IDENTIFICATION OF TISSUE DISTRIBUTION AND REGULATION OF BOVINE STEAROYL-COA DESATURASE BY HORMONES AND NUTRIENTS

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Stearoyl-CoA Desaturase: Tissue Distribution and Regulation

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ABSTRACT

Studies were conducted to investigate the tissue distribution of stearoyl-CoA desaturase-1 (SCD) and the regulation of SCD1 protein expression by dietary fat, insulin, polyunsaturated fatty acids (PUFA), and linoleic acid (*cis*-9, *cis*-12 18:2). The first study examined tissue distribution of SCD1 protein in Holstein calves (n=6/diet) fed one of four milk replacer diets for a nine wk period after which they were sacrificed. Milk replacer diets varied in fat content and were formulated and administered as follows: 0.4 kg/d 20% protein, 20% fat (20:20; **CON**), 0.97 kg/d (28:20; **HPLF**), 0.97 kg/d (28:28; **HPHF**), or 1.46 kg/d (28:28; **HPHF+**). Samples of subcutaneous adipose tissue **(AT)**, perirenal AT, omental AT, duodenum, proximal jejunum, distal jejunum, ileum, and liver were collected from calves fed the HPHF+ diet to determine SCD1 tissue distribution. Tissue homogenates were prepared and used for Western blotting. Additionally, dietary effects were analyzed on tissues expressing SCD1 protein for all 24 calves. The second study investigated the regulation of SCD1 protein expression by insulin, fatty acids increasing in degree of unsaturation, and increasing concentrations of linoleic (18:2) acid. Subcutaneous AT was collected from Smith Valley Meats in Rich Creek, VA and used to prepare explants cultured in treatment media for 24 h. Treatments consisted of insulin at 0, 7, 14, and 21 nM; stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids at 100 µM; and linoleic (18:2) acid at concentrations of 0, 25, 50, 75, and 100 µM. Tissue explant homogenates were used for Western blotting to detect SCD1. In the first study, we found that SCD1 protein was detectable in subcutaneous AT, perirenal AT, and omental AT; however, it was not detectable in liver or small intestine samples. Also, the HPHF+ diet increased SCD1 protein expression in subcutaneous AT and perireanl AT. In the second study, SCD1 protein expression increased linearly with insulin concentration. There was no fatty acid treatment effect, but there was a negative linear effect with increase in degree of unsaturation. Finally, there was no effect on SCD1 protein expression with linoleic acid increasing in concentration. In conclusion,

results indicate that SCD1 protein expression was detected in bovine AT depots, regulated by dietary fat, insulin, and by PUFA .

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CHAPTER 1: INTRODUCTION

Consumers are increasingly concerned with the healthfulness of food components, especially fat content and quality. Consumers have progressively reduced their intake of products that contain fat and increased their consumption of products that contain no fat, are low fat, or lean. Producers of animal products are interested in increasing the nutritional quality of their products by improving their fatty acid composition to appeal to consumers (Lock and Bauman, 2004). Dairy products and beef are enriched in a wide variety of nutrients, but both contain a large amount of saturated fatty acids in contrast to monounsaturated or polyunsaturated fatty acids. This also differs from plant oils that contain greater amounts of mono- and polyunsaturated fatty acids (Table 1.1). The saturated fat content of ruminant derived foods is due to ruminal biohydrogenation (Harfoot, 1988).

Many consumers have negative perceptions of animal products due to the high saturated fatty acid content. Saturated fatty acids are associated with coronary heart disease, increased risk of cardiovascular disease, and increased low-density-lipoprotein (LDL) cholesterol (Williams, 2000, Xu et al., 2006). Unlike saturated fatty acids, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) decrease the likelihood of coronary heart disease and lower LDL cholesterol (Williams, 2000). Monounsaturated fatty acids have been shown to decrease serum lipids, lipoproteins, and prevent the oxidation of LDL (Krauss et al., 1996) and a diet rich in MUFA decreased plasma LDL greater than PUFA (Mensink and Katan, 1989). Similarly, omega-3 and omega-6 PUFA have been shown to decrease coronary heart disease (Wijendran and Hayes, 2004, Williams, 2000). Polyunsaturated fatty acids in the

omega-3 family, found in fish oils, have properties that protect against coronary heart disease including anti-inflammatory properties and hypolipidemic properties that inhibit atherosclerosis (Connor, 2000). Scientists and some producers are trying to improve the fatty acid composition of these products to make them more appealing to consumers. Enriching animal products with MUFA and PUFA improves the fatty acid composition of these products. Thus, converting saturated fatty acids in ruminant fats to MUFA would be an appropriate strategy.

In addition to saturated fatty acids, ruminant fats also contain conjugated linoleic acids (CLA). These fatty acids are isomers of linoleic acid that result from ruminal biohydrogenation of linoleic acid or from the desaturation of vaccenic acid (Lock and Bauman, 2004). The most abundant CLA isomer in ruminant fats is the *cis*-9, *trans*-11 isomer. CLA isomers have several health benefits in animal models that include antiatherogenic, antiobesity, and anticarcinogenic effects (Williams, 2000). These anticarcinogenic effects have been demonstrated with a wide variety of cancers. Dietary CLA reduced the risk of mammary cancer by 50% (Ip et al., 1999) and decreased the number of forestomach tumors compared to diets containing linoleic acid (Ha et al., 1990). Increasing the concentration of *cis*-9, *trans*-11 CLA in ruminant fats may increase their appeal to consumers.

To convert saturated fatty acids to monounsaturated fatty acids and to produce *cis*-9, *trans*-11 CLA from vaccenic acid (*trans*-11 18:1), stearoyl-CoA desaturase (SCD) is required. This enzyme is located in the endoplasmic reticulum and it inserts a *cis* double bond between carbons 9 and 10 (Ntambi, 1999). This enzyme helps to maintain the ratio of saturated to monounsaturated fatty acids. Alterations in this ratio are associated

with cardiovascular disease, obesity, diabetes, insulin sensitivity, atherosclerosis, and cancer (Ntambi, 1999, Ntambi and Miyazaki, 2004, Ntambi et al., 2004). Better understanding of SCD and its regulation in ruminants may lead to methods and technologies that improve the fatty acid composition of ruminant fats, increasing consumer demand for these products.

Table 1.1 Fatty acid compositions of animal and plant products.

Fatty acids	**Milk fat[1]**	**Beef fat[2]**	**Pork fat[3]**	**Sunflower Oil[4]**	**Peanut Oil[4]**	**Olive Oil[5]**
	-------------- *g/100g* Fatty Acid --------------					
4:0-12:0	17.1					
14:0	9.6	3.3	1.3			
14:1, *cis*-9	0.6	0.9				
15:0	0.8	0.5				
16:0	26.3	26.3	24.5	4.0	12.3	8.4
16:1, *cis*-9	0.7	3.6	2.1			
17:0	0.5	1.2				
18:0	14.3	14.1	14.1	5.4	3.2	3.3
18:1, *cis*-9	20.7	39.3	39.9	21.2	51.5	77.6
18:1, *trans*	4.1	2.7				
18:2, *cis*-9,12	4.0	1.6	12.8	69.4	30.2	8.0
CLA, *cis*-9, *trans*-11	0.7	0.7				
18:3, *cis*-9,12,15	0.5	0.2	0.9			0.8
Total Saturated	68.6	45.4	39.9	9.4	15.5	11.7
Total Monounsaturated	26.1	46.5	42.0	21.2	51.5	77.6
Total Polyunsaturated	5.2	2.5	13.7	69.4	30.2	8.8

[1](Corl et al., 2001) [2](Madron et al., 2002)[3](Weber et al., 2006)[4](Kelly et al., 1998) [5](Ruiz-Gutierrez et al., 1998)

CHAPTER 2: LITERATURE REVIEW

Fatty Acid Structure and Synthesis

Fatty acids have a unique structure that includes a carboxyl group and a hydrocarbon chain. Fatty acids are categorized according to their structure and can be saturated, with all carbon-carbon bonds linked by single bonds, or unsaturated, with one or more double bonds in the hydrocarbon chain. Fatty acids with a single double bond are monounsaturated fatty acids and fatty acids with more than one double bond are polyunsaturated. The double bonds in the hydrocarbon chain are usually in the *cis* configuration with alkyl groups on the same side, but double bonds can also occur in the *trans* configuration with alkyl groups on opposite sides of the hydrocarbon chain. Fatty acids are stored as triacylglycerols and exist structurally as phospholipids (Garrett, 1999).

Fatty acid synthesis occurs in the cytosol of the cell and substrates needed for fatty acid synthesis include acetyl-CoA, malonyl-CoA, and NADPH. Acetyl-CoA sources include degradation products of amino acids, oxidation of fatty acids, pyruvate, and acetate produced in the rumen through fermentation. Acetate is activated to acetyl-CoA by acyl-CoA synthetase and malonyl-CoA is produced through the carboxylation of acetyl-CoA. NADPH for fatty acid synthesis in ruminants is produced by the pentose phosphate pathway and through the conversion of isocitrate to α-ketoglutarate by NADPH-isocitrate dehydrogenase (Garrett, 1999).

Fatty acid synthesis involves the polymerization of malonyl-CoA with acetate to create the fatty acid chain. The growing chain continues until it is 16 carbons in length producing palmitic acid. The first step in fatty acid synthesis is the carboxylation of

acetyl-CoA by acetyl-CoA carboxylase. Acetyl-CoA carboxylase (ACC) contains domains that include a biotin carboxyl carrier moiety, biotin carboxylase, and transcarboxylase activity. To form malonyl-CoA, carboxylation of biotin causes CO_2 to be transferred to acetyl-CoA. The carboxylase adds the CO_2 and the transcarboxylase transfers the activated CO_2 to acetyl-CoA. The acetyl- and malonyl-CoA bind to fatty acid synthase. Fatty acid synthase carries out 6 separate enzymatic activities. Initially, acetyl-O-enzyme is formed between the acetyl-CoA and the acetyl transferase moiety, and malonyl-O-enzyme is formed between the malonyl-CoA and the malonyl transferase moiety. The acetyl group is combined with acyl carrier protein from the acetyl transferase activity by interacting with the –SH group on acyl carrier protein. The acetyl group is then transferred to the β-ketoacyl-ACP synthase that allows for the acyl carrier protein to attach to the malonyl group from the malonyl transferase. At this point, decarboxylation occurs leaving a carbon anion that reacts with the carbonyl of the acetyl group located on the β-ketoacyl synthase. The carbonyl is then converted to an alcohol, dehydrated to make a *trans*-α, β double bond, and finally reduced to a saturated bond. This process of acetate addition continues until the chain reaches 16 carbons. Thioesterase activity releases the newly formed fatty acid by interacting with the carbonyl on the fatty acyl thioester (Garrett, 1999).

Biohydrogenation of Fatty Acids in the Rumen

The diet of ruminants consists of plants that contain PUFA, but ruminant products contain large amounts of saturated fatty acids, and small amounts of *trans* fatty acids and CLA. These components are produced through ruminal biohydrogenation (Harfoot 1988). Biohydrogenation is a process carried out by two populations of bacteria in the

rumen that convert PUFA from the diet to saturated fatty acids. Group A bacteria hydrogenate linoleic and linolenic acids to vaccenic acid. Group B bacteria hydrogenate a variety of fatty acids to stearic acid (Griinari, 1999).

The first step in biohydrogenation is the hydrolysis of the glycerolipids that contain PUFA. The main PUFA found in plants are linoleic (*cis*-9, *cis*-12 18:2) and linolenic (*cis*-9, *cis*-12, *cis*-15 18:3) acids. Biohydrogenation of linoleic acid produces two intermediates. The first step in the biohydrogenation of linoleic acid is isomerization of the *cis*-12 double bond to form *cis*-9, *trans*-11 CLA. Next, the *cis*-9 double bond is hydrogenated to form vaccenic acid (*trans*-11 18:1). Finally, the *trans*-11 double bond of vaccenic acid is hydrogenated to form stearic acid (Griinari, 1999).

Biohydrogenation of linolenic acid involves three intermediates. The first step involves the isomerization of the *cis*-12 double bond to form *cis*-9, *trans*-11, *cis*-15 octadecatrienoic acid. Next, the *cis*-9 double bond is hydrogenated to form *trans*-11, *cis*-15 octadecadienoic acid. Subsequently, the *cis*-15 double bond is hydrogenated to form vaccenic acid. Finally, vaccenic acid is hydrogenated to stearic acid (Griinari, 1999).

Vaccenic acid is a common intermediate of the biohydrogenation of linoleic and linolenic acids and the final product for both pathways is stearic acid. Ruminant products contain a large amount of saturated fatty acids due to biohydrogenation, but small amounts of these biohydrogenation intermediates escape complete biohydrogenation and can be found in ruminant fats (Griinari, 1999).

Biohydrogenation increases the saturated fatty acid concentration in ruminant products by the conversion of PUFA from the diet to saturated fatty acids. There are two

populations of bacteria that convert PUFA to saturated fatty acids. Finally, intermediates of biohydrogenation can escape complete biohydrogenation resulting in small levels of *trans* fatty acids and CLA.

Stearoyl-CoA Desaturase Pathway

Stearoyl-CoA desaturase (SCD) is an iron containing enzyme located on the endoplasmic reticulum (Kato et al., 2006) that is responsible for the production of monounsaturated fatty acids from saturated fatty acids by inserting a *cis* double bond between carbons 9 and 10 (Ntambi, 1995, 1999). Stearoyl-CoA desaturase prefers palmitoyl- and stearoyl-CoA as substrates (Enoch et al., 1976, Heinemann and Ozols, 2003), but it also utilizes other substrates including vaccenic acid and *trans*-7 18:1 to produce *cis*-9, *trans*-11 and *trans*-7, *cis*-9 CLA isomers (Corl et al., 2001). Stearoyl-CoA desaturase uses an electron transport chain that requires NADH, oxygen, cytochrome b_5 reductase, and cytochrome b_5 (Ntambi and Miyazaki, 2004). Electrons are transferred from NADH to cytochrome b_5 by cytochrome b_5 reductase resulting in oxidation of cytochrome b_5. The non-heme iron (Fe^{+3}) located in stearoyl-CoA desaturase is then reduced by accepting the pair of electrons from cytochrome b_5 and creating a *cis* double bond (Ntambi, 1995). Diatomic oxygen accepts four electrons, two that are transferred from cytochrome b_5 and two released from substrate during double bond formation, producing two water molecules (Ntambi, 1995). The formation of the double bond is the committed step in the synthesis of unsaturated fatty acids (Ntambi, 1995).

Stearoyl-CoA desaturase is responsible for the production of MUFA by inserting a *cis* double bond between carbons 9 and 10 through an electron transport chain. The pathway requires NADH, oxygen, cytochrome b_5 reductase, and cytochrome b_5.

Stearoyl-CoA Desaturase Isoforms

Four isoforms of SCD have been identified in mice, two in rats, and two in humans (Miyazaki et al., 2003, Ntambi et al., 2004). The isoforms found in mice vary in tissue expression profile, but are similar in structure. Stearoyl-CoA desaturase isoforms 1-3 have transcript lengths of 4.9 kilobases (kb), produce a protein 41 kiloDaltons (kD) in size, and have genes that are 15 kb that contain six exons and five introns and are all found on chromosome 19 (Kaestner et al., 1989, Ntambi et al., 1988, Zheng et al., 2001). The SCD4 isoform is slightly different from the other isoforms. Stearoyl-CoA desaturase 4 has a shorter transcript of 3.1 kb and the gene is 14 kb, but it still contains six exons and five introns (Miyazaki et al., 2003).

Stearoyl-CoA desaturase 2 has 87% amino acid homology with SCD1 and is expressed mostly in the brain, but is also found in kidney, adipose, and lung tissues (Kaestner et al., 1989). The third isoform is located in sebaceous glands and demonstrates 91 and 88% homology in mRNA to SCD1 and SCD2, respectively (Zheng et al., 2001). Stearoyl-CoA desaturase 4 is expressed only in the heart and has 83% homology with the other SCD isoforms (Miyazaki et al., 2003). SCD isoforms in mice are virtually identical in gene structure, but vary in their tissue expression.

Only one SCD isoform has been identified in goats, cattle, and sheep. Ward et al. (1998) identified the SCD1 gene in sheep. Clones of ovine SCD1 were isolated and all were from a single mRNA and the transcript was 5 kb. In goats, the transcript was also

5 kb and the gene was 15 kb, consisting of six exons and five introns similar to the gene structure described in mice. The goat and bovine SCD1 genes are located on chromosome 26 and the sheep SCD1 gene is located on chromosome 22. The SCD1 mRNA of goats is similar to sheep and bovine with 98 and 95% homology and the protein has 98 and 94% homology, respectively (Bernard et al., 2001).

Recently, a second bovine SCD isoform has been identified and termed bovine SCD5 due to its high homology with human SCD5 (Lengi and Corl, 2007). Bovine SCD5 contains four transmembrane domains similar to SCD proteins in other species. The gene structure is similar to human SCD5 with five exons and four introns, but differs from mice isoforms and SCD1 in other species. In addition, the transcript is 2.6 kb, smaller than the mice SCD isoforms and other SCD1 transcripts. The bovine SCD5 that is mostly expressed in the brain has 90.1% homology to human SCD5, but only 65% homology to bovine SCD1 (Lengi and Corl, 2007).

Mice have four SCD isoforms that are similar in structure, but one SCD isoform has been identified in sheep and two in bovine. Bovine SCD1 has similarities to mice, but the bovine SCD5 is similar to human SCD5.

Stearoyl-CoA Desaturase-1 Tissue Distribution

Of the stearoyl-CoA desaturase isoforms, SCD1 has been most extensively studied. The structure of SCD1 has been examined to determine similarities between isoforms and to provide insight into function. Stearoyl-CoA desaturase 1 (SCD1) consists of three domains: an extracellular membrane domain, a transmembrane domain, and an intracellular membrane domain (Mziaut et al., 2000). The membrane spanning sections contain the active site, the N terminus, and the C terminus. The

molecular weight of the protein is 37 kDa. There is a segment rich in histidines that is a key functional section of the protein (Thiede et al., 1986). It also contains two arginyl residues that might be important for the protein function. When these residues are alkylated, the activity of the protein is decreased (Enoch and Strittmatter, 1978).

The tissue distribution of SCD1 has been studied in mice, rats, and sheep. Rat SCD1 is located in liver, kidney, lung, heart, adipose tissue, and spleen (Mihara, 1990). In the mouse, SCD1 is located in adipose tissue, but can be induced in liver and kidney (Heinemann and Ozols, 2003, Ntambi et al., 1988). Ovine SCD1 mRNA was located in subcutaneous adipose tissue, liver, and mammary gland (Ward et al., 1998), but adipose and mammary gland expression were greater than liver. Desaturase activity has been found in the sheep intestinal mucosa as well (Bickerstaffe and Annison, 1969).

The tissue distribution of SCD1 in cattle has not been studied extensively and results from existing studies are inconsistent. Bovine SCD1 mRNA was first extracted from subcutaneous adipose tissue (Chung et al., 2000). St. John et al. (1991) measured SCD activity in bovine subcutaneous adipose tissue and liver and found that activity was present in adipose tissue, but was not detectable in liver microsomes. Activity was compared between bovine subcutaneous adipose tissue and rat liver and was shown to be similar (St John et al., 1991). Similar results were observed by Page et al. (1997) for subcutaneous adipose tissue, but liver SCD activity in this study was detectable although it was drastically lower compared to adipose tissue activity. Chang et al. (1992) collected samples of duodenum, liver, longissimus dorsi muscle, perianal adipose tissue, and subcutaneous adipose tissue from calves fed different diets. SCD

activity was detected in the liver and duodenum of one calf and the muscle of two others fed the control diet. Activity was detected in all tissue samples of the animals fed a high fat diet indicating potential regulation of SCD tissue distribution by diet. In addition to these tissues, SCD activity has been detected in mammary tissue from lactating cows, but was low in tissue from non-lactating cows (Kinsella, 1972).

Stearoyl-CoA desaturase 1 in mice is detected in mostly subcutaneous adipose tissue, liver, and kidney. Ovine studies have detected SCD1 in subcutaneous adipose tissue, liver, mammary gland, and intestinal mucosa. Unlike mice and ovine, bovine studies provide inconsistent results. Bovine SCD1 has been detected in subcutaneous adipose tissue, liver, duodenum, perianal adipose tissue, and longissimus dorsi muscle.

Regulation of Stearoyl-CoA Desaturase-1 by Fatty Acids

Fatty acids regulate SCD activity and mRNA. Saturated fatty acids and MUFA supplemented in animal diets or cell culture media have little or no effect on SCD activity or mRNA, but usually increase SCD activity if effects are observed (Heinemann and Ozols, 2003, Ntambi, 1999). Diets supplemented with tripalmitin (16:0), tristearin (18:0), and triolein (*cis*-9 18:1) had no effect on liver SCD1 mRNA in mice (Ntambi, 1992), but other studies have observed slight reductions in SCD1 mRNA with triolein (Landschulz et al., 1994, Ntambi, 1992). Diets supplemented with cocoa butter, a fat rich in saturated fatty acids, had no effect on SCD1 mRNA in mice unlike diets supplemented with olive oil, which is rich in oleic acid that decreased SCD1 mRNA (Landschulz et al., 1994). Saturated and monounsaturated fatty acids exhibit similar effects on SCD1 mRNA *in vitro*. Stearic acid did not affect SCD1 mRNA in HepG2 hepatocytes (Waters et al., 1997) and stearic and oleic acid did not affect SCD1 mRNA

in 3T3-L1 adipocytes (Sessler et al., 1996). Overall, saturated and MUFA do not consistently affect SCD1 mRNA, but slight reductions with MUFA have been observed in some studies.

Unlike saturated fatty acids, PUFA have been shown to decrease SCD activity and mRNA. Rats fed a diet containing linoleic acid (*cis*-9, *cis*-12 18:2) had decreased SCD activity (Jeffcoat and James, 1978), and linoleic, linolenic, and arachidonic acids decreased SCD1 mRNA compared to palmitic, stearic, and oleic acids (Ntambi, 1992). Sunflower and corn oils are high in PUFA and rats fed diets containing these oils had reduced hepatic SCD activity (Jeffcoat and James, 1978, Weekes et al., 1986). Similarly, rats fed a high PUFA diet had decreased SCD1 mRNA in adipose tissue and liver (Jones et al., 1996). Similar results are observed with mice. Diets containing safflower oil decreased SCD1 mRNA in mice compared to mice fed a high-carbohydrate fat-free diet (Landschulz et al., 1994). Trilinolein, trilinolenin, and triarachidonin reduced SCD1 mRNA in mice compared to tristearin and the greatest reductions were observed with the triarachidonin diet (Landschulz et al., 1994, Ntambi, 1992, Waters and Ntambi, 1996). The triarachidonin (*cis*-5, 8, 11, 14 20:4) diet decreased SCD1 mRNA to a greater extent due to the degree of unsaturation.

As observed *in vivo*, stearoyl-CoA desaturase mRNA is decreased when cells are cultured in media containing PUFA. SCD1 mRNA abundance was reduced in primary hepatocytes cultured with unsaturated fatty acids and the reduction increased with unsaturation. The greatest reduction was observed with arachidonate and eicosapentaenoate, twenty carbon fatty acids with four and five double bonds, respectively (Landschulz et al., 1994). HepG2 cells incubated in media containing

linolenic acid had reduced SCD1 mRNA compared to control (Waters et al., 1997). In 3T3-L1 cells, SCD1 activity and mRNA were reduced with arachidonic, linoleic, and linolenic acids compared to untreated cells. Also, as the concentration of arachidonic acid was increased, SCD activity was reduced in association with reductions in SCD1 mRNA (Jones et al., 1996, Ntambi, 1999, Sessler et al., 1996).

In bovine mammary epithelial cells (MAC-T), stearic and vaccenic acids did not alter promoter activation of the bovine SCD1 gene, but CLA isomers decreased bovine SCD1 promoter activation in MAC-T cells (Keating et al., 2006). The *trans*-10, *cis*-12 CLA isomer decreased the activation of SCD drastically, but linoleic and linolenic acids had no effect (Keating et al., 2006).

PUFA may modulate SCD activity and mRNA through transcriptional or posttranscriptional mechanisms (Waters and Ntambi, 1996). One mechanism for PUFA regulation of SCD1 is through posttranscriptional regulation that involves destabilization of SCD1 mRNA. 3T3-L1 cells incubated with aracidonic acid had a reduction in the half life of SCD1 mRNA from 8 to 4 hr (Ntambi, 1999, Sessler et al., 1996, Sessler and Ntambi, 1998). A better defined mechanism involves regulation of transcription. Stearoyl-CoA desaturase transcription was decreased when measured by run-on transcription assays when mice were fed a diet containing trilinolein (Landschulz et al., 1994). The promoter of SCD1 contains a PUFA-responsive element (PUFA-RE) that could bind factors that block or may be needed for transcription. The PUFA-RE is a 60 bp region on the SCD1 promoter (Ntambi, 1999, Sessler and Ntambi, 1998, Waters et al., 1997) and nuclear proteins bind this region (Waters et al., 1997). Several transcription factors regulated by PUFA are known and these include peroxisome

proliferator activated receptor (PPAR)α and sterol regulatory element binding protein SREBP-1c. Although PPARα binds PUFA and controls transcription, the SCD1 promoter PPRE is located in a region different from the PUFA-RE. However, the PUFA-RE does have a binding region for SREBP-1 and CCAAT-binding factor/nuclear factor Y (NF-Y) (Ntambi, 1999, Sessler and Ntambi, 1998).

Polyunsaturated fatty acids decrease activation of the SCD promoter by decreasing the mature forms of SREBP-1c. Only the mature form of SREBP-1c promotes transcription of the genes it regulates. SREBP-1c is bound to the endoplasmic reticulum in its inactive, precursor form and is cleaved at the N terminus by SREBP cleavage-activating protein (SCAP), Site-1 protease (S1P), or Site-2 protease (S2P) to become active. Once cleaved, the SREBP-1 protein translocates to the nucleus where it binds to the sterol response element (SRE) of promoters (Heinemann and Ozols, 2003). Polyunsaturated fatty acids have been shown to decrease SREBP-1c mRNA, the precursor form, and the active nuclear form and decreased SREBP-1c binding to the SCD1 promoter is a mechanism for reducing SCD1 transcription (Heinemann and Ozols, 2003).

Saturated and monounsaturated fatty acids do not have an effect on SCD activity or mRNA, but increase SCD activity if effects are observed. Unlike saturated and monounsaturated fatty acids, polyunsaturated fatty acids decrease SCD activity and mRNA. Polyunsaturated fatty acids might regulate SCD1 through several mechanisms that include: destabilize SCD1 mRNA, down regulate transcription of SCD1, and decrease the activation of the SCD1 promoter.

Regulation of Stearoyl-CoA Desaturase-1 by Carbohydrates

Carbohydrate diets regulate SCD activity and mRNA in rodents. Rats and mice fed high carbohydrate diets have increased liver SCD1 activity and mRNA consistently (Ntambi, 1995). Specific sugars have varying effects as well. Diets containing fructose increased SCD activity in rats (Jeffcoat and James, 1977) and in diabetic mice (Prasad and Joshi, 1979), which have low levels of SCD1 expression. However, the increase in SCD mRNA was greater in normal mice fed a fructose diet along with injection of insulin (Ntambi, 1992, 1995, Waters and Ntambi, 1994). A diet containing glucose increased SCD activity in rats, but no increase was observed in diabetic rats until insulin was injected (Jeffcoat and James, 1977, Prasad and Joshi, 1979). Rats fed sucrose, fructose, or glucose diets had increased SCD1 activity compared to controls fed starch, but the activity was greatest in rats fed glucose (Jeffcoat and James, 1977).

Carbohydrates increase SCD1 mRNA and activity *in vitro*. Fructose added to the media of primary hepatocytes increased SCD1 activity, and the increase was greater with the addition of insulin. No increase in SCD1 activity with glucose was observed until insulin was added to culture media (Spence and Pitot, 1982). Unlike primary hepatocytes, there was an increase in SCD1 mRNA with glucose in 3T3-L1 adipocytes (Jones et al., 1998).

Carbohydrates and insulin may affect SCD1 mRNA through SREBP-1c. Glucose and fructose increased SREBP-1c mRNA, but fructose increased SREBP-1c mRNA to a greater extent than glucose (Miyazaki et al., 2004). However, the carbohydrate regulation of SCD1 transcription does not depend on SREBP-1c because SCD1 mRNA was increased in fructose fed SREBP-1c knockout mice (Miyazaki et al., 2004).

Carbohydrates increase SCD1 mRNA and activity *in vivo* and *in vitro*. Carbohydrates such as fructose, glucose, and sucrose increased SCD activity and mRNA. Fructose increased SCD1 mRNA and activity to a greater extent when insulin was added.

Regulation of Stearoyl-CoA Desaturase-1 by Insulin

Insulin has been shown to increase SCD activity, mRNA abundance, and promoter activation in rats, mice, sheep, and cows. Mice and rats have been studied extensively to understand the regulation of SCD1 by insulin. The activity of SCD was decreased in livers and adipose tissues of mice treated with alloxan, which decreases plasma insulin concentrations, indicating the importance of insulin for the induction of SCD activity (Enser, 1979, Prasad and Joshi, 1979). Injection of insulin in diabetic mice increased hepatic SCD activity (Prasad and Joshi, 1979). Diabetic mice injected with insulin had a 22-fold increase in hepatic SCD1 mRNA within 20 hr of injection (Waters and Ntambi, 1994). Taken together these data indicate that insulin positively regulates SCD activity and that insulin regulates SCD1 mRNA expression, possibly through transcriptional regulation.

Studies conducted *in vitro* have shown similar results regarding the regulation of SCD activity by insulin. Primary hepatocytes and 3T3-L1 cells incubated in media containing insulin had increased SCD1 activity (Jeffcoat et al., 1979, Kasturi and Joshi, 1982, Spence and Pitot, 1982). Similarly, when 3T3-L1 cells were cultured in media containing anti-insulin serum there was a reduction in SCD activity, further demonstrating the regulation of SCD activity by insulin (Kasturi and Joshi, 1982). Insulin induced increases in activity are mediated by increases in SCD1 mRNA abundance *in*

vitro. When 3T3-L1 and H2.35 cells were cultured in media that did not contain insulin, SCD1 mRNA was not detected (Weiner et al., 1991), whereas cells cultured in media containing insulin had detectable levels of SCD1 mRNA (Kim and Ntambi, 1999, Ntambi et al., 1996, Weiner et al., 1991). This insulin induced up regulation of SCD1 mRNA expression explains activity measurements and correlates with *in vivo* studies.

Insulin has also been shown to increase SCD1 mRNA abundance in sheep adipose tissue explants. Subcutaneous, perirenal, and omental adipose tissue explants cultured in media containing insulin had increased SCD1 mRNA compared to untreated controls (Daniel et al., 2004a, Ward et al., 1998). The greatest insulin-induced increase in SCD1 mRNA was seen in the subcutaneous adipose tissue explants (Daniel et al., 2004a). The increase in SCD1 mRNA correlated with the fatty acid profiles, which had increased MUFA, indicating increased SCD activity (Daniel et al., 2004a). Overall, SCD1 mRNA was increased with insulin treatment with explants of sheep adipose tissue.

Insulin has been shown to increase SCD1 promoter activation in a bovine mammary epithelial cell line (MAC-T) and increase SCD1 mRNA expression in bovine primary stromal-vascular cells. When MAC-T cells were transfected with a reporter construct containing the SCD1 promoter and cultured in media containing insulin, there was an increase in the activation of the promoter (Keating et al., 2006). Similarly, primary cells cultured in media containing insulin had an increase in SCD1 mRNA expression (Smith, 2006). The insulin-induced up regulation of SCD1 promoter activity and mRNA expression in bovine cells suggests that insulin may act on the promoter of SCD1.

Thus, multiple studies have shown that SCD1 mRNA is increased by insulin, which indicates that insulin regulates SCD1 through transcriptional activation (Ntambi, 1995). Insulin is a ligand for a tyrosine kinase receptor that activates the PI3-kinase pathway resulting in the activation of SREBPs (Foufelle and Ferre, 2002, Jump, 2006). Fasted animals exhibit a reduction in the mature form of SREBP-1c, likely due to the low levels of insulin in these animals (Foufelle and Ferre, 2002, Horton et al., 2002). Insulin regulates hepatic genes by changing transcription of SREBP-1c (Foufelle and Ferre, 2002). SREBP-1c was determined to be the isoform of SREBP that regulates SCD1 using knock out mice (Shimano et al., 1997). This transcription factor increases SCD1 mRNA and activity by binding to the sterol response element (SRE) on the SCD1 promoter and initiating transcriptional activation (Foufelle and Ferre, 2002, Heinemann and Ozols, 2003, Nakamura and Nara, 2002). Stearoyl-CoA desaturase-1 mRNA expression and activity were both increased when SREBP-1c was overexpressed (Horton et al., 2002, Moon et al., 2001, Ntambi, 1999, Shimomura et al., 1998). Insulin also activates other transcription factors such as liver X receptors (LXRs) (Heinemann and Ozols, 2003). Mice deficient in LXR have a reduction in SCD1 expression indicating another mechanism by which insulin might regulate SCD1. In conclusion, insulin regulates SCD1 mRNA expression and activity through the activation of SREBP-1c, a transcription factor that binds to the promoter of the SCD1 gene causing the induction of transcription.

Insulin increases SCD activity and mRNA in rats, mice, sheep, and bovine cells. This has been shown in *in vivo* and *in vitro* studies. Insulin regulates SCD1 through transcriptional activation by increasing the mature form of SREBP-1c or increasing the

transcription of SREBP-1c, which binds to the sterol response element on the SCD1 promoter.

Degradation of Stearoyl-CoA Desaturase-1

Regulation of SCD1 protein concentration is mediated through degradation. SCD1 rapidly turns over and its short half life facilitates rapid transcriptional regulation of SCD activity. Recently, sophisticated methodology for measuring SCD1 degradation in real-time has been developed using green fluorescent protein (GFP) technology. This protein fluoresces when exposed to light of the correct wavelength and allows for non-invasive measurement of protein abundance when fused to another protein. To measure SCD half-life in real-time, CHO cells were transfected with a construct that produced a fusion protein of SCD1 and GFP. Cells were then exposed to cycloheximide to eliminate protein translation leaving only SCD-GFP proteins formed prior to cessation of protein translation. This treatment does not influence protein degradation and degradation proceeds at its normal rate. Fluorescence decreased indicating degradation of the SCD-GFP fusion protein with a half life of 4 hr. The half-life was increased to 6 hours by treatment with MG132, a proteasome inhibitor (Heinemann and Ozols, 1998, 2003, Kato et al., 2006, Mziaut et al., 2000).

The signal initiating degradation functions on the cytosolic side, but not on the lumenal side of the ER membrane and is found at the N terminus of the protein (Mziaut et al., 2000). An N terminus lysine residue is important for degradation. When this residue is mutated to alanine, protein stability is significantly increased. This lysyl residue is a tag for the ubiquitin degradation pathway (Heinemann and Ozols, 2003, Mziaut et al., 2000). The N terminus also contains PEST sequences that target the

protein for degradation (Mziaut et al., 2000) by the ubiquitin proteasome pathway (Kato et al., 2006). Degradation is regulated through the endoplasmic reticulum associated protein degradation (ERAD) pathway that identifies polyubiquitylated substrates. Substrates are ubiquitylated by E2 ubiquitin-conjugating enzymes and E3 ubiquitin ligases. The polyubiquitylated protein is then translocated to the cytosol and degraded by proteasomes (Kato et al., 2006).

During degradation, a 20-kDa fragment of SCD1 is formed. This fragment could be produced from a microsomal protease (Heinemann and Ozols, 1998). This protease has properties similar to an integral membrane protein and is similar in structure to plasminogen (Heinemann and Ozols, 1998). SCD1 protein was degraded when incubated with plasminogen and urokinase or a plasminogen activator. Plasmin can degrade SCD protein and it might be due to its location on the ER membrane that influences the activation of the protease (Heinemann et al., 2003).

Stearoyl-CoA desaturase 1 is quickly turned over with a half life of 4hr. The degradation sequence is located on the N terminus of the protein. Degradation of the protein is regulated by the ERAD pathway.

Overall, SCD1 produces the double bond formation using an electron transport chain that involves NADH, oxygen, cytochrome b_5 reductase, and cytochrome b_5. There are four isoforms identified in mice, but only two isoforms have been identified in bovine. Tissue distribution studies with bovine SCD activity are inconsistent. Stearoyl-CoA desaturase 1 activity and mRNA is increased with carbohydrate diets, saturated fatty acids, MUFA, and insulin, but decreased with PUFA. Finally, SCD1 is quickly turned over and has a half life of 4 hr.

CHAPTER 3: TISSUE DISTRIBUTION OF STEAROYL-COA DESATURASE IN CALVES AND REGULATION BY DIETARY FAT

Introduction

Consumers are increasingly concerned with the healthfulness of food components and are especially concerned with fat content and quality. Consumers have progressively reduced their intake of products that contain fat and increased their consumption of products that contain no fat, are low in fat, or lean. Producers of animal products want to increase the nutritional quality of their products to appeal to consumers and are interested in manipulating fatty acid composition (Lock and Bauman, 2004). Ruminant products contain a large amount of saturated fatty acids. For example, milk contains 70% saturated fatty acids, 25% monounsaturated fatty acids (MUFA), and 5% polyunsaturated fatty acids (PUFA) compared to plant oils that contain a higher percentage of MUFA and PUFA (Grummer, 1991, Lock and Bauman, 2004). The saturated fatty acid content of ruminant products concerns consumers due to the increased risk of heart disease and increased low-density-lipoprotein (LDL) cholesterol associated with these fatty acids. The saturated fat content of ruminant fats is due to biohydrogenation. Biohydrogenation is a process carried out by two populations of bacteria in the rumen that convert PUFA from the diet to saturated fatty acids (Harfoot, 1988).

One protein involved in manipulating fatty acid composition in tissues and milk is stearoyl-CoA desaturase (SCD). Stearoyl-CoA desaturase is an enzyme located in the membrane of the endoplasmic reticulum, and converts saturated fatty acid substrates to monounsaturated fatty acids by inserting a *cis* double between carbons 9 and 10. Substrates include palmitoyl-CoA and stearoyl-CoA, which are converted to

palmitoleoyl-CoA and oleoyl-CoA, respectively (Heinemann and Ozols, 2003, Man et al., 2006, Ntambi, 1999). Stearoyl-CoA desaturase also converts *trans*-11 18:1 to *cis*-9, *trans*-11 CLA (Bauman, 2003), a fatty acid with beneficial health properties (Belury, 2002).

Stearoyl-CoA desaturase is expressed in a variety of tissues in mice and sheep. Four isoforms of SCD have been identified in mice, two in rats, and two in humans (Miyazaki et al., 2003, Ntambi et al., 2004). In mice, SCD1 mRNA is detected in adipose tissue constitutively, but can be induced in liver and kidney by feeding a fat-free, high carbohydrate diet (Miyazaki and Ntambi, 2003, Ntambi, 1999, Ntambi et al., 1988). In sheep, SCD1 mRNA was detected in subcutaneous adipose tissue, liver, intestinal mucosa and mammary gland (Bickerstaffe and Annison, 1969, Ward et al., 1998). In the bovine, there has been little research to expressly determine the tissue distribution of SCD and results are inconsistent. For example, SCD activity was detected in subcutaneous adipose tissue, but liver had little or no activity (Page et al., 1997, St John et al., 1991); however, SCD activity was detected in subcutaneous adipose tissue, liver, duodenum, and longissimus dorsi muscle in another study (Chang et al., 1992).

Stearoyl-CoA desaturase expression is regulated by diet (Ntambi, 1995). Mice fed a high-carbohydrate diet had increased SCD activity (Prasad and Joshi, 1979); however, carbohydrate diets supplemented with fat fed to mice reduced SCD1 mRNA (Ntambi, 1992). Mice fed a high calorie high-fat diet had increased fat accumulation and increased SCD1 mRNA compared to mice fed a low-fat diet (Hu et al., 2004). SCD

activity is also correlated with body fat content independent of the diet. Obese mice have greater SCD activity compared to lean mice (Enser, 1979).

The long term goal of this research is to improve the fatty acid composition of ruminant products. Stearoyl-CoA desaturase is a target enzyme that could be manipulated to reach this goal. To achieve this long term goal, the tissues expressing SCD1 must be established. Once the tissue distribution is identified, then regulation can be investigated to develop methods to increase SCD1 protein expression and activity. In the present study we examined the tissue distribution of SCD1 protein expression in calves, and investigated the effect of increasing dietary fat on SCD1 protein expression.

Materials and Methods

Animals

All procedures involving animals were approved by the Virginia Tech Animal Care and Use Committee. Experimental details have been previously reported (Hill, 2006). A total of twenty-four Holstein heifer calves were fed one of four milk replacer diets for 63 d: control (CON; 20% protein, 20% fat at 0.350 kg DM/d), high protein, low fat (HPLF; 28% protein, 20% fat at 0.764 kg DM/d DM), high protein, high fat (HPHF; 28% protein, 28% fat at 0.782 kg DM/d), and high protein, high fat with increased intake (HPHF+28% protein, 28% fat at 1.177 kg DM/d). Starter was available throughout the experiment and consisted of 40% corn, 40% soybean meal, and 20% cottonseed hulls. Six calves were allotted to each treatment diet, and diets were produced by Land O'Lakes Animal Milk Products Co. (Arden Hills, MN) and fed twice a day at 0700 and 1900. Calves arrived in one of three groups (n=8/group) and the dates of arrival were: August 20, September 10, and September 25, 2005. Calves were randomly assigned to treatments with equal representation in each group. The calves were housed in hutches at the Virginia Tech Dairy Center. Weights and heights were measured weekly, starter orts recorded at evening feeding, and intake was measured at each feeding.

Sampling

Calves were euthanized after 63 d on treatment diets with an overdose of sodium pentobarbital and tissue samples were harvested. Subcutaneous adipose tissue was removed from the inner, rear leg. The tissue was cut into small pieces less than 1 cm in diameter, snap frozen in liquid nitrogen, and stored at -80℃. Perirenal and omental adipose tissue samples and liver samples were also collected. The intestines were

removed and the mesentery separated from the small intestine. Sixty cm lengths of the duodenum and ileum were collected from the terminal ends of the intestine. One 60 cm length of the jejunum located 2-3 m distal of the duodenum and one 60 cm length located 2-3 m proximal to the ileo-cecal junction were collected and rinsed with water. All four sections were placed on ice. The lengths were opened longitudinally on a piece of ice cold glass and the mucosa was scraped from the serosa with a microscope slide. Scraped mucosa was snap frozen in liquid nitrogen and stored at -80℃.

Immunoblotting

Tissue samples (1 g) were homogenized with Pro200 homogenizer (Pro Scientific, Oxford, CT) at setting 3 for ~30 sec in 2 ml of lysis buffer (500 mM Tris pH 7.4, 0.5% Triton X-100, 300 mM NaCl, 2 mM EDTA pH 8, mammalian protease inhibitor cocktail (Sigma, St. Louis, MO). The homogenate was centrifuged at 14,000xg, 4℃ for 10 minutes to remove cell debris. The supernatant was centrifuged again at 14,000xg, 4℃ for 10 minutes and the secondary supernatant st ored at -20℃. Protein concentration was measured using the Bradford assay (Bio-Rad, Hercules, CA).

Supernatants were mixed with an equal volume of 2X Laemmli sample buffer (Sigma, St. Louis, MO) and heated at 95℃ for 5-10 minutes. Samples were electrophoresed on a 12% SDS-PAGE gel and transferred using Bio-Rad trans-blot SD semi-dry transfer cell (Bio-Rad, Hercules, CA) to a PVDF membrane. Membranes were blocked to reduce background staining by incubation in Tris-buffered saline containing Tween-20 (TBST; 0.05 M Tris pH 7.4, 0.2 M NaCl, 0.1% Tween) containing 5% dried nonfat milk for one hour at room temperature on a shaking platform. This was followed by incubation in a solution (TBST with 5% dried nonfat milk) with antibodies specific to

bovine SCD1 (1:1000) in ~20-30 ml per membrane for one hour. Antiserum against the C terminus of bovine SCD1 was prepared by immunizing a rabbit with a 15 amino acid peptide corresponding to amino acids 345-359 of bovine SCD1 (LARIKRTGEESYKSG) conjugated to Keyhole limpet hemocyanin (KLH) (Pacific Immunology, Ramona, CA). Membranes were washed with ~20 ml of TBST then incubated in TBST with 5% dried nonfat milk containing goat anti-rabbit conjugated HRP secondary antibody (1:1000) in ~20-30 ml per membrane for one hour. Membranes were then washed with ~20 ml of TBST and developed using ECL-Plus Reagents (Amersham Biosciences, Pittsburgh, PA) according to the manufacturer's instructions. Chemiluminescence was visualized using a Chemidoc XRS digital imaging system, and densitometry (net intensity) was measured using Quantity One software (Bio-Rad, Hercules, CA).

Fatty acid analysis

Milk replacer fatty acids were extracted following Folch et al. (1957). Lipids were transesterfieid with 0.5N NaOH in methanol and 14% BF_3 (Park, 1994). Undecenoic acid was included as an internal standard. For tissue fatty acid analysis, subcutaneous, omental, and perirenal adipose tissue samples (100 mg) were homogenized in 3:2 hexane:isopropanol and extracted according to Hara and Radin (1978). A total of 40 mg of lipid from each sample was transferred to extraction tubes and used for methylation. Lipids were methylated by base-catalyzed transesterfication using sodium methoxide (Christie, 1982). Fatty acid methyl esters were analyzed using a gas chromatograph equipped with a flame ionization detector (Agilent 6890N, Santa Clara, CA) and a CP-Sil 88 column (100 m x 0.25 mm i.d. with 0.20 μm film thickness, Varian, Inc., Palo Alto, CA). Milk replacer fatty acid methyl esters were analyzed using a temperature program

started at 70℃ for 1 min, increased to 100℃ at 5° C/min for 3 min, increased to 175℃ at 10℃/min for 45 min, and finally increased to 22 0℃ at 5℃/min and held for 15 min. Injector and detector temperatures were 250℃ and 3 00℃, respectively. Gas flow rate through the column was 1.4 ml/min and the split ratio was 80:1. For adipose tissue fatty acid methyl esters, the temperature program started at 80℃ and increased by 2℃/min until it reached 190℃ and remained at this tempera ture for 15 min. Injector and detector temperatures were 250℃. Hydrogen flow rate to the detector was 25 ml/min, air flow rate was 400 ml/min, nitrogen flow rate was 40 ml/min, gas flow rate through the column was 1ml/min, and the split ratio was 100:1. Recovery and correction factors for adipose tissue fatty acids were calculated using a certified reference standard (Beef/pork fat blend; CRM 163; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium).

The indices of SCD activity were determined using products and substrates for SCD that included *cis*-9 14:1 and 14:0, *cis*-9 16:1 and 16:0, *cis*-9 18:1 and 18:0, and *cis*-9, *trans*-11 CLA and *trans*-11 18:1. The indices were calculated as: (product)/(product + substrate) (Kelsey et al. 2003).

Statistical Analysis

Data are reported as least square means ± SEM. Gauss trace (densitometry) and fatty acid data were analyzed using the Mixed procedure of SAS (SAS for Windows Version 9.1, SAS Institute Inc., Cary, NC). The model included block, diet, and block by diet interaction. Block indicates the time of arrival for the calves since the calves arrived in one of three groups. Diet is defined by the milk replacer diets. If a significant

treatment effect was observed, Tukey’s multiple comparison procedure was used to separate treatment means.

Table 3.1 Milk replacers fed to 24 Holstein calves.

	Control (CON)	**High Protein, Low Fat (HPLF)**	**High Protein, High Fat (HPHF)**	**High Protein, High Fat 1.5% (HPHF+)**
Milk Replacer (kg DM/d)	0.350	0.764	0.782	1.177
Fat (%DM)	20	20	25	25
Protein (%DM)	24	32	31	31

Table 3.2 Fatty acid composition of milk replacer diets fed to 24 Holstein calves.

Fatty Acid	CON	HPLF	HPHF and HPHF+
	--------*% of total fat*--------		
14:0	1.51	1.51	1.49
16:0	23.85	23.68	23.63
16:1, *cis*-9	1.96	1.96	1.96
17:0	0.31	0.32	0.30
18:0	12.93	12.87	12.85
18:1, *cis*-9	34.87	34.78	35.00
18:1, *cis*-11	2.20	2.21	2.23
18:1 *trans*-6 and 7	0.1	0.1	0.1
18:1 *trans*-9	0.2	0.2	0.2
18:1 *trans*-10	0.2	0.2	0.2
18:1 *trans*-11	0.1	0.1	0.1
18:2 *cis*-9, *cis*-12	17.05	17.39	17.20
20:1, *cis*-11	0.67	0.66	0.67
18:3 *cis*-9,12,15	0.86	0.88	0.87
18:2, *cis*-9, *trans*-11	0.14	0.16	0.16
20:2 *cis*-11,14	0.99	0.94	0.98
20:4 *cis*-5, 8,11,14	0.22	0.22	0.22

Results

Tissue Distribution

The first objective was to determine the tissue distribution of SCD1 by protein expression using an antibody specific to bovine SCD1. SCD1 protein expression was measured in samples from calves fed the HPHF+ diet. SCD1 was detected at a similar concentration in subcutaneous, omental, and perirenal adipose tissues, but was undetectable in duodenum, jejunum, ileum, or liver samples (Figure 3.1). Comparing tissues, subcutaneous adipose tissue had numerically greater protein expression than perirenal or omental adipose tissues.

Dietary Fat

The second objective was to determine the effect of dietary fat on SCD1 protein expression. Dietary regulation was determined by measuring SCD1 protein expression in subcutaneous, omental, and perirenal adipose tissues from calves fed one of four diets: CON, HPLF, HPHF, or HPHF+. One calf from the HPLF diet group was not included in the analysis due to the death unrelated to treatment during the experiment. One sample was not included in the analysis of subcutaneous adipose tissue in the CON diet group due to insufficient tissue.

The measured treatment diet compositions (excluding starter), milk replacer intakes, and the fatty acid composition of the milk replacer diets are reported in Tables 3.1 and 3.2. The fat content of the HPHF and HPHF+ diets was less than formulated. The protein content was greater for all diets and intakes were lower than intended. Calculated fat intakes for the 4 diets were: 0.07, 0.153, 0.196, and 0.29 kg/d for the

CON, HPLF, HPHF, and HPHF+ diets, respectively. The fatty acid compositions were similar throughout all milk replacer diets.

Growth and body composition measurements of the calves are presented in Table 3.3 and more complete results have been previously reported (Hill, 2006). Body weight was significantly lower in the CON diet compared to the HPLF and HPHF+ diets ($p < 0.05$). The average daily gain was 0.41, 0.68, 0.63, and 0.82 kg/d for CON, HPLF, HPHF, and HPHF+, respectively. Average daily gain was significantly greater in the HPLF, HPHF, and HPHF+ diets compared to the CON diet. Calves fed the HPHF and HPHF+ diets had the greatest amount of fat in the empty body compared to the other diets, with the lowest amount in calves fed the CON diet ($p < 0.001$). Fat percent was similar between the HPHF and HPHF+ diets ($p > 0.05$), but both of these diets were significantly different from the CON ($p < 0.05$).

The fatty acid profiles of perirenal, omental, and subcutaneous adipose tissues from calves fed the four diets are presented in Tables 3.4, 3.5, and 3.6. Several fatty acids followed a similar trend in all tissues. Calves fed the CON diet had greater concentrations of 15:0, *trans*-6-8 18:1, *trans*-10 18:1, and *trans*-11 18:1 in all adipose depots. Greater concentrations of *cis*-9 18:1 and *cis*-9, *cis*-12 18:2 were present in the adipose tissue of calves fed the HPHF+ diet. Heptadecenoic acid (17:0) was lower in subcutaneous and perirenal adipose tissue from calves fed the HPHF+ diet compared to the CON diet. *Cis*-9, *trans*-11 CLA was increased in subcutaneous adipose tissues from the CON diet.

The *cis*-9 18:1 and *cis*-9, *trans*-11 CLA desaturase indices had similar trends in all tissues. The *cis*-9 18:1 desaturase index was increased in tissues from calves fed

the HPHF+ diet compared to the CON diet. The *cis*-9, *trans*-11 CLA desaturase index was increased in all tissues of the CON diet fed calves compared to the other diets. The *cis*-9 16:1 desaturase index was increased 31 and 36% in omental and subcutaneous adipose tissues from the HPHF+ diet compared to the CON diet, respectively. Finally, *cis*-9 14:1 desaturase index was 50% greater in subcutaneous adipose tissue from the HPHF+ diet compared to the CON diet.

SCD protein expression was increased in subcutaneous, omental, and perirenal adipose tissues of calves fed the HFHP+ diet compared to the other diets (Figures 3.2, 3.3, and 3.4). Stearoyl-CoA desaturase protein expression increased with the increase in fat content and intake. Significant treatment effects were observed in subcutaneous ($p < 0.05$) and perirenal ($p < 0.05$) adipose tissues. Using Tukey's multiple comparison, means were not significantly different in perirenal adipose tissue, but the pattern of response was similar. The HPHF+ diet increased SCD protein expression significantly in subcutaneous adipose tissue compared with calves fed the CON, HPLF, or HPHF diets.

Table 3.3 Nutrient intake, growth measurements, and body composition from Holstein calves fed milk replacers[1].

	CON	HPLF	HPHF	HPHF+	SEM	P <
Starter Intake, kg/d[2]	0.89	0.60	0.40	0.32	0.15-0.17	0.08
Energy, Mcal[3]	1.64	3.74	4.10	6.17		
Final Body Weight, kg	50.42[a]	63.65[bc]	55.69[ab]	64.79[c]	2.13-2.46	0.002
Fat of EBW, kg DM[4]	6.89[a]	10.32[b]	11.17[b]	16.05[c]	0.74-0.85	<0.0001
Fat, % of EBW[5]	16.83[a]	19.50[a]	23.83[b]	27.17[b]	0.01	<0.0001

[1] (Hill, 2006)
[2] Mean over the 63 d study.
[3] Energy of the milk replacer diets of calves fed equal amounts daily.
[4] Mean at slaughter.
[5] Mean at slaughter.
[a, b, c] significantly different $p < 0.05$.

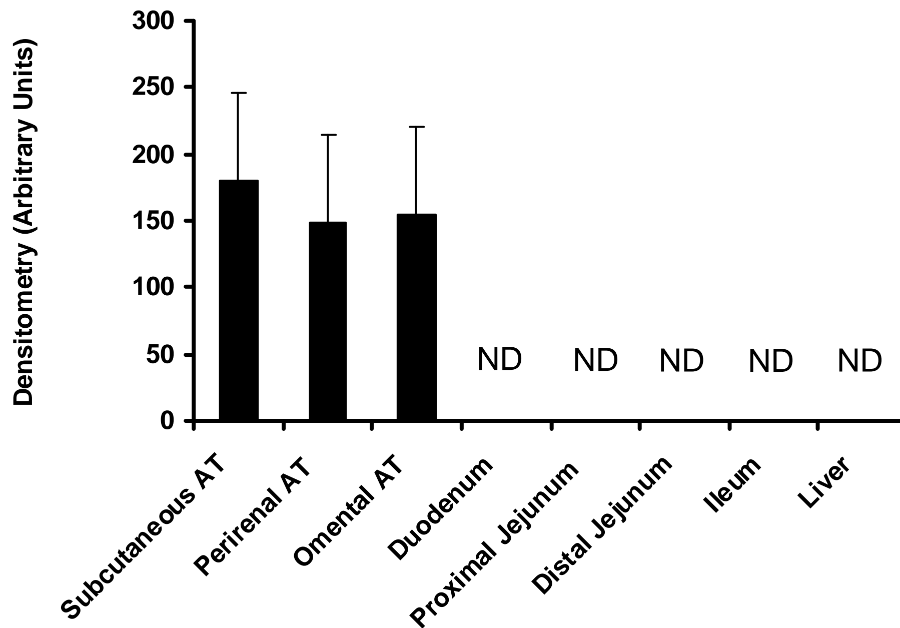

Figure 3.1 Stearoyl-CoA desaturase protein expression in tissues from calves (n = 6) fed the high protein, high fat with increased intake (HPHF+) diet. AT (adipose tissue) and ND (not detected).

Table 3.4 Fatty acid composition of perirenal adipose tissues from calves fed control (CON; n=6), high protein, low fat (HPLF; n=5), high protein, high fat (HPHF; n=6), and high protein, high fat with increased intake (HPHF+; n=5).

Fatty Acids	CON	HPLF	HPHF	HPHF+	SEM	P <
		------------*g/100 g Fatty Acid*------------				
14:0	1.41	1.18	1.16	1.16	0.08-0.09	0.16
14:1 *cis*-9	0.13	0.10	0.11	0.12	0.02-0.03	0.83
15:0	0.14^{a}	0.09^{b}	0.09^{b}	0.07^{b}	0.01	0.001
16:0	20.53	17.89	19.85	18.41	0.80-0.93	0.17
16:1 *cis*-9	1.91	1.48	1.88	2.14	0.18-0.21	0.23
17:0	0.82^{a}	0.68^{ab}	0.55^{bc}	0.42^{c}	0.47-0.06	0.002
18:0	19.76	21.45	18.14	17.42	0.86-0.99	0.059
18:1 *trans*-6-8	0.14^{a}	0.09^{ab}	0.07^{b}	0.06^{b}	0.01-0.02	0.012
18:1 *trans*-9	0.15	0.15	0.15	0.14	0.01	0.56
18:1 *trans*-10	3.67^{a}	0.91^{b}	0.45^{b}	0.26^{b}	0.25-0.29	<0.001
18:1 *trans*-11	0.29^{a}	0.23^{ab}	0.18^{b}	0.16^{b}	0.01-0.02	0.001
18:1 *trans*-12	0.16^{a}	0.11^{b}	0.09^{b}	0.08^{b}	0.01	<0.001
18:1 *cis*-9	35.82^{a}	40.24^{b}	41.10^{b}	42.78^{b}	0.52-0.60	<0.001
18:2 *cis*-9,12	10.22^{a}	10.56^{ab}	11.42^{bc}	11.85^{c}	0.21-0.24	0.002
20:0	0.18	0.19	0.17	0.17	0.01-0.02	0.72
18:3, *cis*-9, 12, 15	0.64	0.54	0.58	0.63	0.03-0.04	0.20
CLA, *cis*-9, *trans*-11	0.11	0.13	0.13	0.13	0.01-0.04	0.11
Others	3.94	3.99	3.90	4.00	0.06-0.07	0.67
Desaturase Indices						
cis-9 14:1	0.08	0.08	0.08	0.07	0.02	0.96
cis-9 16:1	0.08	0.08	0.09	0.10	0.01	0.16
cis-9 18:1	0.65^{a}	0.65^{ab}	0.69^{bc}	0.71^{c}	0.01	0.006
cis-9, *trans*-11 CLA	0.72^{a}	0.64^{b}	0.57^{b}	0.56^{b}	0.02	<0.001

Desaturase Index = (Product)/(Product + Substrate).
Comparisons analyzed using Tukey's multiple comparison.
[a, b, c] significantly different $p < 0.05$.

Table 3.5 Fatty acid composition of omental adipose tissues from calves fed control (CON; n=6), high protein, low fat (HPLF; n=5), high protein, high fat (HPHF; n=6), and high protein, high fat with increased intake (HPHF+; n=4).

Fatty Acids	CON	HPLF	HPHF	HPHF+	SEM	P <
		------------*g/100 g Fatty Acid*------------				
14:0	1.67[a]	1.50[ab]	1.43[b]	1.50[ab]	0.04-0.05	0.0273
14:1 *cis*-9	0.14	0.18	0.14	0.19	0.02	0.1457
15:0	0.22[a]	0.13[b]	0.12[b]	0.10[b]	0.01	<0.0001
16:0	22.42	21.15	22.20	21.59	0.42-0.47	0.2274
16:1 *cis*-9	1.92	2.23	2.31	2.50	0.12-0.14	0.0718
17:0	0.34	0.03	0.30	0.14	0.10-0.12	0.2241
18:0	16.96[ab]	17.33[a]	15.36[b]	15.10[b]	0.40-0.45	0.146
18:1 *trans*-6-8	0.20[a]	0.11[ab]	0.10[b]	0.08[b]	0.20	0.0157
18:1 *trans*-9	0.39[a]	0.22[ab]	0.16[b]	0.15[b]	0.05-0.06	0.0390
18:1 *trans*-10	4.17[a]	0.89[b]	0.46[b]	0.22[b]	0.36-0.41	0.0004
18:1 *trans*-11	0.29[a]	0.20[b]	0.17[b]	0.18[b]	0.01	0.0008
18:1 *trans*-12	0.16[a]	0.07[b]	0.08[ab]	0.07[b]	0.02	0.0247
18:1 *cis*-9	34.33[a]	39.23[b]	39.69[b]	40.58[b]	0.54-0.60	0.0004
18:2 *cis*-9, 12	10.80[a]	11.20[b]	12.10[c]	12.10[c]	0.04-0.05	<0.0001
20:0	0.15	0.13	0.13	0.12	0.01	0.1768
18:3 *cis*-9, 12,15	0.69[ab]	0.64[a]	0.67[ab]	0.72[b]	0.02	0.0453
CLA *cis*-9, *trans*-11	0.12	0.14	0.13	0.14	0.005	0.0524
Others	5.05	4.62	4.47	4.56	0.18-0.20	0.1883
Desaturase Indices						
cis-9 14:1	0.08	0.11	0.09	0.11	0.01	0.0818
cis-9 16:1	0.08[a]	0.10[ab]	0.09[ab]	0.10[b]	0.004	0.124
cis-9 18:1	0.67[a]	0.69[ab]	0.72[b]	0.73[b]	0.01	0.0032
cis-9, *trans*-11 CLA	0.71[a]	0.59[b]	0.56[b]	0.56[b]	0.01-0.02	0.0003

Desaturase Index = (Product)/(Product + Substrate).
Comparisons analyzed using Tukey's multiple comparison.
[a, b, c] significantly different $p < 0.05$.

Table 3.6 Fatty acid composition of subcutaneous adipose tissues from calves fed control (CON; n=6),high protein, low fat (HPLF; n=5), high protein, high fat (HPHF; n=6), and high protein, high fat with increased intake (HPHF; n=5).

Fatty Acids	CON	HPLF	HPHF	HPHF+	SEM	P <
		------------*g/100 g Fatty Acid*------------				
14:0	1.74	1.59	1.50	1.57	0.06-0.07	0.1597
14:1 *cis*-9	0.16^{a}	0.21^{ab}	0.18^{ab}	0.23^{b}	0.01-0.02	0.0234
15:0	0.22^{a}	0.12^{b}	0.11^{b}	0.09^{b}	0.01	0.0002
16:0	21.59	21.85	22.50	21.07	0.48-0.55	0.366
16:1 *cis*-9	2.15^{a}	2.50^{ab}	2.49^{ab}	2.96^{b}	0.10-0.12	0.0052
17:0	1.11^{a}	0.76^{b}	0.60^{bc}	0.53^{c}	0.04-0.05	<0.0001
18:0	17.81^{a}	17.01^{ab}	15.81^{b}	14.98^{b}	0.42-0.48	0.0074
18:1 *trans*-6-8	0.20^{a}	0.11^{ab}	0.11^{ab}	0.06^{b}	0.02	0.0078
18:1 *trans*-9	0.56	0.20	0.17	0.12	0.12-0.14	0.1031
18:1 *trans*-10	4.56^{a}	0.95^{b}	0.50^{b}	0.29^{b}	0.36-0.41	<0.0001
18:1 *trans*-11	0.34^{a}	0.19^{b}	0.18^{b}	0.17^{b}	0.02	0.0009
18:1 *trans*-12	0.16	0.11	0.09	0.08	0.02	0.0889
18:1 *cis*-9	33.39^{a}	38.19^{b}	39.13^{bc}	40.80^{c}	0.43-0.50	<0.0001
18:2 *cis*-9, 12	10.49^{a}	10.78^{ab}	11.58^{bc}	12.10^{c}	0.19-0.22	0.0015
20:0	0.16	0.14	0.14	0.13	0.01	0.3578
18:3 *cis*-9, 12, 15	0.74	0.74	0.71	0.82	0.04-0.05	0.4142
CLA *cis*-9, *trans*-11	0.13	0.16	0.12	0.16	0.01	0.0362
Others	4.53	4.4	4.09	3.85	0.34-0.39	0.5845
Desaturase Indices						
cis-9 14:1	0.08^{a}	0.12^{b}	0.10^{ab}	0.13^{b}	0.01	0.0072
cis-9 16:1	0.09^{a}	0.10^{a}	0.1^{a}	0.12^{b}	0.003	0.0002
cis-9 18:1	0.65^{a}	0.69^{b}	0.71^{bc}	0.73^{c}	0.01	<0.0001
cis-9, *trans*-11 CLA	0.72^{a}	0.55^{b}	0.59^{b}	0.51^{b}	0.03	0.0019

Desaturase Index = (Product)/(Product + Substrate).
Comparisons analyzed using Tukey's multiple comparison.
[a, b, c] significantly different $p < 0.05$.

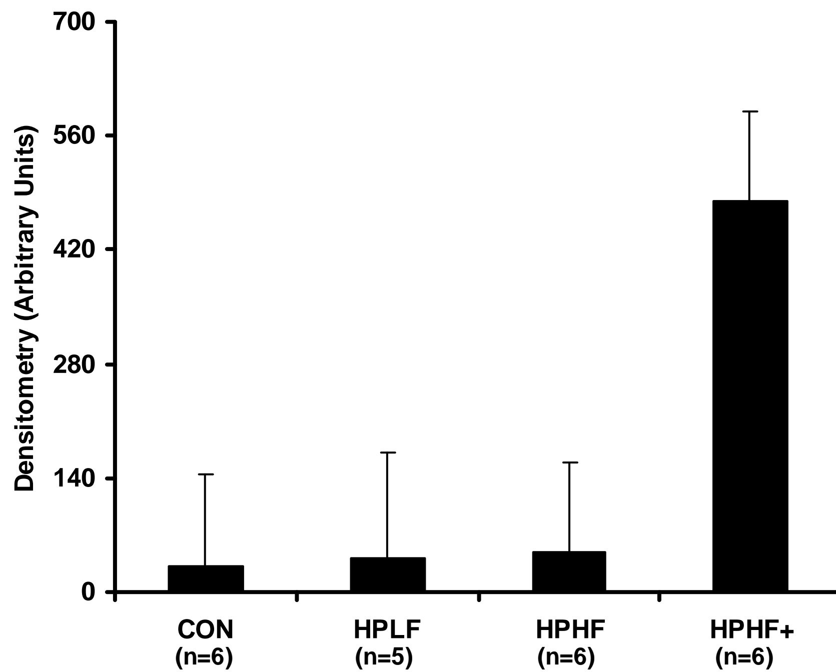

Figure 3.2 Stearoyl-CoA desaturase-1 protein expression in perirenal adipose tissue from calves (n = 23) fed control (CON), high protein, low fat (HPLF), high protein, high fat (HPHF), and high protein, high fat with increased intake (HPHF+). Treatment $p < 0.05$.

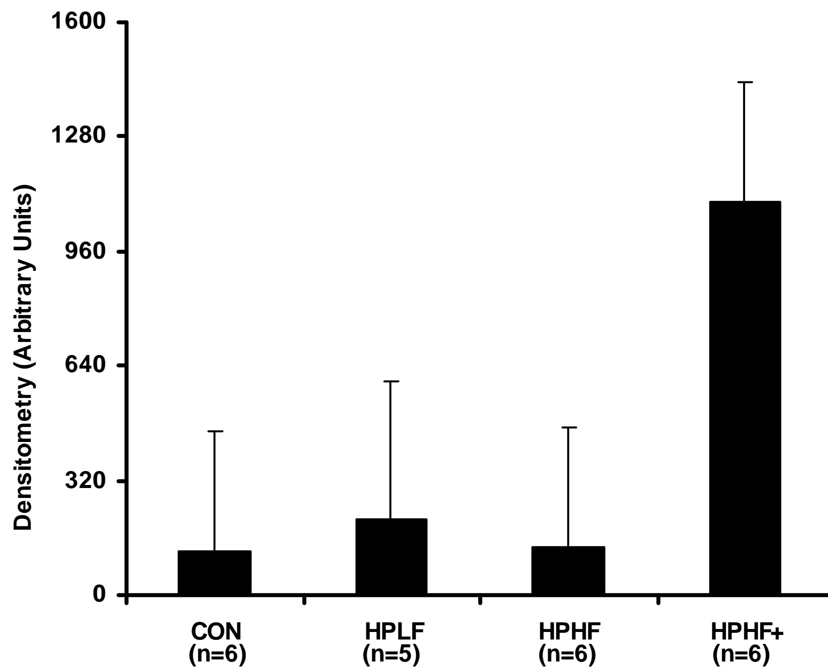

Figure 3.3 Stearoyl-CoA desaturase-1 protein expression in omental adipose tissue from calves (n = 23) fed control (CON), high protein, low fat (HPLF), high protein, high fat (HPHF), and high protein, high fat with increased intake (HPHF+). Treatment $p > 0.05$.

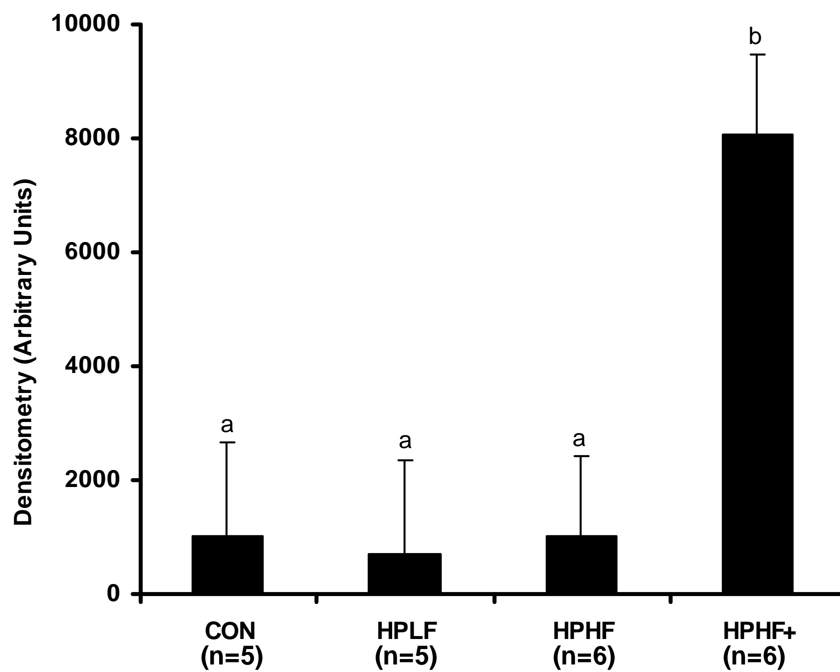

Figure 3.4 Stearoyl-CoA desaturase-1 protein expression in subcutaneous adipose tissue from calves (n = 23) fed control (CON), high protein, low fat (HPLF), high protein, high fat (HPHF), and high protein, high fat with increased intake (HPHF+). Treatment $p < 0.05$. [a, b] significantly different $p < 0.05$.

Discussion

Stearoyl-CoA desaturase protein expression was detected in perirenal, omental, and subcutaneous adipose tissue, but not in the liver, duodenum, jejunum, or ileum. SCD1 expression in most species is associated with lipogenic tissues. In mice and rats, SCD1 mRNA is normally found in adipose tissue, but can be induced in the liver and kidney by feeding a fat-free, high-carbohydrate diet (Ntambi et al., 1988). In ruminants, adipose tissue is lipogenic and the liver is not, unlike the liver of mice. The detection of SCD1 protein expression in subcutaneous, omental, and perirenal adipose tissues supports other studies. In mice and sheep, SCD1 mRNA was detected in subcutaneous adipose tissue (Ntambi et al., 1988, Ward et al., 1998). Others have measured SCD activity in bovine subcutaneous adipose tissue (Page et al., 1997, St John et al., 1991), (Chang et al., 1992).

We did not detect SCD1 protein expression in liver or small intestine in support of St. John et al. (1991). In contrast, Page et al. (1997) detected SCD activity in the liver, but at drastically reduced levels compared to subcutaneous adipose tissue. Similarly, Chang et al. (1992) detected SCD activity in both bovine liver and duodenum samples. SCD activity has been reported for ovine intestinal mucosa (Bickerstaffe and Annison, 1969).

One potential explanation for conflicting findings with respect to SCD activity in the bovine liver is dietary. For example, in the study by St. John et al. (1991) where SCD activity was not detected, animals were fed a high-energy diet that contained 33% corn and 33% ground milo diet, while Chang et al. (1992) compared a corn based control diet and a diet containing 10% sunflower seed. A corn based diet has shown to

decrease SCD1 mRNA in cattle and corn oil decreased SCD1 mRNA in rats (Chung et al., 2007, Jones et al., 1996). St. John et al. (1991) could have down regulated SCD1 mRNA affecting the activity, whereas Chang et al. (1992) up regulated SCD1 mRNA and activity. These experiments relied on activity measurements for determining the presence of SCD and consequently do not distinguish between isoforms. With the recent discovery of SCD5 in bovine, it maybe they were detecting SCD5 activity in the liver and small intestine rather than SCD1 (Lengi and Corl, 2007). However, SCD5 is minimally expressed in liver.

The HPHF+ diet increased SCD protein expression in subcutaneous, omental, and perirenal adipose tissues. The treatment effect was only statistically significant in subcutaneous adipose tissue but increases in all three depots were of a similar magnitude. The desaturase indices positively correlate with the SCD1 protein expression data. The *cis*-9 18:1 desaturase index was significantly higher in the HPHF+ diet in all adipose tissues and the *cis*-9 16:1 desaturase index was significantly higher in subcutaneous and omental adipose tissues from calves fed the HPHF+ diet compared to the calves fed the CON diet. This indicates that calves fed the HPHF+ diet had increased SCD1 protein expression, and the increase in MUFA concentrations in the subcutaneous, omental, and perirenal adipose tissues indicates positive correlation with activity.

The increased SCD1 protein expression in the adipose tissues of the HPHF+ diet may be due to adiposity and increased energy intake. Body weight and fat percent of EBW were significantly greater in calves fed the HPHF+ diet compared to all other diets. The fat percent of EBW was significantly greater in calves fed the HPHF+ diet

compared to the calves fed the CON and the HPLF diets. Calves fed the CON, HPLF, HPHF, and HPHF+ milk replacer diets had average daily energy intakes of 1.64, 3.74, 4.10, and 6.17 Mcal from milk replacer alone, respectively. Hu et al. (2004) reported that mice fed a high calorie, high-fat diet, exhibited increased body weight accumulation of fat mass, and increased liver SCD1 mRNA, and increased insulin concentrations compared to mice fed a low calorie, low-fat diet (Hu et al., 2004). The energy expenditure in these mice was also reduced (Hu et al., 2004). Obese Zucker rats also have increased SCD1 mRNA abundance compared to lean Zucker rats (Jones et al., 1996).

In contrast to this study, the desaturase indices from other studies did not correlate with activity (Archibeque et al., 2005). This could be due to the time of tissue collection. Over time, the activity of SCD could fluctuate, but the fatty acid composition represents the integration of activity over time. Since our study lasted 63 d, shorter than Archibeque et al. (2005), fatty acid composition more likely correlates with the SCD1 protein expression. Our data did correlate with a study in sheep fed a concentrate diet. With the concentrate diet, the sheep had higher concentrations of oleic acid in the livers and omental, perirenal, and subcutaneous adipose tissues compared to sheep fed dehydrated grass pellets. This increase in oleic acid correlated with an increase in SCD1 mRNA and also indicated increased activity (Daniel et al., 2004b).

The CLA desaturase index was decreased in perirenal, omental, and subcutaneous adipose tissues in the HPHF+ diet compared to the CON diet and *cis*-9, *trans*-11 CLA concentrations were increased in the adipose tissues of calves fed the CON diet. The higher concentrations of *cis*-9, *trans*-11 CLA and its desaturase index in

adipose tissue of CON fed calves is due ruminal production of CLA. The fatty acid composition data from perirenal, omental, and subcutaneous adipose tissues indicates that some bacterial fermentation was occurring in all calves. The increased fermentation occurring in the CON diet could be accounted for by calves consuming more starter. There was a reduction in *cis*-9 18:1 and *cis*-9, *cis*-12 18:2 in the control diet compared to the HPHF+ diet indicating biohydrogenation of these fatty acids. The presence of 15:0, 17:0, and *trans* fatty acids in the tissues of calves fed the CON diet also indicates fermentation and biohydrogenation, as these fatty acids are produced through the fermentation by bacteria (Jensen, 2002). Unlike eukaryotes bacteria produce odd numbered carbon fatty acids. Eukaryotes only use acetate or butyrate to initiate chain elongation of fatty acids, resulting in even carbon numbered fatty acids. Bacteria can also use odd carbon precursors for chain elongation producing odd chain fatty acids.

In conclusion, SCD1 protein expression was detected in subcutaneous, perirenal, and omental adipose tissues, but not in the liver and small intestine samples. Stearoyl-CoA desaturase-1 protein expression was increased in the calves fed the HPHF+ diet and the protein expression correlated with the desaturase indices. These data indicate that SCD1 protein expression occurs in adipose tissue of prepubertal calves and expression can be changed due to variations in diet suggesting that fatty acid composition could be modified to meet consumer demands by diet or management.

CHAPTER 4: REGULATION OF STEAROYL-COA DESATURASE BY INSULIN AND FATTY ACIDS

Introduction

Stearoyl-CoA desaturase (SCD), an enzyme located in the membrane of the endoplasmic reticulum, converts saturated fatty acid substrates to monounsaturated fatty acids (MUFA) by inserting a *cis* double bond between carbon 9 and 10. Substrates include palmitoyl-CoA (16:0) and stearoyl-CoA (18:0), which are converted to palmitoleoyl-CoA and oleoyl-CoA, respectively (Heinemann and Ozols, 2003, Man et al., 2006, Ntambi, 1999). Ruminant products have high concentrations of saturated fatty acids. Understanding the regulation of SCD1 may contribute to improving the fatty acid composition of ruminant products (Grummer, 1991).

Stearoyl-CoA desaturase 1 is regulated by diet and hormones. The enzyme is positively impacted by insulin with increases in mRNA and activity. Activity was 7-fold greater in diabetic rats injected with insulin (Prasad and Joshi, 1979). A similar effect was observed in primary hepatocytes cultured in media containing insulin, and furthermore activity was reduced by 80% when insulin was removed from the media (Jeffcoat et al., 1979, Spence and Pitot, 1982). Insulin alters SCD1 transcription. Insulin increased SCD1 mRNA 4-fold in diabetic mice within 4 hr of administration (Waters and Ntambi, 1994). Insulin also increased SCD mRNA in omental, perirenal, and subcutaneous adipose tissue explants from sheep and 3T3-L1 cells cultured in media containing insulin (Daniel et al., 2004a, Smith, 2006).

Unlike insulin, dietary polyunsaturated fatty acids (PUFA) reduce SCD activity and mRNA abundance. Activity was decreased in rats fed a diet supplemented with corn oil high in PUFA (Jeffcoat and James, 1978). Stearoyl-CoA desaturase activity was

also decreased in 3T3-L1 (mouse preadipoctye cell line) cells cultured in the presence of arachidonic acid (Sessler and Ntambi, 1998). Mice fed diets supplemented with trilinolenin and triarachidonin had decreased SCD1 mRNA compared to mice fed diets supplemented with oleic and stearic acids (Sessler and Ntambi, 1998, Waters and Ntambi, 1996).

This study investigated the regulation of SCD1 protein expression in bovine adipose tissue by selected hormones and fatty acids. Understanding the regulation of SCD1 protein expression will contribute to strategies to increase the MUFA concentration in tissues and milk.

Materials and Methods

Animals and Explants

Subcutaneous adipose tissue was harvested from the flank region of cattle harvested at a packing plant (Smith Valley Meats, Rich Creek, VA). The tissue was transported in media (DMEM/Ham's Nutrient Broth F-12, 10% fetal bovine serum (FBS), and antibiotics) back to the laboratory. Unless indicated differently explants were incubated in media supplemented with 10 ml of antibiotic cocktail/ L of media (10,000 units/ml penicillin, 10mg/ml streptomycin, and 25 µg/ml amphotericin B; Sigma, St. Louis, MO). DMEM/Ham's Nutrient Broth F-12 media, M199 media, Hank's Balanced Salts (HBSS), sodium acetate, and insulin were obtained from Sigma (St. Louis, MO). Fetal bovine serum was obtained from Atlanta Biologicals (Lawrenceville, GA). Bovine serum albumin (BSA) was obtained from Fisher Scientific. Fatty Acids were obtained from Nu-Chek Prep (Elysian, MN). The tissue and media were maintained at 37℃ during transport. The tissue was washed in 20% betadine solution (v/v in HBSS) for 10 min in a biological safety cabinet and then washed in HBSS containing antibiotics. A Stadie-Riggs microtome was used to prepare tissue slices (100 – 200 mg) which were cultured overnight in M199 supplemented with 2 mM sodium acetate, BSA (1 g/L), and 10 ml/L antibiotic cocktail. A total of 1 g of tissue was placed in each petri dish. After overnight culture, tissue slices were randomly allocated to treatments.

Treatments

The effect of treatment on SCD protein expression was analyzed by culturing explants in media containing treatments. Treatments consisted of increasing concentrations of insulin, fatty acids increasing in unsaturation, and increasing

concentrations of linoleic acid (*cis*-9, *cis*-12 18:2). Insulin was included at concentrations of 0, 7, 14, and 21 nM. Fatty acids analyzed included: stearic (18:0), oleic (*cis*-9 18:1), linoleic (*cis*-9, *cis*-12 18:2), and alpha-linolenic (*cis*-9, *cis*-12, *cis*-15 18:3) and increasing concentrations of linoleic acid were 0, 25, 50, 75, and 100 μM. Sodium salts of fatty acids were prepared and complexed to BSA. Fatty acids increasing in unsaturation were applied at 100 μM in M199 supplemented media with 21 nM insulin. The linoleic fatty acid treatments were applied in M199 supplemented media with insulin (21 nM). There were 3 replications for each treatment with a total of 500 mg of tissue added to each petri dish. The tissue was maintained in treatment media for 24 hrs at 37℃ and 5% CO_2 in air.

Immunoblotting

Tissue samples (~500 mg) were homogenized using a Pro200 Hand-Held or Post-Mounted laboratory homogenizer at setting ~3 for ~30 sec in 1 ml of lysis buffer (500 mM Tris pH 7.4, 0.5% Triton X-100, 300 mM NaCl, 2 mM EDTA pH 8, mammalian protease inhibitor cocktail; Sigma, St. Louis, MO). The homogenate was centrifuged at 14,000xg at 4℃ for 10 minutes to remove cell debri s. The supernatant was centrifuged again at 14,000xg at 4℃ for 10 minutes and the sup ernatant stored at -20℃. Protein concentration was determined using the Bradford assay using BSA as a standard (Bio-Rad, Hercules, CA).

Supernatants were mixed with an equal volume of 2X Laemmli sample buffer (Sigma, St. Louis, MO) and heated at 95℃ for 5-10 minutes. Samples were electrophoresed on a 12% SDS-PAGE gel and transferred using a Bio-Rad trans-blot SD semi-dry transfer cell (Bio-Rad, Hercules, CA) to a PVDF membrane. Membranes

were blocked to reduce background staining by incubating in Tris-buffered saline containing Tween-20 (TBST; 0.05 M Tris pH 7.4, 0.2 M NaCl, 0.1% Tween) containing 5% dried nonfat milk) for one hour at room temperature on a shaking platform. This was followed by incubation in a solution (TBST containing 5% dried nonfat milk) with antibodies specific to bovine SCD1 (1:1000) in ~20 ml for one hour. Antiserum against the C terminus of bovine SCD1 was prepared by immunizing a rabbit with a 15 amino acid peptide corresponding to amino acids 345-359 of bovine SCD1 (LARIKRTGEESYKSG) conjugated to keyhole limpet hemocyanin (KLH) (Pacific Immunology, Ramona, CA). Membranes were washed with ~ 20 ml of TBST then incubated in ~20-30 ml of TBST containing 5% dried nonfat milk with goat anti-rabbit conjugated HRP secondary antibody (1:1000). Membranes were then washed with ~20 ml of TBST and developed using ECL-Plus Reagents (Amersham Biosciences, Pittsburgh, PA) according to the manufacturer's instructions. Chemiluminescence was visualized using a Chemidoc XRS digital imaging system, and densitometry (net intensity) was measured using Quantity One software (Bio-Rad).

Statistical Analysis

Data are reported as least square means ± SEM. The densitometry data were analyzed using the Mixed procedure of SAS (SAS for Windows Version 9.1, SAS Institute, Cary, NC). The model included treatment, animal, and the animal by treatment interaction. Treatments includes insulin at concentrations of 0,7, 14, and 21 nM, fatty acids increasing in unsaturation, and linoleic acid at concentrations of 0, 25, 50, 75, and 100 μM. Animals included bulls, steers, and cows which subcutaneous adipose tissue

samples were collected. Linear contrasts were conducted on the densitometry data from the insulin and fatty acid studies.

Results

The first objective of this study was to determine if expression of SCD protein was influenced by insulin. This was determined by measuring SCD1 protein expression in explants cultured in media containing insulin at concentrations of 0, 7, 14, and 21 nM. Insulin increased SCD1 protein expression with a significant linear effect; SCD protein expression increased as insulin concentration increased (Figure 4.1; $p < 0.05$).

The second objective of this study was to examine the regulation of SCD protein expression by PUFA. Regulation was determined by measuring SCD protein expression in explants cultured in media containing individual fatty acids increasing in unsaturation (18:0, 18:1, 18:2, and 18:3) at 100 μM. There was no significant treatment effect with fatty acids increasing in unsaturation on SCD protein expression, but as the degree of fatty acid unsaturation increased, SCD protein expression decreased with a significant linear fashion (Figure 4.2; $p < 0.05$).

The final objective of this study was to determine the concentration of 18:2 that would affect SCD protein expression. The effect of increasing concentrations of 18:2 on SCD protein concentration was determined by culturing explants in media containing 18:2 at 0, 25, 50, 75, or 100 μM. No significant treatment effect of 18:2 concentration on SCD protein expression was found (Figure 4.3).

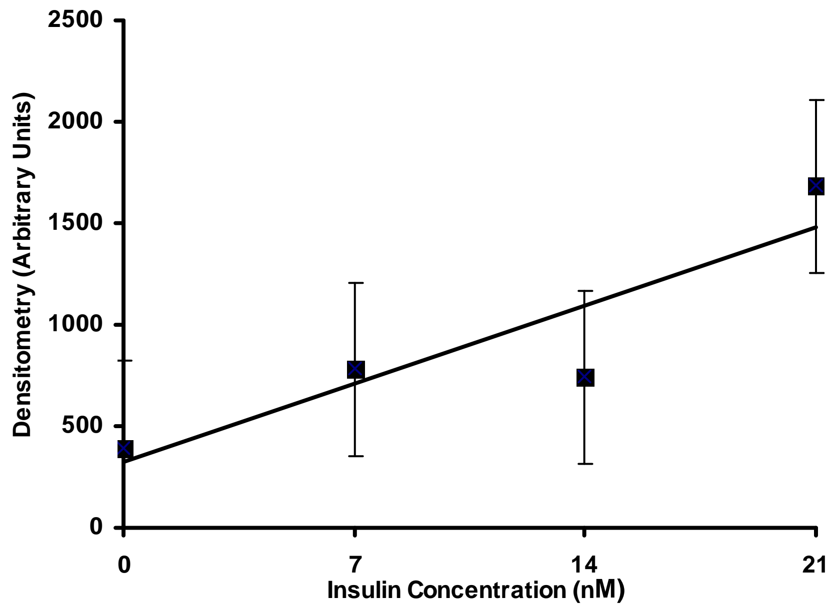

Figure 4.1 Insulin increases stearoyl-CoA desaturase expression. Subcutaneous adipose tissue explants collected from 4 cows, 2 steers, and 1 bull were maintained for 24 h in M199 media supplemented with insulin at increasing concentrations. Treatment $p < 0.05$. There was a linear effect with a $p < 0.05$.

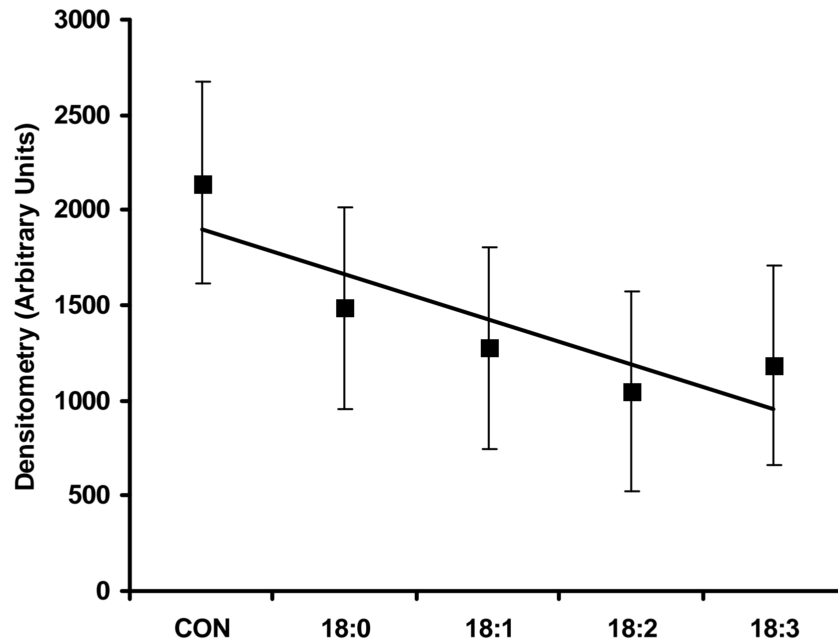

Figure 4.2 Effects of fatty acids increasing in double bonds on stearoyl-CoA desaturase expression. Subcutaneous adipose tissues explants from 4 steers, 2 cows, and 1 bull were maintained for 24 h in M199 media supplemented with fatty acids increasing in double bonds or with BSA and water (CON). Treatment effect $p > 0.05$. There was a linear effect $p < 0.05$.

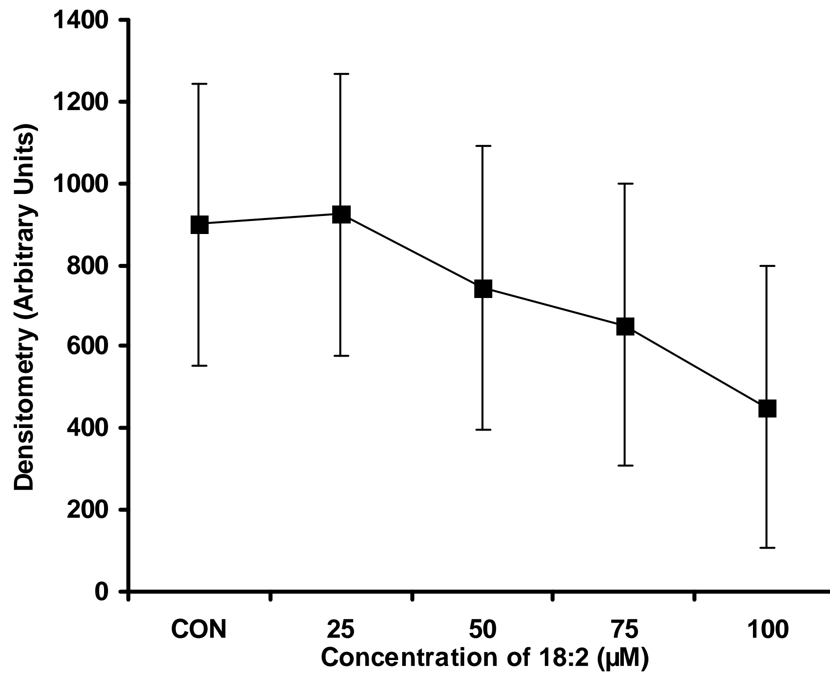

Figure 4.3 Effects of 18:2 fatty acid at different concentration on stearoyl-CoA desaturase expression. Subcutaneous adipose tissue explants collected from 3 cows, 2 steers, and 1 bull were maintained for 24 h in M199 media supplemented with 18:2 fatty acid at increasing concentration or with BSA and water (CON). Treatment $p > 0.05$.

Discussion

Stearoyl-CoA desaturase-1 protein expression was increased linearly in bovine adipose tissue explants cultured with increasing concentrations of insulin. This observation supports studies with rodents, sheep, and cattle (Daniel et al., 2004a, Keating et al., 2006, Prasad and Joshi, 1979, Ward et al., 1998, Waters and Ntambi, 1994) that demonstrated an increase in SCD1 activity and mRNA expression with insulin treatment. Diabetic rats injected with insulin had increased liver SCD activity compared to non-diabetic rats (Prasad and Joshi, 1979) and insulin injection also increased SCD1 mRNA in livers of diabetic mice (Waters and Ntambi, 1994). Subcutaneous, perirenal, and omental adipose tissue explants from sheep cultured in media containing insulin had an increase in SCD1 mRNA compared to controls incubated in media containing no insulin (Daniel et al., 2004a). Furthermore, the activation of the SCD1 promoter in bovine mammary epithelial cells was increased when insulin was added to culture media (Keating et al., 2006).

Insulin regulation of SCD1 is mediated through the transcription factor sterol regulatory element binding protein (SREBP)-1c (Foufelle and Ferre, 2002). This transcription factor is bound to the ER membrane as an inactive precursor. Cleavage of its N-terminus by SREBP cleavage-activating protein (SCAP) leads to translocation of the active form to the nucleus where it binds to the sterol response element (SRE) of SCD1, and thus transcription (Heinemann and Ozols, 2003). Insulin positively regulates transcription of SREBP-1c through the PI 3-kinase pathway. The promoter of SCD1 contains a sterol response element (SRE) where SREBP-1c binds to activate transcription (Foufelle and Ferre, 2002).

Stearoyl-CoA desaturase 1 protein expression was decreased in bovine adipose tissue explants cultured with PUFA (Figure 4.2). The respone of SCD1 to fatty acids increasing in unsaturation was linear. These results are similar to studies with rodents fed PUFA and cells cultured in media containing PUFA (Jones et al., 1996, Landschulz et al., 1994, Ntambi, 1992, Waters and Ntambi, 1996). Obese Zucker rats fed a PUFA-enriched diet had reductions in SCD1 mRNA in liver and adipose tissues compared to rats fed a control diet with 12% corn oil (Jones et al., 1996). Mice fed a diet supplemented with PUFA also had reductions in hepatic SCD1 mRNA (Ntambi, 1992) and SCD1 mRNA was reduced in 3T3-L1 cells cultured in media containing linoleic acid (18:2) (Jones et al., 1996). Similarly, primary hepatocytes cultured in media containing unsaturated fatty acid had a decrease in SCD1 mRNA, and the reduction increased with the degree of unsaturation (Landschulz et al., 1994). In contrast to this study and previous studies, linoleic and linolenic acids did not affect SCD1 transcription in bovine mammary epithelial cells (Keating et al., 2006). Also, a 15% whole cottonseed diet did not decrease SCD activity in steers; however, increases in adipose tissue PUFA content were not observed with these diets (Archibeque et al., 2005).

Polyunsaturated fatty acids negatively regulate SCD1 through SREBP-1c. One potential mechanism for the reduction is reduced transcription of SREBP-1c. It has been shown that culturing HEK-293 cells in the presence of PUFA reduces SREBP-1c transcription (Yoshikawa et al., 2002), as well as quantity of mature form of SREBP-1c in the nucleus (Nakamura and Nara, 2002). Another proposed mechanism is a PUFA responsive element (PUFA-RE) in the promoter of SCD1 that a transcription factor binds to prevent transcription of SCD1 (Waters et al., 1997). This 60 bp region of the

bovine SCD1 promoter has been shown to bind nuclear proteins (Keating et al., 2006). The murine PUFA-RE contains binding sites for both SREBP and nuclear factor-Y (NF-Y) (Shimomura et al., 1998, Tabor et al., 1999). A third possible mechanism is PUFA binding to a PUFA-binding protein (PUFA-BP) which inhibits transcription by binding to the PUFA-RE region on the promoter of SCD1 (Ntambi, 1999, Sessler and Ntambi, 1998). A mechanism unrelated to the PUFA-RE involves possible PUFA induced reductions in SCD1 mRNA stability, thereby decreasing the half life of SCD mRNA (Ntambi, 1999, Sessler et al., 1996).

Stearoyl-CoA desaturase-1 protein expression in bovine adipose tissue explants did not decrease with increasing concentration of linoleic acid (18:2) (Figure 4.3). This result is not similar to rodent cells cultured in media containing PUFA, specifically linoleic acid (Jones et al., 1996, Landschulz et al., 1994). 3T3-L1 cells treated with linoleic acid at increasing concentrations had a 60% reduction in SCD1 mRNA (Jones et al., 1996), and rat primary hepatocytes cultured in linoleic acid also had a reduction in SCD1 mRNA (Landschulz et al., 1994). One possible explanation for the discrepancy is the different concentrations of the fatty acid used. Both of these studies only showed significant effects after culturing cells with 100 and 300 μM of linoleic acid, while the highest concentration used in the present study was 100 μM. There is also variability between the animals within treatment. The variability could be due to age or sex of the animals. Studies have determined that age can effect SCD expression (Martin et al., 1999).

In conclusion, using subcutaneous adipose tissue explants insulin increased SCD1 protein expression, whereas PUFA decreased SCD1 protein expression. Overall, linoleic acid had no significant effect on SCD1 protein expression.

CHAPTER 5: CONCLUSIONS AND IMPLICATIONS

Ruminant products contain a large amount of saturated fatty acids due to biohydrogenation, but ruminant products do contain a small amount of unsaturated fatty acids and CLA. Stearoyl-CoA desaturase converts saturated fatty acids to monounsaturated fatty acids and converts vaccenic acid (*trans*-11 18:1) to *cis*-9, *trans*-11 CLA. Strategies to reduce the saturated fat content of ruminant products and increase the monounsaturated fatty acid and *cis*-9, *trans*-11 CLA content may include increasing stearoyl-CoA desaturase activity.

In the first experiment, SCD1 protein expression was detected in subcutaneous, omental, and perirenal adipose tissues, but it was not detected in the liver or small intestinal samples. Identifying tissue distribution of SCD1 protein expression is important because regulation studies can focus on the specific tissues that express SCD1 protein. Strategies to express stearoyl-CoA desaturase in tissues such as intestine and liver could augment activity present in terminal tissues such as adipose. Activity could be induced in these tissues by diet as demonstrated in mice fed a fat-free, high carbohydrate diet (Ntambi et al., 1988). However, our study did not indicate that diet could induce SCD1 protein expression as has been shown previously in Chang et al. (1992).

Subcutaneous, perirenal, and omental adipose tissue samples from the calves fed HPHF+ had increased SCD1 protein expression. Stearoyl-CoA desaturase-1 protein expression was significantly higher in subcutaneous adipose tissue from calves fed the HPHF+ diet compared to the control. Increasing intake and fat increases SCD1 protein expression. Several factors could have contributed to the up regulation of SCD1 protein

expression and these include variation in fat intake, energy intake, and body weight gain between treatments. This study demonstrates that feeding strategies could increase SCD1 protein expression possibly through energy intake and fat content.

Insulin treatment had a positive linear effect on SCD1 protein expression. Unlike insulin, fatty acids increasing in unsaturation decreased SCD1 protein expression. Understanding the regulation of bovine SCD1 can contribute information that could be used to manipulate fatty acid composition of ruminant products. Feeding could be adjusted to increase insulin concentration that would then increase SCD1 protein expression. An example could be to feed a high-carbohydrate diet to increase insulin concentrations. In addition to increasing the MUFA and CLA content of ruminant fats, there is also interest in increasing the PUFA content. These studies indicate that PUFA can decrease SCD1 protein expression. Feeding a diet high in PUFA may increase tissue PUFA content, but could also decrease SCD1 protein expression causing a reduction in the MUFA content of the products.

Future research should involve identification of other transcription factors that could be involved in the PUFA regulation of SCD1 transcription. Also, the stability of bovine SCD1 mRNA in response to PUFA should be determined to examine if this is a possible mechanism of regulation. Possible diet induction of SCD1 expression in tissues other than adipose could also be examined.

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