## Nutritional regulation of stearoyl-CoA desaturase in the bovine mammary gland

## **Antoon Jacobs**

Increasing the proportion of unsaturated fatty acids (UFA) in milk is believed to be beneficial in terms of human health, thereby increasing the nutritional quality of milk. In the Netherlands however, the proportion of UFA in milk decreased in the last decade, which is most likely related to changes in composition of diets fed to dairy cows (Heck et al., 2009). The proportion of UFA in milk is mainly dependent on the proportion of UFA in the diet, the degree of biohydrogenation of UFA in the rumen, and on activity of the stearoyl-CoA desaturase (SCD) enzyme in the mammary gland. Stearoyl-CoA desaturase is a membrane-bound, endoplasmic enzyme which introduces a *cis*-double bond between carbons 9 and 10 in a wide range of fatty acids (FA). The preferred substrates of SCD are C18:0 and, to a lesser extent, C16:0, which are converted to C18:1 cis-9 and C16:1 cis-9, respectively (Ntambi & Miyazaki, 2004). The oxidative reaction catalysed by SCD requires the electron acceptor cytochrome b5, NAD(P)-cytochrome b5 reductase and molecular oxygen. The electrons flow from NAD(P)H via cytochrome b5 reductase, to cytochrome b5, to SCD, and finally to O<sub>2</sub>, which is reduced to H<sub>2</sub>O (Paton & Ntambi, 2009). SCD catalyses the critical committed step in the biosynthesis of monounsaturated fatty acids (MUFA), predominately C18:1 cis-9. These MUFA serve as the main substrates for the synthesis of membrane phospholipids, triglycerides, cholesterol esters, wax esters and alkyldiacylglycerols (Paton & Ntambi, 2009). In addition to MUFA, SCD can also produce *cis*-9, *trans*-11 conjugated linoleic acid (CLA) by desaturation of C18:1 trans-11. The cis-9, trans-11 CLA isomer has been associated with numerous health benefits for consumers including prevention of atherosclerosis, different types of cancer and hypertension (Wahle et al., 2004; Bhattacharya et al., 2006). In bovine, two isoforms of SCD have been characterized, i.e. SCD1 and SCD5. In lactating ruminants, SCD1 is abundantly expressed in the mammary gland and plays an important role in the production of milk fat (McDonald & Kinsella, 1973; Bernard et al., 2008; Bionaz & Loor, 2008). The recently discovered SCD5 appears to be predominantly expressed in brain and pancreas (Lengi & Corl, 2007). Contrary to rodents, not much is known about the nutritional regulation of bovine SCD.

We carried out studies that focused on SCD in the mammary gland of dairy cows, and how SCD can be influenced by nutrition. More specifically, the effect of short- and longchain fatty acids on mRNA expression of SCD was investigated in the bovine mammary gland. The purpose of this research was to explore nutritional strategies that could increase the activity of SCD in the mammary gland of dairy cows, thereby improving the FA profile of milk. The objective of the first experiment was to compare the effects of various FA typically present in dairy cow rations, on the expression of both SCD1 and SCD5 (the two known bovine isoforms of SCD) in the mammary gland of dairy cows. Twenty-eight Holstein-Friesian cows were randomly assigned to one of the four dietary treatments being a basal diet supplemented (DM basis) with either 2.7% rapeseed oil as a source of C18:1 *cis*-9, 2.7% soybean oil as a source of C18:2 *cis*-9,12, 2.7% linseed oil as a source of C18:3 *cis*-9,12,15 or 2.7% of a 1:1:1 mixture of the three oils. After the treatment period of 23 days, all cows were switched to a control diet for an additional 28 days. At the end of both the treatment period and the control period, tissue from the mammary gland was taken by biopsy and analysed for mRNA expression of SCD1 and SCD5 by using quantitative real-time polymerase chain reaction (qRT-PCR). Milk yield as well as milk protein and fat content did not differ between the four dietary treatments. Mammary SCD1 expression was significantly down-regulated in dairy cows by feeding soybean oil compared with rapeseed oil or linseed oil, and this was partially reflected by the lower desaturase indices in the milk, which are frequently used as proxies for mammary SCD activity. In contrast, SCD5 expression in the mammary gland was much lower (<10<sup>3</sup>) than that of SCD1 and did not differ amongst the four treatments, indicating that mammary expression of SCD5 is less sensitive to changes in FA supply compared with SCD1.

In a second experiment, a bovine mammary cell line (MAC-T) was used to assess the effect of acetic acid (Ac) and  $\beta$ -hydroxybutyric acid (BHBA) on the mRNA expression of SCD via gRT-PCR, and to compare this to the effect of various LCFA on SCD expression, as well as expression of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). In addition, expression of sterol regulatory binding protein 1 (SREBP-1), insulin-induced gene 1 protein (INSIG-1) and peroxisome proliferator-activated receptors (PPARs) were measured to examine if these transcription factors are involved in the regulation of SCD expression in bovine mammary epithelial cells. MAC-T cells were treated for 12 h without FA additions (CON) or with either 5 mM Ac, 5 mM BHBA, a combination of 5 mM Ac + 5 mM BHBA, 100  $\mu$ M palmitic acid (PA), 100  $\mu$ M stearic acid (SA), 100  $\mu$ M oleic acid (OA), 100  $\mu$ *M trans*-vaccenic acid (TVA), 100  $\mu$ *M* linoleic acid (LA) or 100  $\mu$ *M* a-linolenic acid (ALA). In comparison with CON, expression of SCD1 was increased by Ac (+61%) and reduced by OA (-61%), LA (-84%) and ALA (-88%). Contrary to SCD1, MAC-T cells did not express SCD5 mRNA. Expression of ACC was also increased by Ac (+44%) and reduced by LA (-48%) and ALA (-49%). Compared with CON, FAS expression was not significantly affected by the treatments. The mRNA level of SREBP-1 was not affected by Ac or BHBA, but was reduced by OA (-44%), TVA (-42%), LA (-62%) and ALA (-68%) compared with CON. Expression of INSIG-1 was down-regulated by SA (-37%), OA (-63%), TVA (-53%), LA (-81%) and ALA (-91%). Both PPARa and PPAR $\delta$  expression was not significantly affected by the treatments. These results show that Ac up-regulates expression of SCD1 and ACC in MAC-T cells, which indicates that Ac may increase desaturation and *de novo* synthesis of FA in the bovine mammary gland. Furthermore, the results strengthen the support for the role of SREBP-1 and INSIG-1 as central regulators of lipogenesis in the bovine mammary gland.

Overall, it can be concluded that saturated LCFA have little or no effect on SCD1 expression in the bovine mammary gland, whereas unsaturated LCFA inhibit mammary SCD1 expression. The inhibitory effect of unsaturated LCFA on mammary SCD1 expression appears to increase proportionally with the amount of double bonds in the LCFA (i.e., more double bonds results in higher inhibition of SCD1 expression). Therefore, it seems difficult to enhance SCD1 expression in the mammary gland by supply of LCFA. In order to limit inhibition of mammary SCD1 expression, supply of poly unsaturated fatty acids (PUFA) to the mammary gland should be restricted. The regulation of SCD1 in the bovine mammary gland by LCFA appears to be, at least partly, regulated by the transcription factors SREBP-1 and INSIG-1. Based on the *in vitro* research it appears that short-chain FA, in particular Ac, upregulate mammary SCD1 expression, although further research is needed to verify if short-chain FA induce SCD1 expression in the bovine mammary gland. The recently discovered isoform SCD5 is expressed in bovine mammary tissue, although contribution to  $\Delta$ 9-desaturation of FA appears to be quite low.

## References

- Bernard, L., C. Leroux, and Y. Chilliard. 2008. Expression and nutritional regulation of lipogenic genes in the ruminant lactating mammary gland. Adv. Exp. Med. Biol. 606: 67-108.
- Bhattacharya, A., J. Banu, M. Rahman, J. Causey, and G. Fernandes. 2006. Biological effects of conjugated linoleic acids in health and disease. J. Nutr. Biochem. 17: 789-810.
- Bionaz, M., and J. J. Loor. 2008. Gene networks driving bovine milk fat synthesis during the lactation cycle. BMC Genomics 9: 366.
- Heck, J. L. M., H. J. F. van Valenberg, J. Dijkstra, and A. C. M. van Hooijdonk. 2009.Seasonal variation in the Dutch bovine raw milk composition. J. Dairy Sci. 92: 4745-4755.
- Lengi, A. J., and B. A. Corl. 2007. Identification and characterization of a novel bovine stearoyl-CoA desaturase isoform with homology to human SCD5. Lipids 42: 499-508.
- McDonald, T. M., and J. E. Kinsella. 1973. Stearoyl-CoA desaturase of bovine mammary microsomes. Arch. Biochem. Biophys. 156: 223-231.
- Ntambi, J. M., and M. Miyazaki. 2004. Regulation of stearoyl-CoA desaturases and role in metabolism. Prog. Lipid Res. 43: 91-104.
- Paton, C. M., and J. M. Ntambi. 2009. Biochemical and physiological function of stearoyl-CoA desaturase. Am. J. Physiol. Endocrinol. Metab. 297: E28-E37.
- Wahle, K. W., S. D. Heys, and D. Rotondo. 2004. Conjugated linoleic acids: are they beneficial or detrimental to health? Prog. Lipid Res. 43: 553-587.