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Long-chain fatty acids differentially alter lipogenesis in bovine and caprine mammary slices

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Indirect comparisons from studies in vivo have suggested that caprine mammary tissue is less sensitive than bovine mammary tissue to the anti-lipogenic effect of long-chain fatty acids (LCFA), including specific rumen biohydrogenation (RBH) intermediates of polyunsaturated fatty acids (PUFA). Our objective was to investigate the effects on lipogenesis of 18-carbon LCFA differing in the degree of unsaturation and/or double bond conformation using cultured slices of bovine and caprine mammary tissues. Mammary tissues were collected from five multiparous Holstein × Normande cows and six multiparous Alpine goats in mid lactation. The expression of genes involved in milk component synthesis was measured in tissues collected at slaughter and after slice preparation: *FASN, SCD1, CD36, SREBF1* and *PPARG1* mRNA levels were higher in bovine than caprine samples, whereas the opposite was observed for *CSN2* mRNA levels. Bovine and caprine mammary slices were incubated for 20 h in a medium with BSA (control), cis-9-18 : 1, 18 : 2n-6, 18 : 3n-3, cis-9, trans-11-CLA, or trans-10, cis-12-CLA (the latter at 3 increasing concentrations: C1 (0·11 mM), C2 (0·16 mM), C3 (0·37 mM)). Lipogenesis was estimated by measuring the incorporation of ^{14}C-acetate into total lipid. Significant differences of individual LCFA ($P<0·05$) were observed between species: bovine tissue showed a decrease in total lipogenesis with 18 : 2n-6, 18 : 3n-3, trans-10,cis-12-CLA (C2 and C3) while caprine tissue showed an increase after treatment with 18 : 3n-3, cis-9, trans-11-CLA or trans-10, cis-12-CLA (C3). These results were not related to the mRNA abundance of our set of genes in the mammary slices after incubation. In conclusion, this study demonstrates that caprine mammary slices reacted differently from bovine mammary slices to the anti-lipogenic activity of specific LCFA and suggests that regulation of lipogenesis via other genes and/or at protein level and enzyme activity may be involved.

Keywords: Long-chain fatty acids, lipogenic activity, mammary slices, lactating cow, lactating goat.

The lipid fraction of ruminant diets contains mainly unsaturated fatty acids (FA) with 18 carbons, namely linolenic, linoleic and oleic acids. These FA are largely modified in the rumen, leading to the synthesis of specific rumen biohydrogenation (RBH) intermediates such as conjugated linoleic acid (CLA). These RBH intermediates, together with a proportion of dietary FA escaping metabolism in the rumen, may have direct effects on mammary tissue. Nutritional studies in lactating cows (Baumgard et al. 2000) identified the RBH intermediate trans-10, cis-12-CLA as a potent inhibitor of mammary FA synthesis that acts in a dose-dependent manner (Shingfield et al. 2010). However, studies in vivo suggested that the sensitivity of mammary lipogenesis to the inhibitory effects of trans-10, cis-12-CLA is much lower in goats than in cows (Shingfield et al. 2009). Elsewhere, studies on bovine mammary epithelial cell lines in vitro (Peterson et al. 2004; Kadegowda et al. 2009) demonstrated that trans-10, cis-12-CLA decreases de-novo lipogenesis, and these studies attempted to decipher the mechanisms underlying this effect. So far, no data are available on the effect of trans-10, cis-12-CLA on de-novo lipogenesis using in-vitro models in goats. However, earlier studies in bovine and caprine mammary epithelial cells in vitro demonstrated that LCFA alter the de-novo synthesis of both short- and medium-chain FA, with the effects being more potent during incubations with PUFA containing a

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longer carbon chain and/or a higher degree of unsaturation (Barber et al. 1997).

Here we examined the effects of oleic, linoleic and linolenic acids, as well as two CLA isomers, on lipogenesis in bovine and caprine mammary slices, with the objective of determining the effects of 18-carbon LCFA differing in the degree of unsaturation and/or double bond conformation. The two CLA isomers studied were cis-9, trans-11- and trans-10, cis-12- (the latter at three increasing concentrations) because these isomers have been shown to elicit different responses in bovine mammary lipid synthesis: the former previously showed no effect while the latter showed a dose-dependent, negative effect (Loor & Herbein, 2003; Harvatine & Bauman, 2006). Our hypothesis was that the regulation of mammary lipogenesis differs between ruminant species and that inhibition of lipogenesis by LCFA, and in particular by trans-10, cis-12-CLA, is more effective in bovine than in caprine mammary tissues. Additionally, in order to elucidate the mechanism of the differences among species, we compared the expression of genes involved in lipogenesis (*FASN, SCD1, GPAM, CD36*) and protein synthesis (*CSN2*), as well as two transcription factors (*SREBF1, PPARG1*) in mammary tissues of lactating goats and cows at the same lactation stage at slaughter, and in the subsequent mammary slices prior to and after their incubation in a culture medium supplemented or not with LCFA.

Materials and methods

Tissue sources

Five non-pregnant multiparous [mean parity 4·2 (SD 0·5)] Holstein × Normande cows and six non-pregnant multiparous [mean parity 4·3 (SD 0·8)] Alpine goats at 98 (SD 6) and 97 (SD 7) days in lactation, respectively, were used as sources of mammary tissue. The week before slaughter, milk yield, fat content and protein content for the cows were 25·8 kg/d (SD 4·66), 45·6 g/kg (SD 7·25) and 27·8 g/kg (SD 1·66), respectively; for the goats these parameters were 4·0 kg/d (SD 0·98), 31·8 g/kg (SD 1·44) and 30·5 g/kg (SD 2·68), respectively. The body weights of the cows and goats were 606 kg (SD 45·8) and 58·5 kg (SD 3·89), respectively. The animals were fed an experimental diet with a similar forage: concentrate ratio (mean 60 : 40 on a dry matter basis) during the last 4 weeks prior to slaughter to minimize any carry-over effect of diet on the tissue used for mammary slice preparation. For the cows, the diet was composed of grass silage [45% of dry matter intake (DMI)], natural grassland hay (15% DMI) and a concentrate containing sugar beet pulp (12% DMI), wheat (9·2% DMI), barley (8% DMI), canola meal (6% DMI) and soybean meal (3% DMI). For the goats, the diet was composed of natural grassland hay (60% DMI) and a concentrate containing rolled barley (16% DMI), maize grain (16% DMI) and soybean meal (7% DMI). Concentrates were offered as two equal meals at 8.30 and 16.30. Animals were milked at 8.00 and 16.00. All experimental procedures including slaughtering were approved by the Animal Care Committee of INRA in accordance with the 1985 Use of Vertebrates for Scientific Purposes Act. Animals from both species were slaughtered in the INRA experimental abattoir (Saint-Genès Champanelle, France). Immediately prior to slaughter, animals were milked to remove most of the milk in the mammary glands. Immediately after death, slices of mammary secretory tissues, from the upper part of a rear quarter in the cow, were collected under sterile conditions and immediately transported on ice to the laboratory. For both species mammary secretory tissues sampling and transport to the laboratory were carried out within less than 15 min post mortem. Samples of mammary tissue were immediately frozen in liquid nitrogen and stored at −80 °C until RNA extraction was performed.

Mammary slice culture

In the laboratory, an incision was made aseptically into the thick secretory tissue to collect thin slices that were immediately rinsed in a 37 °C, pH 7·4 basal culture medium composed of M199 medium (M2154, Sigma, St. Quentin Fallavier, France) supplemented with sodium acetate (10·6 mM final concentration; S5636, Sigma), glucose (15·5 mM final concentration; G8644, Sigma) and glutamine (0.68 mM final concentration; G7513, Sigma). Mammary slices approximately 1 mm thick were cut and placed in a 25-ml flask containing the basal medium that was saturated with oxygen and maintained at 37 °C during slice preparation which took approximately 90–120 min (for the rest of the manuscript, t0 refers to the time immediately following the end of slice preparation). At the end of slice preparation (t0), aliquots of the mammary slices were collected and frozen in liquid nitrogen and then, stored at −80 °C until RNA extraction was performed. Aliquots of fresh mammary slices (approximately 100 mg, 5–6 slices) were placed on stainless steel grids positioned in 24-well dishes with 1·8 ml of culture medium, then placed in an incubator for 20 h at 37 °C in a water-saturated atmosphere (95% O_2/5% CO_2). Preliminary kinetic measurements over a 30-h incubation period demonstrated a linear rate of substrate (i.e. acetate and glucose) uptake by mammary slices, allowing the use of a 20-h treatment for this study.

The culture medium (control) was composed of basal medium supplemented with insulin (5 mg/l; I 6634, Sigma), hydrocortisone (1 mg/l; H 0888, Sigma), ovine prolactin (1 mg/l; L 6520, Sigma), antibiotic and antimycotic solution (10 ml/l; A 5955, Sigma) and BSA (0·11 mM; A 6003, Sigma). Treatments consisted of culture medium supplemented with either no fatty acid (control), 0·17 mM-oleic acid (cis-9-18 : 1; Sigma O 1383), 0·16 mM-linoleic acid (cis-9, cis-12-18 : 2; L 1012, Sigma), 0·16 mM-linolenic acid (cis-9, cis-12, cis-15-18 : 3; L 2376, Sigma), 0·20 mM-cis-9, trans-11-CLA (UC-60A, Nu-chek Prep, Inc.) or trans-10, cis-12-CLA (UC-61A, Nu-chek Prep, Inc.) at 0·11 mM (C1), 0·16 mM (C2) or

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Table 1. mRNA abundance of genes encoding for fatty acid synthase (FASN), stearoyl-CoA desaturase (SCD1), glycerol-3-phosphate acyltransferase, mitochondrial (GPAM), platelet glycoprotein 4 (CD36), sterol regulatory element binding transcription factor 1 (SREBF1), peroxisome proliferator-activated receptor gamma 1 (PPARG1) and beta-casein (CSN2) in mammary tissue collected at slaughtering and in mammary slices prepared prior to incubation (t0) and after 20-h incubation in a culture media without addition of fatty acids (control, t20 h) in lactating cows and goats

	Mammary tissue		Mammary slices (t0)		Mammary slices (control, t20 h)	
Species	Cow	Goat	Cow	Goat	Cow	Goat
Samples analysed, *n*	5	6	5	6	5	6
Abundance†						
FASN	7·4 ± 1·44^{a}	3·6 ± 0·50^{b}	6·3 ± 1·56^{a}	3·9 ± 0·44^{b}	0·68 ± 0·17	0·65 ± 0·13
SCD1	25 ± 5·06^{a}	10 ± 1·75^{b}	19 ± 4·20^{a}	8·9 ± 1·79^{b}	5·1 ± 0·98^{a}	2·0 ± 0·56^{b}
GPAM	2·5 ± 0·36	4·3 ± 0·86	1·9 ± 0·03^{b}	3·8 ± 0·68^{a}	0·10 ± 0·04	0·41 ± 0·16
CD36	7·5 ± 0·75^{a}	6·4 ± 87^{b}	7·0 ± 0·64^{a}	6·2 ± 0·85^{b}	1·8 ± 0·22^{a}	1·2 ± 0·17^{b}
SREBF1	33 ± 3·43^{a}	14 ± 3·00 ^{b}	27 ± 2·67^{a}	14 ± 1·39^{b}	10 ± 1·1^{a}	3·3 ± 0·43^{b}
PPARG1	18 ± 2·62^{a}	5·9 ± 0·73^{b}	24 ± 5·4^{a}	7·5 ± 1·07^{b}	6·9 ± 1·14	5·3 ± 0·62
CSN2	9·9 ± 1·80^{b}	38 ± γ3·60 ^{a}	9·2 ± 1·48 ^{b}	37 ± 4·51^{a}	3·3 ± 0·62^{b}	16 ± 2·9^{a}

† mRNA levels expressed in arbitrary units (AU) determined as the mRNA copy number of each gene of interest relative to the geometric mean of 3 housekeeping genes (*PPIA, EIF3 K, UXT1*) mRNA and multiplied by 100

Values are means ± SE. For each tissue type means within a row with unlike superscripts differ ($P < 0·05$)

0·37 mM (C3), complexed to BSA (FA:albumin molar ratio of 4 : 1). The concentrations tested were based upon physiological plasma non-esterified FA concentrations in mid-lactating cows (Gagliostro et al. 1991) and goats (Bernard et al. 2009b). Two wells were respectively prepared per treatment for total lipogenesis measurements and for mRNA measurement.

Measurements and sampling

The mRNA levels of few genes involved in milk component synthesis were measured for both species in mammary tissues collected at slaughter, as well as in slices collected at the end of slice preparation (t0) and after their incubation (t20 h). For this purpose, total RNA was extracted (Invitrogen Life Technologies, Carlsbad CA 92008, USA) and cDNA was generated using the High Capacity Reverse Transcription kit (Applied Biosystems, Villebon-sur-Yvette, France). The mRNA levels of genes involved in the main lipogenic pathways [i.e. uptake and intracellular transport of FA (*CD36*), de-novo FA synthesis (*FASN*), FA desaturation (*SCD1*), FA esterification to glycerol (*GPAM*)] and their regulators (i.e. transcription factors *SREBF1* and *PPARG1*), as well as a major milk protein (*CSN2*), were measured in triplicate using a StepOnePlus real-time PCR system (Applied Biosystems), as previously described (Bernard et al. 2005). The results were expressed as the mRNA copy number of each gene of interest (three repetitions/sample) relative to the geometric mean of three housekeeping genes [peptidyl-prolyl cis-trans isomerase A or cyclophilin A (*PPIA*), eukaryotic translation initiation factor 3 subunit K (*EIF3K*), and ubiquitously expressed transcript (*UXT1*)] as determined using GeNorm software (Vandesompele et al. 2002) accounting for technical variations in RT-qPCR experiment (Bonnet et al. 2001; 2012).

After a 20-h incubation, total lipogenesis in duplicate per treatment was estimated by the replacement of the culture medium with one supplemented with 1-^{14}C-acetate (0·5 µCi/ml = 0·0185 MBq/ml, 2·07 GBq/mmol, Amersham) for 2 h followed by the measurement of radioactivity incorporated into the lipids by extraction (slices and culture medium), according to the method of Folch et al. (1957) and as described by Graulet et al. (2000).

Statistical analysis

Measurements of mRNA levels in mammary tissue and in mammary slices prior to incubation (t0) were subjected to ANOVA using the General Linear Models Procedure of SAS (SAS Institute, Cary NC, USA) with a model that included the effect of species. Species means were compared using the least-squares mean procedure (*t* test; SAS Institute), with differences declared significant at $P < 0·05$.

Measurements of total lipogenesis and mRNA levels after incubation of mammary slices (t20 h) were subjected to ANOVA using the General Linear Models Procedure of SAS (SAS Institute) with a model that included the effects of species, treatment, and their interaction. Differences between means were evaluated using the 'pdiff option' of the 'LS means' (SAS Institute) statement of the General Linear Models procedure, with differences declared significant at $P < 0·05$.

Results

FASN, SCD1, CD36, SREBF1, PPARG1 and *CSN2* mRNA levels in mammary tissue and mammary slices prior to incubation (t0) differed ($P < 0·05$) between cows and goats (Table 1). *FASN, SCD1, CD36, SREBF1* and *PPARG1* mRNA

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Table 2. Effect of species and long-chain fatty acids (LCFA) treatment on mRNA abundance relative to control (without addition of fatty acids) in mammary tissue slices after 20-h incubation time

	Species		Treatment†								P value			
mRNA relative abundance‡	Cow	Goat	Control	c-9–18:1 (0·17 mM)	c9c12–18:2 (0·16 mM)	c9c12c15–18:3 (0·16 mM)	c9t11–18:2 (0·20 mM)	t10c12–18:2 (0·11 mM)	t10c12–18:2 (0·16 mM)	t10c12–18:2 (0·37 mM)	SEM	Species	Treatment	Treatment ×Species
FASN	−25·4	−19·7	0	−17·1	−20·21	−16·1	−26·2	−30·8	−38·0	−32·3	7·35	0·28	0·018	0·99
SCD1	−11·5	−19·2	0	−9·87	−17·6	−14·3	−13·8	−24·1	−23·1	−20·1	6·19	0·082	0·14	0·91
CPAM	−17·5 [b]	−43·7[a]	0[b]	−34·4[a]	−37·7[a]	−26·4[a]	−31·8[a]	−43·7[a]	−35·6[a]	−35·1[a]	8·15§	<0·0001	0·017	0·77
CD36	−5·02[b]	−19·7[a]	0	−11·9	−14·0	−18·7	−17·2	−20·0	−8·49	−8·52	5·04§	0·0001	0·12	0·72
SREBF1	−15·0	−19·4	0	−13·8	−13·7	−15·6	−13·9	−20·7	−28·7	−30·9	7·06	0·38	0·083	0·97
PPARG1	−0·005	−3·14	0	−1·42	−3·28	−5·91	−0·76	−4·75	3·63	−0·096	7·01	0·53	0·99	0·85
CSN2	−10·9	−0·066	0	1·18	0·003	−4·3	−12·1	−14·8	−6·86	−7·12	7·35	0·041	0·73	0·91

†Refers to culture medium supplemented with either no fatty acid (control), 0·17 mM oleic acid (cis-9-18:1), 0·16 mM linoleic acid (cis-9, cis-12-18:2), 0·16 mM linolenic acid (cis-9, cis-12, cis-15-18:3), 0·20 mM-cis-9, trans-11-CLA or trans-10, cis-12-CLA at 0·11 mM (C1), 0·16 mM (C2) or 0·37 mM (C3)

‡Expressed as per cent difference between LCFA treatment and control without FA; *FASN* = fatty acid synthase, *SCD1* = stearoyl-CoA desaturase, *GPAM* = glycerol-3-phosphate acyltransferase, mitochondrial, *CD36* = platelet glycoprotein 4, *SREBF1* = sterol regulatory element binding transcription factor 1, *PPARG1* = peroxisome proliferator-activated receptor gamma 1, *CSN2* = beta-casein

§The statistical model was significant ($P<0·05$) only for *GPAM* and *CD36*: means within a row among species or treatment with unlike superscripts differ ($P<0·05$)

levels were higher in cows, whereas *CSN2* mRNA abundance was higher in goats (Table 1).

During the incubation in a culture medium without addition of FA for 20 h (control), the mRNA abundance of *FASN, SCD1, CD36, SREBF1, PPARG1* and *CSN2* genes in mammary slices decreased sharply, whereas *SCD1, CD36* and *SREBF1* mRNA abundances remained higher in cows than in goats, and *CSN2* mRNA abundance remained higher in goats than in cows (Table 1).

After LCFA treatments for 20 h, mRNA levels in mammary tissue slices, expressed as per cent difference relative to the control (without added FA) are presented in Table 2. LCFA treatments did not change the mRNA levels significantly.

Lipogenesis as measured by ^{14}C-acetate incorporation into the lipid fraction of mammary slices was significantly ($P<0·05$) affected by LCFA treatment, species and interaction among these factors. Indeed, although lipogenesis in control conditions did not differ between cows and goats, LCFA treatments had a significant effect ($P<0·05$) on lipogenesis in both species and this effect differed between species. Compared with control treatments, lipogenesis in cows mammary slices was not affected by 0·17 mM-oleic acid, 0·20 mM-cis-9, trans-11-CLA or 0·11 mM (C1) trans-10, cis-12-CLA, but was significantly decreased by 0·16 mM-linoleic acid, 0·16 mM-linolenic acid, or trans-10, cis-12-CLA at 0·16 mM (C2) or 0·37 mM (C3) (Fig. 1). In contrast, lipogenesis in goat mammary slices was not affected by oleic acid, linoleic acid, or trans-10, cis-12-CLA at 0·11 mM (C1) or 0·16 mM (C2), but was significantly increased by 0·16 mM-linolenic acid, 0·20 mM-cis-9, trans-11 CLA and 0·37 mM (C3) trans-10, cis-12-CLA (Fig. 1).

Discussion

This study shows that mRNA levels of genes involved in milk component synthesis differ between mammary tissues from cows and goats [either collected after slaughter or after preparation of tissue slices (t0)] suggesting differences between ruminant species in mammary metabolism. *CSN2* mRNA level was lower in cows than in goats whereas the opposite was observed for *FASN, SCD1, CD36, SREBF1* and *PPARG1* mRNA levels (Table 1). These results may suggest (i) a lower protein synthesis in cows compared with goats which cannot be related to milk protein content, which was similar in cows and goats (27·8 vs. 30·5 g/kg; NS) and (ii) a higher lipid metabolic activity in cows compared with goats which could be related to the higher milk fat content observed in the cows (45·6 vs. 31·8 g/kg, $P<0·01$) in this study. These differences in mRNA abundances between species may be partly due to differences in the nature of a part of the forage fraction of the diet: grass silage (45% DMI) plus natural grassland hay (15% DMI) in cows vs. natural grassland hay (60%) in goats. Although in-vivo data showed that the effects of grass silage or natural grassland hay on milk FA secretions are of small magnitude (Chilliard et al. 2007), an effect of the nature of the forage and preservation mode

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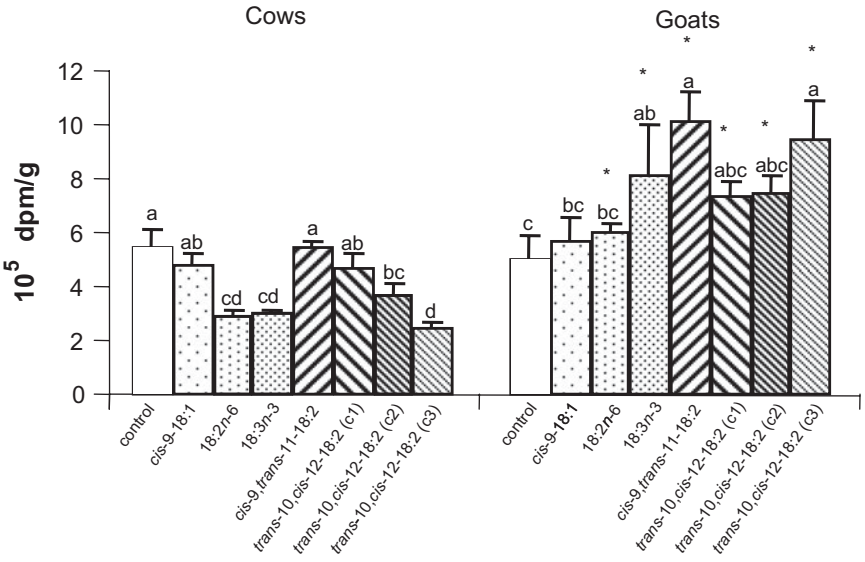

Fig. 1. Lipogenic activity measured by the incorporation of ^{14}C-acetate into the lipid fraction of mammary slices incubated for 20 h without (control) or with long-chain fatty acids, either cis-9-18 : 1 (0·17 mM), 18 : 2n-6 (0·16 mM), 18 : 3n-3 (0·16 mM), cis-9, trans-11-CLA (0·20 mM), and trans-10, cis-12-CLA (at C1 : 0·11 mM; C2 : 0·16 mM and C3 : 0·37 mM) from cows and goats. Values are means ± SE for $n = 5$ cows and $n = 6$ goats. Significant effect of species, long-chain fatty acids treatment and interaction among them were observed ($P < 0·05$). Uncommon letters above bars within a panel indicate differences between long-chain fatty acid treatments within each species ($P < 0·05$). Stars (*) above bars within a panel indicate differences between species for similar long-chain fatty acid treatment ($P < 0·05$)

on nutrient availability for mammary metabolism may not be ruled out. However, these effects are probably of limited magnitude and species specificities are likely to be the main reason for the differences in gene expression.

With the objective of specifying changes in lipogenesis in response to LCFA in cow and goat, respectively, we incubated bovine and caprine mammary slices for 20 h with different LCFA.

In-vitro systems have been developed previously to investigate the role of specific FA on the regulation of mammary lipogenesis, including the following: (i) dispersed bovine mammary epithelial cells (Hansen et al. 1986; Hansen & Knudsen, 1987) or primary bovine mammary epithelial cells (Matitashvili & Bauman, 2000); (ii) bovine mammary epithelial cell lines [MAC-T (Jayan & Herbein, 2000; Peterson et al. 2004; Kadegowda et al. 2009), BME-UV (McFadden et al. 2008)] or the cloned bovine mammary epithelial cells, bMEC (Liu et al. 2006; Yonezawa et al. 2008); and (iii) mammary slices (Matitashvili et al. 2001). These in-vitro systems offer an advantage over nutritional studies in vivo because the effects of specific FA can be evaluated under controlled conditions. However, while mammary epithelial cells and cultured mammary slices have a limited lifetime, modified cell lines often have abnormal characteristics and low secretory activity, which was an argument in favour of the choice of mammary slices in the present study. In addition, cultured mammary slices preserve the three-dimensional structure of mammary tissue and thus resemble conditions in vivo in terms of cellular composition and extracellular matrix. We found that lipogenesis as measured by ^{14}C-acetate incorporation into the lipid fraction of mammary slices was differently affected by LCFA in goat and cow. Indeed, culturing bovine mammary slices for 20 h with certain LCFA [i.e. oleic acid; cis-9, trans-11-CLA and (C1) trans-10, cis-12-CLA] had no effect on lipogenesis, while others LCFA [i.e. linoleic acid, linolenic acid and (C2) and (C3) trans-10, cis-12-CLA] decreased lipogenesis, compared with controls. As shown in Fig. 1, in bovine the observed decreases in lipogenesis [32 and 55%, respectively, for (C2) and (C3) trans-10, cis-12-CLA; Fig. 1] paralleled the 45–82% decreases in lipogenesis induced by trans-10, cis-12-CLA in other studies using bovine tissue, including mammary epithelial cells in primary culture (Matitashvili & Bauman, 2000) and mammary epithelial cell lines (Peterson et al. 2004; McFadden et al. 2008; Kadegowda et al. 2009), as well as mammary slices (Matitashvili et al. 2001). These data are consistent with bovine studies in vivo using abomasal (Baumgard et al. 2000, 2002) or intravenous (Gervais et al. 2009) infusion of trans-10, cis-12-CLA, which induced a dramatic, dose-dependent decrease in milk fat yield (Shingfield et al. 2010) with de-novo synthesized FA (C416) accounting for 78% of the FA reduction (mmol basis; Baumgard et al. 2000). However, studies in vivo in cows showed that the cis-9, trans-11-CLA isomer had no anti-lipogenic effect (Baumgard et al. 2000). Similarly, the decrease in lipogenesis we observed for linoleic and linolenic acid in bovine mammary slices is consistent with the decreased de-novo FA synthesis found in studies using post-ruminal infusion of plant oils rich in either linoleic (Christensen et al. 1994) or linolenic

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acid (Chilliard et al. 1991). Linoleic acid also decreased lipogenesis in a bovine mammary cell line model (BME-UV; McFadden et al. 2008).

In contrast to what was observed in bovine tissue, inclusion of certain LCFA in the culture medium of caprine mammary slices had either no effect on lipogenesis [i.e. oleic acid, linoleic acid; (C1) and (C2) trans-10, cis-12-CLA] or increased lipogenesis [linolenic acid; cis-9, trans-11-CLA; and (C3) trans-10, cis-12-CLA] as compared with controls (Fig. 1b). Our work is the first reporting in-vitro data on caprine mammary lipogenesis, and thus our results can only be compared with data obtained from lactating goats in vivo. In published studies where goats were fed diets supplemented with plant oils rich in either oleic (oleic-rich sunflower oil; Bernard et al. 2005), linoleic (sunflower oil; Bernard et al. 2009a) or linolenic acid (linseed oil; Bernard et al. 2009b), the reductions in milk C10–C16 : 0 output were moderate (17, 27 and 32%, respectively) and much lower than the corresponding decreases in cows fed diets with similar levels of linoleic and linolenic acid (46 to 69%, respectively) (Roy et al. 2006). These findings suggest that goats are less sensitive than cows to the anti-lipogenic activity of dietary FA (including PUFA that escaped ruminal metabolism and specific trans FA arising from biohydrogenation) (Bernard et al. 2008). By supplementing animals with calcium salts of a mixture of CLA isomers, indirect comparisons between lactating cows and goats have demonstrated that the inhibitory effects of trans-10, cis-12-CLA on mammary lipogenesis are dose-dependent for both species but several-fold lower in goats than in cows (Shingfield et al. 2009). By using three concentrations of pure trans-10, cis-12-CLA in cultured mammary slices, our study demonstrates that goat mammary tissue is not sensitive to the antilipogenic effect of trans-10, cis-12-CLA in vitro conversely to cow mammary tissue.

Our study using cultured mammary slices in vitro allows direct comparisons between bovine and caprine mammary metabolism. Our results not only confirm our hypothesis that caprine mammary tissue is less sensitive than bovine mammary tissue to the anti-lipogenic activity of specific LCFA observed in vivo but also show that lipogenesis in caprine mammary slices is not affected and may even be stimulated by LCFA. These differences cannot be related in the present study to the mRNA abundance of lipogenic genes due to the observed sharp decrease of these mRNA abundances during 20-h incubation time without addition of FA (control, Table 1). It is likely that other regulatory mechanisms may be involved in the differences between cow and goat responses to LCFA, such as the expression of other genes, and/or at a protein level (translation efficiency or regulation of the enzyme activities). Our results in vitro outline the specificity of the caprine mammary tissue response to trans-10, cis-12-CLA which was neutral or stimulatory compared with its anti-lipogenic effect as previously observed in cows (Baumgard et al. 2000 and present study), as well as in mice (Lin et al. 2004; Kadegowda et al. 2010).

In conclusion, the current study demonstrates for the first time, using a mammary slice model, that de-novo lipogenesis is differentially regulated in cows and goats. PUFA (18 : 2n-6 and 18 : 3n-3) and trans-10, cis-12-CLA were anti-lipogenic in cows, whereas in goats, these FA had either no effect or promoted lipogenesis (18 : 3n-3, cis-9, trans-11-CLA and high concentrations of trans-10, cis-12-CLA). These results could not be explained by differences in the mRNA abundance of our set of lipogenic genes and probably that other genes and/or level of regulation may be involved. Our results in vitro support the previously observed differences from experiments in vivo suggesting that goats differ from cows in term of their sensitivity to the antilipogenic LCFA. Further studies are required to deepen our understanding of the mechanistic events that explain differences between ruminant species.

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