Short communication: In vivo deposition of [1-¹³C]vaccenic acid and the product of its Δ^9 -desaturation, [1-¹³C]rumenic acid, in the body tissues of lactating goats fed oils

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ABSTRACT

This study was conducted in lactating goats with the aim of measuring the deposition of *trans*-11 18:1 (vaccenic acid, VA) and the product of its Δ^9 -desaturation, *cis*-9, *trans*-11 18:2 (rumenic acid, RA), in the major tissues that are involved in lipid metabolism in the lactating ruminant (i.e., mammary secretory tissue, liver, and omental and perirenal adipose tissues) and examining its potential link with variations in the expression of genes encoding Δ^9 -desaturase [stearoyl-CoA desaturases 1 and 5 (*SCD1* and *SCD5*)]. Eight lactating goats were fed a diet supplemented with sunflower oil ($n = 4$) or sunflower oil plus fish oil and additional starch ($n = 4$), based on the hypothesis that these dietary treatments could affect Δ^9 -desaturase gene expression in specific tissues. A chemical tracer, 1.5 g of [1-¹³C] VA as nonesterified fatty acid, was delivered by jugular injection. Goats were slaughtered 4 d later, and tissue samples were collected for the measurement of [¹³C]VA and [¹³C]RA enrichment and *SCD1* and *SCD5* expression. The addition of fish oil and additional starch to a diet containing sunflower oil was associated with several changes in [¹³C]VA and [¹³C]RA enrichment. These results support previous studies suggesting that mammary secretory tissue is the primary site of Δ^9 -desaturation in lactating goats. In adipose tissues, the [¹³C]VA + [¹³C]RA enrichment was consistent with a net uptake of circulating fatty acids to reconstitute body reserves at the end of the lactation cycle. The putative uptake of [¹³C]RA synthesized by other tissues precludes any conclusion from being drawn regarding potential Δ^9 -desaturation in the liver of goats, despite the detection of *SCD1* and *SCD5* mRNA in this tissue. Finally, no significant effect of dietary treatment was observed for *SCD1* or *SCD5* mRNA abundance in the mammary secretory tissue or other body tissues.

Key words: adipose tissue, liver, mammary secretory tissue, stearoyl-coenzyme A desaturase

Short Communication

In ruminants, the nutritional value of milk fat can be modulated naturally and effectively by changes in live-stock feeding (Chilliard et al., 2007). Special attention has been paid to improving the concentrations of some bioactive FA in milk, mainly conjugated linoleic acid (CLA). The consumption of dairy products represents approximately 70% of daily CLA intake, with the *cis*-9, *trans*-11 isomer (rumenic acid, RA) constituting 70 to 91% of the total CLA in milk (Palmquist et al., 2005; Bernard et al., 2009). High levels of RA are found in ruminant milk because this CLA isomer is synthesized either in the rumen as an intermediate in the biohydrogenation of 18:2n-6 or in the mammary gland and other body tissues from the Δ^9 -desaturation of *trans*-11 18:1 (vaccenic acid, VA) via stearoyl-CoA desaturase (*SCD*) activity (Palmquist et al., 2005).

Tracer methodology has been used to quantify the relative contribution of endogenous synthesis to milk RA in cows (83%; Mosley et al., 2006) and goats (63 to 73%; Bernard et al., 2010). These data suggest that the mammary gland is the major site of Δ^9 -desaturation. However, other body tissues, such as hepatic tissue and abdominal adipose tissues, are actively involved in nutrient partitioning and lipid mobilization during lactation, but their actual contribution to the in vivo conversion of VA to RA in lactating ruminants is not known. Furthermore, dietary treatments that have been tested in nutritional assays aiming to modify milk FA composition, such as feeding fish oil (Ahnadi et al., 2002) or different levels and types of starchy concentrates (Bernard et al., 2012), could affect the expression of genes encoding Δ^9 -desaturase in ruminant tissues (i.e., *SCD1* and *SCD5*; Lengi and Corl, 2007), but the effect of these putative changes on endogenous RA synthesis in body tissues is also unknown.

The present study in lactating goats was conducted with the aim of measuring the in vivo deposition of VA

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and the product of its Δ^9 -desaturation, RA, in the major tissues involved in lipid metabolism in the lactating ruminant (i.e., mammary secretory tissue, liver, and omental and perirenal adipose tissues) and examining its potential link with variations in the expression of genes encoding the Δ^9 -desaturases 1 and 5 (*SCD1* and *SCD5*). A chemical tracer strategy was used in goats fed a diet supplemented with sunflower oil or sunflower oil plus fish oil and additional starch to test the hypothesis that these dietary treatments could affect *SCD* gene expression in various tissues.

All experimental procedures were approved by the Animal Care Committee of INRA in accordance with the Use of Vertebrates for Scientific Purposes Act of 1985. Eight multiparous Alpine goats (287 ± 2.5 DIM) were divided into 2 groups balanced for milk yield, parity, and stage of lactation and allocated at random to 2 experimental diets consisting of grassland hay and a concentrate mixture (forage:concentrate ratio of 67:33) supplemented with 90 g of sunflower oil/d or a combination of 60 g of sunflower oil/d and 30 g of fish oil/d plus additional starch from rolled barley. The detailed compositions of these diets are described in Bernard et al. (2010). Goats were milked daily at 0730 and 1530 h, and fresh diets were offered in 2 equal meals after the milking. After 18 d of the treatments, 1.5 g of [^{13}C] VA as NEFA was delivered by jugular injection after the morning milking (Bernard et al., 2010). Four days later, the goats were slaughtered, and samples of mammary secretory tissue, liver, and omental and perirenal adipose tissues were immediately collected under sterile conditions, frozen in liquid N_2 , and stored at -80°C until analysis.

Total lipids were extracted from each tissue, and FA were converted to FA methyl esters (Roy et al., 2007) and quantified by gas chromatography (Loor et al., 2005). To measure [^{13}C]VA and [^{13}C]RA enrichment, FA methyl esters were converted, respectively, to dimethyl disulfide derivatives and 4-methyl-1,2,4-triazoline-3,5-dione derivatives and analyzed by GC-MS (Mosley et al., 2006). The fragment ions m/z 245 and 246 for VA and m/z 322 and 323 for RA were examined using selective ion monitoring.

Total RNA was isolated from each tissue, purified, and evaluated for concentration and integrity as described previously (Bernard et al., 2010). Quantitative reverse transcription-PCR was carried out (Bernard et al., 2005) using specific primers for *SCD1* (Bernard et al., 2005) and *SCD5* (Lengi and Corl, 2007). Gene transcript abundance was assessed as mRNA copy number relative to the geometric mean of cyclophilin A (*PPIA*), ubiquitously expressed transcript (*UXT*), and eukaryotic translation initiation factor 3 subunit K

(*EIF3K*), which were used as reference genes to account for variations in RNA integrity, RNA quantification, and cDNA synthesis. Specific primers were previously described for *PPIA* (Bonnet et al., 2000), *UXT*, and *EIF3K* (Kadegowda et al., 2009). Details on primer and probe sequences and real-time PCR conditions and performance can be found in the Supplemental Materials (available online at <http://www.journalofdairyscience.org>). Data were analyzed by 1-way ANOVA using the MIXED procedure of the SAS 9.1 software package (SAS Inst. Inc., Cary, NC). The statistical model included fixed effects for sampling site, diet, and their interaction. Means were separated using the PDIF option of the LSMEANS statement of the MIXED procedure, and differences were considered significant at $P < 0.05$.

Enrichment in [^{13}C]VA was higher ($P < 0.001$) in the liver than in the mammary or adipose tissues (Figure 1), most likely because of the active uptake and incorporation of plasma NEFA into the liver lipid fraction in ruminants and the low amount of long-chain FA released in very low density lipoproteins in these animals (Bell, 1979). The putative uptake of [^{13}C]RA synthesized by other tissues precludes any conclusion from being drawn regarding the potential Δ^9 -desaturation of VA in the liver of goats, despite the detection of *SCD1* and *SCD5* mRNA in this tissue. Previous studies in the bovine and ovine have reported inconsistent Δ^9 -desaturation in the liver (see review by Palmquist et al., 2005).

The desaturase index of [^{13}C]VA, [^{13}C]RA/([^{13}C]RA + [^{13}C]VA), measured in the mammary secretory tissue was relatively high compared with those in other tissues (0.38; Figure 2), but its value did not differ from that in perirenal adipose tissue of goats fed SO. Furthermore, higher *SCD1* and *SCD5* mRNA levels were observed in mammary tissue than in liver or adipose tissues (Figure 2), which is consistent with the present data on [^{13}C]RA enrichment in mammary tissue, in addition to the conversion of [^{13}C]VA into [^{13}C]RA that is subsequently secreted in milk (Bernard et al., 2010). Taken together, these results support previous studies suggesting that mammary secretory tissue is a major site of Δ^9 -desaturation of VA in lactating goats (Bernard et al., 2010) and cows (Mosley et al., 2006).

In this study, the goats were in late lactation and in positive energy balance (Bernard et al., 2010), and lipomobilization was therefore expected to be low. Because [^{13}C]VA and [^{13}C]RA concentrations are expressed as percentages of total FA in the tissue, the lower enrichments in adipose tissues than in mammary or hepatic tissue (Figure 1) can be explained by the dilution of

Figure 1. $[^{13}\text{C}]$ vaccenic acid (VA), $[^{13}\text{C}]$ rumenic acid (RA), and $[^{13}\text{C}]$ VA + $[^{13}\text{C}]$ RA concentration (mg/100 g of total FA) in mammary secretory tissue, liver, omental adipose tissue, and perirenal adipose tissue in dairy goats fed a diet containing sunflower oil (white bars) or sunflower oil and fish oil plus additional starch (gray bars). The values shown represent the means ($n = 4$), and the vertical bars represent the standard error of the difference. The statistical probabilities (P) of the effects of sampling site, diet, and their interaction are shown below each panel. Different letters (a–e) indicate significant differences ($P < 0.05$) for the effects of the site $\times$ diet interaction.

$[^{13}\text{C}]$ VA and $[^{13}\text{C}]$ RA in the high lipid content of fat deposits. However, the $[^{13}\text{C}]$ VA and $[^{13}\text{C}]$ RA enrichment in adipose tissues was consistent with a net uptake of circulating FA to reconstitute body reserves at the end of the lactation cycle. In perirenal adipose tissue, the average desaturase index of $[^{13}\text{C}]$ VA (27%) was higher than that of the omental fat (2%) and of previous estimates for the conversion of VA to RA in mesenteric adipose tissue in growing lambs (9 to 16%; Palmquist et al., 2004) but similar to in vivo estimates for adipose tissue in rats (28%; Kraft et al., 2006). However, it is worth noting that *SCD1* and *SCD5* were expressed at similar levels in the 2 adipose tissues studied (Figure 2), suggesting a posttranscriptional regulation of *SCD* in these tissues.

In the present study, 2 dietary treatments were tested based on the hypothesis that they would differentially affect Δ^9 -desaturase gene expression in the mammary gland or other body tissues (Ntambi, 1999; Ahnadi et al., 2002; Gervais et al., 2009; Bernard et al., 2012). The addition of fish oil and additional starch to a diet containing sunflower oil was associated with increases in $[^{13}\text{C}]$ VA and $[^{13}\text{C}]$ RA enrichment in liver and mammary tissue, respectively (Figure 1). The increase in mammary tissue $[^{13}\text{C}]$ RA is in line with observations in the milk fat of the same animals (Bernard et al., 2010), although no significant differences were observed for *SCD1* or *SCD5* mRNA abundance between mammary tissue of animals fed the 2 different diets (Figure 2). Similarly, dietary treatment did not affect *SCD* gene expression in adipose tissues, despite the reduction in the Δ^9 -desaturation index in the perirenal adipose tissue of goats fed 60 g of sunflower oil/d and 30 g of fish oil/d plus additional starch from rolled barley (Figure 2). However, it is possible that differences in the uptake of $[^{13}\text{C}]$ RA synthesized and recirculated by other body tissues have influenced the Δ^9 -desaturation index calculated in the perirenal site because of its low $[^{13}\text{C}]$ VA enrichment.

In conclusion, these results support previous studies suggesting that mammary tissue is the primary site of in vivo Δ^9 -desaturation of $[^{13}\text{C}]$ VA in lactating goats. In adipose tissues, the $[^{13}\text{C}]$ VA + $[^{13}\text{C}]$ RA enrichment was consistent with a net uptake of circulating FA to reconstitute body reserves at the end of the lactation cycle. The putative uptake of $[^{13}\text{C}]$ RA synthesized by other tissues precludes any conclusion from being drawn regarding potential Δ^9 -desaturation of VA in the liver of goats, despite the detection of *SCD1* and *SCD5* mRNA in this tissue. Finally, no significant effect of dietary treatment was observed for *SCD1* or *SCD5* mRNA abundance in the mammary secretory tissue or other body tissues.

Figure 2. The Δ^9 -desaturase index of $[^{13}\text{C}]$ vaccenic acid (VA), $[^{13}\text{C}]$ RA/ $[^{13}\text{C}]$ RA + $[^{13}\text{C}]$ VA, where RA = rumenic acid, and the relative abundance of mRNA encoding stearoyl-CoA desaturases 1 and 5 (*SCD1* and *SCD5*) in mammary secretory tissue, liver, omental adipose tissue, and perirenal adipose tissue in dairy goats fed a diet containing sunflower oil (white bars) or sunflower oil and fish oil plus additional starch (gray bars). For each gene, mRNA levels are expressed in arbitrary units after normalization to the geometric mean of 3 housekeeping genes. The values shown represent the means ($n = 4$), and the vertical bars represent the standard error of the difference. The statistical probabilities (P) of the effects of sampling site, diet, and their interaction are shown below each panel. Different lowercase letters (a–c) indicate significant differences ($P < 0.05$) for the effect of the site $\times$ diet interaction. Different uppercase letters above the bars (A–C) indicate significant differences ($P < 0.05$) for the effect of sampling site.

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