CHAPTER 5

OVERALL CONCLUSION AND IMPLICATIONS

Under normal dietary situations in dairy cows, 16:0 and 18:0 account for approximately 20% and 50%, respectively, of the fatty acids available for absorption into the circulatory system and consequent delivery to the mammary gland. The major fatty acid present in bovine milk fat is 16:0, and it accounts for approximately 28% of total milk fatty acids and 44% of the saturated milk fatty acids. Part of the 16:0 in milk is synthesized *de novo* in the mammary epithelial cells, and the remaining is derived from diet *via* blood. An effective method for obtaining desirable changes in bovine milk fatty acid profile would include reduced dietary 16:0, reduced *de novo* synthesis of 16:0, and enhanced desaturation of 18:0 to 18:1.

Results of the present study indicated that *cis* and *trans* isomers of 18:1 and 18:2 influenced *de novo* fatty acid synthesis in mouse and bovine mammary epithelial cells. In both cell types, treatment with 18:1 and 18:2 isomers reduced cellular 16:0 content and cellular fatty acid synthetase (FAS) activity, when compared with 18:0 treatment. Stearic acid (18:0) is a long-chain saturated fatty acid. Presence of higher amounts of 18:0 in the cell membrane would decrease membrane fluidity, when compared with the presence of equal amounts of short- and medium-chain saturated fatty acids or long-chain unsaturated fatty acids. Therefore, increased FAS activity in response to 18:0 treatment could be a cellular response to synthesis of greater amounts of short- and medium-chain saturated

fatty acids for incorporation into cell membranes, so as to maintain normal membrane fluidity. Results from bovine cells further indicated that 18:1 and 18:2 isomers reduced the abundance of FAS and acetyl-CoA carboxylase (ACC) mRNA and inhibited activity of cellular ACC. Depression of ACC and FAS activities by 18:1 and 18:2 could be a cellular response to reduce the synthesis of short- and medium- chain fatty acids for incorporation into the cell membrane and to maintain normal membrane fluidity, since incorporation of higher amounts of the available 18:1 and 18:2 into cell membranes would have already enhanced the membrane fluidity. In both cell types, CLA and *trans*-vaccenic acid (TVA) appeared to be the most potent inhibitors of ACC and FAS activity and mRNA abundance.

Stearoyl-CoA desaturase (SCD) activity and mRNA abundance was also affected by *cis* and *trans* isomers of 18:1 and 18:2. CLA inhibited SCD activity and SCD mRNA abundance in mouse mammary cells, whereas CLA enhanced SCD activity and mRNA abundance in the bovine cells. Treatment with 18:0 enhanced SCD activity and mRNA abundance in mouse cells. However, in the bovine cells 18:0 did not affect SCD activity although it caused a gradual increase in SCD mRNA abundance in response to increasing 18:0 concentration. When compared with the control low concentrations of CLA enhanced SCD activity and mRNA abundance in bovine and mouse mammary cells. In both cell types TVA increased SCD activity and mRNA abundance when compared with SA. TVA is a long-chain *trans* unsaturated fatty acid and its physical properties would be more similar to that of a long-chain saturated fatty acid than to that of a long-chain *cis* unsaturated fatty acid. Presence of higher amounts of TVA in the cell membrane would

reduce membrane fluidity when compared with the presence of equal amounts of long-chain *cis* unsaturated fatty acids. Therefore, increase in SCD activity caused by TVA-treatment would be a cellular response to synthesize greater amounts of long-chain *cis* unsaturated fatty acids for incorporation into the cell membranes, in order to maintain normal membrane fluidity. Enhanced SCD activity by CLA could also be explained as a cellular response to maintain normal membrane fluidity. CLA is *cis*, *trans*-18:2. It is possible that CLA is competing with LA (which is *cis*, *cis*-18:2) for incroporation into cell membranes. Having one of the double bonds in *trans* configuration would make CLA less fluid in nature when compared with LA. Increase in SCD activity by CLA could be a cellular response to synthesize greater amounts of *cis* unsaturated fatty acids for incorporation into cell membranes.

Results of the present study using mouse and bovine mammary epithelial cells suggest that enhanced delivery of oleic and linoleic acids to the mammary gland will depress *de novo* fatty acid synthesis by the mammary cells. Moreover, enhanced delivery of TVA or CLA to the bovine mammary gland could simultaneously reduce *de novo* saturated fatty acid synthesis and enhance desaturation of 18:0 to 18:1.

Further investigation is necessary to elucidate the mechanisms by which unsaturated fatty acids modulate ACC, FAS, and SCD activities. Changes in ACC activity have been closely related to the degree of lipogenic activity in mammalian tissues. Previous studies have indicated that the activity of this enzyme is acutely controlled by covalent modification through phosphorylation and dephosphorylation. The sensitivity of this

regulation is complemented by allosteric control mechanisms involving various cellular metabolites, such as citrate and fatty acyl-CoA, that act to alter the equilibrium between the inactive protomer and the active polymer forms of the enzyme. It has been established that covalent modification and allosteric control mechanisms account for most of the short-term regulatory aspects of ACC activity. However, the mechanism by which fatty acids cause these effects remains to be established by further investigation.

The present study did not quantitate the actual amounts of cellular ACC, FAS or SCD protein. It is not possible to say whether the responses in enzyme activity were due to changes in the absolute quantity of the protein or due to changes in specific activity of the enzyme molecules. Studies in the past have indicated that regulation of ACC at the gene was an important factor in controlling the activity of the enzyme in certain tissues. In the present study, the effect of exogenous fatty acids on ACC gene transcription was quantified by determining the amount of ACC mRNA in the cells. However, this approach cannot distinguish between the effect on ACC mRNA turnover and the effect on ACC gene transcription. The rate of gene transcription, stability of the mRNA transcript, efficiency of mRNA processing and editing are all factors that can contribute to alteration in the abundance of the mRNA under question. Further investigation is necessary to determine the effects of fatty acids on the ACC gene. The same is true for FAS and SCD gene transcription.