	0.43	–	–	0.37	0.80	–	–	0.43	1.34

Table 4 *Continued*

Species	Caprine		Bovine		Ovine		Bovine		Ovine		Bovine	
Reference	Bernard *et al.* (2009c)		Roy *et al.* (2006)		Hervás *et al.* (2008)		Roy *et al.* (2006)		Gómez-Cortés *et al.* (2008)		Piperova *et al.* (2000)	
Main basal forage in the diet	Maize silage		Maize silage		Dehydrated lucerne hay		Maize silage		Dehydrated lucerne hay		Maize silage	
Oil supplement	None	Sunflower	None	Sunflower	None	Sunflower	None	Sunflower	None	Soya bean	None	Soybean
trans-16 and *cis*-14 18:1	0.13	0.30	0.22	0.52	0.33	0.37	0.19	0.25	0.32	0.31	0.14	0.22
$\sum$ *trans* 18:1	2.55	15.05	3.03	11.99	5.10	17.09	5.37	23.93	6.19	15.57	1.89	15.53
cis-8, *trans*-10 CLA	–	–	–	–	–	–	–	–	–	–	0.008	0.017
cis-9, *cis*-11 CLA	0.001	0.005	0.001	0.003	0.003	0.005	0.000	0.004	0.002	0.003	–	–
cis-9, *trans*-11 CLA	0.816	4.266	0.509	0.769	0.940	3.600	0.551	1.002	1.040	3.440	0.446	0.539
trans-7, *cis*-9 CLA	0.040	0.106	0.033	0.147	0.066	0.171	0.040	0.136	0.120	0.150	0.044	0.222
trans-8, *cis*-10 CLA	0.019	0.107	0.007	0.011	0.022	0.076	0.009	0.023	0.023	0.066	–	–
trans-9, *cis*-11CLA	0.002	0.001	0.000	0.100	0.020	0.070	0.037	0.160	0.030	0.090	–	–
trans-10, *cis*-12 CLA	0.004	0.064	0.003	0.024	0.010	0.070	0.010	0.049	0.010	0.080	0.006	0.096
trans-11, *cis*-13 CLA	0.007	0.009	0.002	0.006	0.003	0.004	0.001	0.005	0.030	0.020	0.004	0.002
trans-7, *trans*-9 CLA	0.005	0.020	0.001	0.004	0.006	0.017	0.001	0.002	0.005	0.008	–	–
trans-8, *trans*-10 CLA	0.003	0.010	0.001	0.002	0.005	0.023	0.001	0.002	0.002	0.004	–	–
trans-9, *trans*-11 CLA	0.015	0.059	0.006	0.008	0.015	0.046	0.007	0.014	0.009	0.030	–	–
trans-10, *trans*-12 CLA	0.003	0.024	0.004	0.015	0.004	0.020	0.005	0.011	0.003	0.009	–	–
Ratio												
cis-9 14:1/14:0	0.019	0.012	0.094	0.132	0.016	0.012	0.116	0.140	0.023	0.018	0.083	0.108
cis-9 18:1/18:0	2.81	1.74	1.92	2.08	2.22	2.12	2.58	3.10	2.51	2.01	2.03[2]	2.19[2]
cis-9, *trans*-11CLA/*trans*-11 18:1	0.70	0.50	0.40	0.53	0.42	0.42	0.53	0.74	0.50	0.55	0.82	0.32
trans-10, *cis*-12 CLA/*trans*-10 18:1	0.009	0.020	0.006	0.003	0.012	0.014	0.003	0.003	0.005	0.013	0.022	0.010
trans-10 18:1/*trans*-11 18:1	0.38	0.38	0.34	5.01	0.38	0.57	2.85	13.69	0.91	0.98	0.48	5.43

DM = dry matter; MFC = milk fat content; CLA = conjugated linoleic acid.
[1]Response calculated as a percentage of the control.
[2]Refers to total *cis* 18:1.

positive associations between *ACACA* and *FASN* mRNA abundance and activity of ACC and FAS in cows (Piperova *et al*., 2000) and goats (Bernard *et al*., 2008 and 2009a) would tend to implicate that the regulation of both genes in ruminant species occurs during transcription.

Fatty acid uptake

Plant oils and oilseeds in the diet consistently enhance mammary secretion of long chain fatty acids in goats, whereas the effects in cows are much more variable. In the bovine, plant oils increase the output of long chain fatty acids on grass hay based diets (Roy *et al*., 2006), whereas inclusion in starch-rich diets that result in MFD either have no effect (Roy *et al*., 2006) or decrease the secretion of preformed fatty acids in milk (Piperova *et al*., 2000; Ahnadi *et al*., 2002; Peterson *et al*., 2003). These findings tend to imply that variation in the availability and uptake of fatty acids from the peripheral circulation may contribute to differences in lipogenic responses to changes in diet composition between ruminant species. Indirect comparisons of milk fat secretion responses to calcium salts of a mixture of CLA isomers in the diet (Figure 5) suggest that the inhibitory effects of *trans*-10, *cis*-12 CLA on fatty acid uptake and incorporation of long chain fatty acids in milk fat TAG are lower in goats compared with cows (Shingfield *et al*., 2009a). In spite of the relatively large increases in the secretion of preformed fatty acids to plant oils in goats these changes are not accompanied by elevated mammary *LPL* transcript abundance and/or activity (Bernard *et al*., 2005a and 2005b; Bernard *et al*., 2009a and 2009b). Studies in cows have shown that mammary *LPL* mRNA abundance is decreased compared with control treatments during MFD on high-concentrate diets containing 10 g/kg DM of sunflower oil (ca. −30%; Peterson *et al*., 2003), total mixed rations comprised of grass silage, maize silage and rolled barley supplemented with 37 g fish oil/kg DM (ca. −70%; Ahnadi *et al*., 2002), high-concentrate diets containing 15 and 30 g/kg DM of fish oil and soya bean oil, respectively, (ca. −60%; Harvatine and Bauman, 2006) or in response to intravenous administration (ca. −25%; Harvatine and Bauman, 2006; ca. −36%. Gervais *et al*., 2009) or abomasal infusion (ca. −48%; Baumgard *et al*., 2002) of *trans*-10, *cis*-12-CLA. Observations thus far would tend to suggest that mammary mRNA and potential activity of LPL is not rate limiting for the uptake of long chain fatty acids in the caprine, but under certain circumstances may contribute to a reduction in milk fat synthesis in the lactating cow. The variable effect of diet on the changes in mammary *LPL* expression would tend to implicate other factors including substrate availability, arterial TAG concentration (Gagliostro *et al*., 1991; Drackley *et al*., 2007) and/or mammary LPL partitioning (Chilliard *et al*., 2003) and the location of long chain fatty acids in milk fat TAG as being more important in the regulation of mammary lipogenesis in the bovine.

Fatty acid desaturation

Relatively few studies have investigated the role of diet on the regulation of *SCD* gene expression and activity. Two *SCD* isoforms have been identified and characterized in the bovine, *SCD1* and *SCD5* that result from the expression of two genes. Prior to the characterization of the bovine *SCD5* gene (Lengi and Corl, 2007), only one *SCD* isoform was thought to be present in this species and therefore the gene was simply referred to as *SCD*. Studies in cows have shown that feeding high concentrate diets containing sunflower oil (Peterson *et al*., 2003) or fish oil and soya bean oil (Harvatine and Bauman, 2006) and causing MFD is not associated with altered mammary *SCD* mRNA abundance. However, expression of *SCD* in the bovine mammary gland has been shown to be significantly downregulated in response to dietary supplements of rumen protected fish oil (Ahnadi *et al*., 2002) or following abomasal (Baumgard *et al*., 2002) or intravenous (Gervais *et al*., 2009) infusions of *trans*-10, *cis*-12 CLA. Recent studies also indicate that intravenous administration of *trans*-10, *cis*-12 CLA has no effect on mammary *SCD5* expression in lactating cows (Gervais *et al*., 2009).

Experiments in goats have shown that supplementing diets based on grass hay with OA enriched sunflower-seed oil (Bernard *et al*., 2005b), sunflower-seed oil or linseed oil decreases SCD activity (Bernard *et al*., 2009b) but has no effect on mammary *SCD* transcript abundance. In contrast, inclusion of sunflower-seed oil and linseed oil in maize silage-based diets was reported to have no effect on *SCD* mRNA or activity in caprine mammary tissue (Bernard *et al*., 2009a). Other experiments in goats have shown that supplementing grass hay-based diets with formaldehyde treated linseed decreases mammary *SCD* mRNA (Bernard *et al*., 2005b), whereas the inclusion of soya beans in lucerne hay-based diets had no effect on mammary *SCD* transcript abundance (Bernard *et al*., 2005a). Overall, these observations highlight the importance of interactions between the composition of the basal diet and lipid supplement in the goat with the implication that specific PUFA escaping metabolism in the rumen or specific biohydrogenation intermediates may inhibit SCD activity via transcriptional or post-transcriptional regulatory mechanisms.

Post-ruminal infusions in lactating cows have established that in amounts above 5 g/day, *trans*-10, *cis*-12 CLA typically decreases milk fat desaturase ratios and also demonstrated that *trans*-9, *trans*-11 CLA and *trans*-10, *trans*-12 CLA modify milk fat composition consistent with the inhibition of SCD (Table 2). *In vivo* (Baumgard *et al*., 2002; Gervais *et al*., 2009) and *in vitro* (Peterson *et al*., 2004; Kadegowda *et al*., 2009) studies indicate that *trans*-10, *cis*-12 CLA decreases *SCD* transcription in the bovine. Similarly, administration of *trans*-10, *cis*-12 CLA at the duodenum was reported to lower milk fat desaturase ratios in the absence of significant changes in milk fat secretion in goats (Andrade and Schmidely, 2006). In some experiments with goats, lipid supplements in the diet have been shown to reduce milk fat desaturase ratios but to have no effect on SCD activity (Bernard *et al*., 2008, 2009a, 2009b and 2009c). It is possible that milk fat desaturase ratios are regulated by factors other than SCD activity, including differential uptake, turnover and utilization of fatty acid substrates in mammary tissue. Furthermore, measurements of SCD activity *in vitro* are made

under optimal conditions and may not be a true reflection of the activity *in vivo*, since the SCD enzyme is known to be regulated by post-translational events and/or cofactors (Bernard *et al*., 2008). For example, *in vitro* incubations have shown that *trans*-10, *cis*-12 CLA decreases SCD activity in the human hepatoblastoma cell line, HepG2, in the absence of changes in *SCD* gene transcription, mRNA or protein (Choi *et al*., 2001). Several experiments in goats have provided evidence to support the involvement of post-translational mechanisms in the regulation of *SCD* activity in ruminants. Inclusion of *cis*-9 18:1 enriched sunflower oil (Bernard *et al*., 2005b), sunflower oil or linseed oil (Bernard *et al*., 2009b) in the diet of goats fed grass hay-based diets has been shown to lower mammary SCD activity measured *ex vivo* but have no effect on mammary *SCD* mRNA abundance.

Role of peripheral tissues

Owing to differences in lipogenic response to changes in diet composition between ruminant species, the possible contribution of non-mammary tissues in the regulation of mammary lipogenesis has been examined. Studies characterizing milk fat secretion responses to plant oils in the diet in mid- (Bernard *et al*., 2005b, 2009a and 2009b) or late-lactation goats (Bernard *et al*., 2005a) offer no support that increases in milk fat synthesis are accompanied by changes in lipogenic gene expression and/or enzyme activity in hepatic or perirenal adipose tissue. An absence of altered lipogenic activity in adipose tissue in goats is consistent with treatments having minimal effects on energy balance or plasma insulin and glucose concentrations that are known to be key components in the regulation of lipogenesis (Chilliard *et al*., 2000).

It has been long recognized that MFD in the lactating cow results in the preferential partitioning of nutrients towards non-mammary tissues including adipose tissue (Bauman and Griinari, 2001). Recent studies in cows have shown that acute or chronic decreases in milk fat secretion are associated with the upregulation of one or more adipogenic genes in subcutaneous adipose tissue. Abomasal infusion of 7.5 g of *trans*-10, *cis*-12-CLA/day over a 4-day period resulting in a 38% decrease in milk fat secretion was shown to increase the expression of *FABP4*, *LPL, FASN* and *SCD*, thyroid hormone responsive spot 14 (*S14*), sterol response element-binding protein 1 (*SREBP1*) and peroxisome proliferator-activated receptor-γ (*PPARG*) genes in subcutaneous adipose tissue (Harvatine *et al*., 2009b). Supplementing maize and lucerne-based diets with 10 g fish oil and 25 g soya bean oil/kg DM over a 21-day period resulting in an overall 25% decrease in milk fat output was also found to result in the increased transcription of adipose differentiation-related protein (*ADFP*), lipin1 (*LPIN1*), *LPL* and *SCD* genes in subcutaneous adipose tissue (Thering *et al*., 2009). These observations are consistent with energy spared for milk fat synthesis during MFD being partitioned towards fat stores, whereas the increase in the expression of one or more genes involved in lipid synthesis in adipose tissues may be an indirect response to energy repartitioning and/or direct effects of long chain fatty acids on gene transcription via their binding with nuclear receptors. However, this interpretation may be an over simplification of a more complicated cause and effect since post-ruminal infusions of rapeseed oil were found to decrease adipose tissue fatty acid synthesis and lipogenic enzyme activity in mid-lactation cows (Chilliard *et al*., 1991).

Nutritional regulation of the ruminant mammary transcriptome

Further advances in molecular techniques have resulted in the development of transcriptomic tools enabling simultaneous changes in the expression of thousand of genes in a wide range of biological samples to be determined. Feeding high-concentrate diets containing soya bean oil was reported to induce MFD in cows and preliminary analysis of mammary biopsies using a bovine oligonucleotides microarray revealed that the reductions in milk fat synthesis were associated with the downregulation of *FASN*, *ACACA*, *SCD*, *LPL*, *FABP3* and *ACSL1* expression (Loor *et al*., 2005c). A recent experiment also reported that reductions in milk fat synthesis in cows fed maize silage and lucerne silage-based diets containing 10 g fish oil and 25 g soya bean oil//kg DM were associated with changes in the expression of several genes involved in lipid metabolism, molecular transport and carbohydrate metabolism (Invernizzi *et al*., 2009) and provided evidence that the changes in mammary lipogenesis are related to the effects on mammary gene networks mediated via different transcription factors/nuclear receptors.

Supplementing high-forage diets with rapeseeds or high-concentrate diets with sunflower oil were shown to increase milk fat synthesis in the goat (Ollier *et al*., 2009). Inclusion of sunflower oil altered the concentrations of most fatty acids in milk characterized by relatively large increases in *trans* 18:1 and 18:2 isomers, whereas rapeseeds resulted in marked reductions in the concentrations of fatty acids synthesized *de novo* and enhanced 18:0 and OA content, in the absence of substantial changes in *trans* 18:1 and CLA isomers. Despite marked differences in milk fatty acid composition and milk fat secretion, none of the experimental treatments induced significant changes in mammary abundance of transcripts encoding for specific genes involved in lipid metabolism. However, a two-way hierarchical clustering highlighted different global mammary expression profiles across diets, suggesting that all experimental treatments could be discriminated based on the expression profile of a broad set of genes. Differential mammary transcriptome responses to lipid supplements in the bovine and caprine is consistent with the known differences in milk fat secretion responses and expression of candidate lipogenic genes between ruminant species.

It has been suggested that owing to the co-ordinate decrease in mammary mRNA encoding for enzymes and proteins in lipogenic pathways during MFD in the bovine, alterations in transcriptional activation of lipogenic genes involves a common regulatory mechanism (Baumgard *et al*., 2002; Peterson *et al*., 2003; Harvatine and Bauman, 2006).

Within the class of transcription factors known as master regulators of lipid synthesis are the SREBP family (Eberlé *et al*., 2004) and peroxisome proliferators-activated receptors (PPARs) (Clarke, 2001). Several studies have demonstrated the involvement of SREBP1 and/or PPAR during diet-induced changes in mammary lipogenic gene expression in lactating cows (Peterson *et al*., 2004; Harvatine and Bauman, 2006; Bionaz and Loor, 2008b), or in response to 3- to 5-day intravenous infusions of *trans*-10, *cis*-12 CLA (Harvatine and Bauman, 2006; Gervais *et al*., 2009) with the implication that these transcription factors are central elements in the overall regulation of milk fat synthesis. Two isoforms of *SREBP1* (a and c) can be differentially expressed in various tissues. *SREBP1c* the major isoform expressed in mammary tissue predominately regulates the transcription of genes encoding for enzymes involved in fat synthesis. The SREBP pathway involves complex interactions with multiple proteins such as: SREBP cleavage activating protein (SCAP), site 1 and 2 proteases (S1P and S2P) and two insulin-induced genes (INSIG1 and 2) to activate target lipogenic genes including *LPL*, *ACACA*, *FASN* and *SCD* (refer to Bernard *et al*., 2008).

A recent study examining the expression of genes associated with lipid synthesis and secretion throughout the lactation cycle (Bionaz and Loor, 2008b) demonstrated that *SREBP1* is constantly regulated suggesting that this transcription factor is ubiquitous for the regulation of milk fat synthesis and point towards a concerted role for *PPARG*, *PPARGC1A* (PPARG coactivator) and *INSIG1* genes. Elsewhere, a role for *PPARG* in regulating bovine milk fat synthesis was supported based on incubations of MAC-T cells with rosiglitazone, a specific PPARG agonist, that leads to a coordinated upregulation in the expression of genes involved in the importation of fatty acids (e.g. *CD36*), *de novo* synthesis (e.g. *ACACA, FASN, SREBP1*) and TAG synthesis (e.g. *LP1N1, SCD*) (Kadegowda *et al*., 2009). In contrast, the expression of the *PPARs* in mammary tissue was reported to be unchanged in cows fed diets causing MFD or in response to 4-day abomasal infusions of *trans*-10, *cis*-12 CLA (Harvatine *et al*., 2009a).

An additional potential mechanism involved in the regulation of milk fat synthesis was disclosed following the identification of changes in the expression of *S14* in mammary tissue of cows fed diets causing MFD or receiving intravenous infusions of *trans*-10, *cis*-12 CLA (Harvatine and Bauman, 2006). Even though the exact biochemical function is not known, *S14* encodes for a nuclear protein that is closely associated with the regulation of fatty acid synthesis in adipose (Harvatine and Bauman, 2006) and mammary tissue (Zhu *et al*., 2005; Anderson *et al*., 2009) in rodents. Collectively, these observations suggest that *S14* may be an important component of the mechanism involved in the decrease in mammary lipogenesis in ruminants fed diets inducing MFD or in response to *trans*-10, *cis*-12 CLA. Furthermore, AMP-activated protein kinase (AMPK) has been implicated in the phosphorylation and inactivation of the ACC protein where activation of AMPK leads to a reduction in the level of transcription factors *SREBP1* and *HNF4a* in the rodent liver (Anderson *et al*., 2007). A recent experiment also demonstrated a role of AMPK in the inhibition of fatty acid synthesis in mammary epithelial cells. Incubation of MAC-T cells with 5-Aminoimidazole-4-carboxamide ribonucleoside (AICAR), which activates AMPK, resulted in the inactivation of ACCα and modified lipogenic expression characterized by a downregulation of *LPL*, *GPAT* and *PPARG* and increase in *FASN*, *FABP3* and *SREBP1* mRNA abundance (McFadden and Corl, 2009).

Future perspectives

The initial steps leading to changes in cellular signalling in response to fatty acids, PUFA and TFA in particular, have not been elucidated but it is probable that other molecules including fatty acid metabolites and proteins are involved. Nutritional regulation of molecules, which have an impact on lipogenesis must be studied in mammary tissue to elucidate a direct cause and effect. Several candidate molecules involved in the regulation of lipid synthesis in rodents have not been investigated in ruminants. These include: (i) protein kinase B (PKB/Akt) with evidence that Akt1-mediated inhibition of GSK3 enhances the stability of *SREBP1* since phosphorylation of SREBP1 by GSK3 enhances the ubiquitination and degradation of SREBP in the nucleus (Anderson *et al*., 2007); (ii) extracellular signal-related kinase (Erk) based on observations that DHA suppresses the expression of *SREBP1* in hepatocytes, changes that are mediated via Erk-dependent and 26S proteasome pathways (Botolin *et al*., 2006); and (iii) endoplasmic reticulum stress-regulated kinase (PERK) based on data in knock out mice models indicating that the *PERK* deletion in murine mammary epithelium inhibits SREBP1 activation and the sustained induction of lipogenic enzymes (Bobrovnikova-Marjon *et al*., 2008). Examination of alterations in the expression of these genes should provide further insight into events upstream of *SREBP1* mediated regulation of lipogenic gene expression.

It could be expected that the development of *in vitro* functional systems of lipid synthesis and secretion combined with the availability of pure TFA isomers would allow further progress in the identification of the inhibitors and activators of fat synthesis and provide a more complete understanding of the differences underlying the regulation of mammary lipogenesis between ruminant and non-ruminant species. Several *in vitro* systems have been developed to allow the role of specific fatty acids on the regulation of mammary lipogenesis to be investigated that include: (i) dispersed bMEC (Hansen *et al*., 1986; Hansen and Knudsen, 1987) or primary bMEC (Matitashvili and Bauman, 2000); (ii) bovine mammary epithelial cell lines: MAC-T (Jayan and Herbein, 2000; Peterson *et al*., 2004; Kadegowda *et al*., 2009); and BME-UV (McFadden *et al*., 2008) or cloned bMEC (Liu *et al*., 2006; Yonezawa *et al*., 2008); and (iii) tissue explants (Matitashvili *et al*., 2001). Development of *in vitro* systems offers the advantage that the effects of relatively small amounts of specific TFA can be evaluated under controlled conditions, but mammary epithelial cells and cultured mammary explants have a short lifetime, whereas

modified cell lines often have abnormal characteristics and a low-secretory activity *in vitro*. Nevertheless, use of these approaches has allowed the underlying mechanisms responsible for the inhibitory effects of *trans*-10, *cis*-12-CLA on *de novo* lipogenesis to be deciphered, at least in part, in primary culture of bMEC (Matitashvili and Bauman, 2000), mammary epithelial cell lines (Peterson *et al*., 2004; McFadden *et al*., 2008; Kadegowda *et al*., 2009) and mammary explants (Matitashvili *et al*., 2001). *Trans*-10, *cis*-12 CLA reduces *ACACA* and *FASN* gene expression in MAC-T cells (Peterson *et al*., 2004; Kadegowda *et al*., 2009) but only the *FASN* gene is downregulated in BME-UV (McFadden *et al*., 2008). Experiments using the MAC-T cell line have demonstrated that the inhibitory effect of *trans*-10, *cis*-12-CLA acts through inhibition of the proteolytic activation of SREBP1, but not *SREBP1* expression, resulting in a reduction in the transcriptional activation of lipogenic genes (Peterson *et al*., 2004). However, studies based on incubations with MAC-T (Kadegowda *et al*., 2009) and BME-UV (McFadden *et al*., 2008) have reported that *trans*-10, *cis*-12 CLA decreases *SREBP1* gene expression. Further investigations with MAC-T cells have shown that *trans*-10, *cis*-12 CLA has no effect on *DGAT1* transcription, increases *DGAT2* and *LPAAT* expression and decreases DGAT activity (Sorensen *et al*., 2009).

Thus far, only two *trans*-18:1 isomers have been tested *in vitro*. Incubation of *trans*-10 18:1 with bovine MAC-T cells decreased *ACACA*, *FASN* and *SCD* transcript abundance (Kadegowda *et al*., 2009), whereas *trans*-11 18:1 enhanced *SCD* mRNA in bMEC (Matitashvili and Bauman, 2000) and increased SCD activity, but decreased ACC and FAS activity in bovine MAC-T cell lines (Jayan and Herbein, 2000). Due to the limited number of studies in ruminants, inferences on the potential anti-lipogenic activity of TFA have also been drawn based on studies involving incubations with adipocytes and hepatocytes *in vitro* (refer to Shingfield and Griinari, 2007). However, *trans*-10, *cis*-12 CLA is known to increase the basal metabolic rate, energy expenditure, fatty acid mobilization, fatty acid oxidation and apoptosis and reduce preadipocyte proliferation and/or differentiation in adipose tissue in rodents, effects that are inconsistent with the effects on mammary lipogenesis in the cow (Bauman *et al*., 2008), highlighting the fact that the lactating ruminant remains a specific model for the examination of cellular signal factors and molecular mechanisms involved in the lipogenic responses to specific TFA.

Conclusions

Trans-10, *cis*-12 CLA formed during metabolism of LA in the rumen is known when infused at the abomasum to inhibit milk fat synthesis in a dose-dependent manner in the cow. Examination of milk fat responses to dietary supplements containing *trans*-10, *cis*-12 CLA suggest that the sensitivity to the anti-lipogenic effects is several-fold lower in the caprine than bovine. Post-ruminal infusions in lactating cows have provided evidence that *trans*-9, *cis*-11 CLA, *cis*-10, *trans*-12 CLA and *trans*-10 18:1 in high amounts inhibit mammary lipogenesis. However, collective increases in ruminal outflow of specific anti-lipogenic CLA isomers in the lactating cow do not entirely explain the decreases in milk fat synthesis during diet-induced MFD. Indirect comparisons indicate that diets causing MFD in the bovine typically increase milk fat secretion in the caprine, whereas limited data in sheep suggest that the responses in the ovine are more similar to the caprine than the bovine. Differences in milk fat secretion responses to changes in diet composition between cows and small ruminants appear to be related to both differences in ruminal lipid metabolism and formation of specific biohydrogenation intermediates and the relative sensitivity of mammary lipogenic processes between ruminant species. Application of molecular approaches has offered an insight into the molecular mechanisms underlying milk fat secretion responses in the bovine, and to a lesser extent in the caprine, and provided evidence that the effects of diet on milk fat synthesis are mediated via alterations in mammary lipogenic gene transcription. Future experiments examining changes in the mammary transcriptome should provide a more comprehensive insight into the role of specific metabolites on milk fat synthesis in ruminant species.

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