METABOLIC AND GENETIC REGULATION IN ADIPOSE TISSUE OF ANGUS AND WAGYU STEERS RAISED TO U.S. AND JAPANESE ENDPOINTS

A Dissertation

by

KI YONG CHUNG

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2006

Major Subject: Nutrition

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Approved by:

Chair of Committee,	Stephen B. Smith
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ABSTRACT

Metabolic and Genetic Regulation in Adipose Tissue of Angus and Wagyu Steers Raised to U.S. and Japanese Endpoints. (May 2006)

Ki Yong Chung, B.S., Yeungnam University;

M.S., Texas A&M University

Chair of Advisory Committee: Dr. Stephen B. Smith

We hypothesized that carcass and fatty acid composition of Angus and Japanese Black (Wagyu) steers would not differ if the steers were fed to a typical U.S. final weight, but that Wagyu steers fed to a typical Japanese endpoint body weight would have greater quality grades and softer fat than Angus steers. Sixteen Angus and 16 Wagyu 8-month-old, weaned steers were assigned to a corn-based diet for 8 or 16 months (n = 4 per breed type and time) or hay-based diet for 12 or 20 months (n = 4 per breed type and time) in a 2 x 2 x 2 factorial arrangement. USDA yield grade was greater at the Japanese endpoint than at the U.S. endpoint in Angus steers (breed x endpoint, P = 0.03). Intramuscular (i.m.) lipid continued to increase to over 20% in the Wagyu steers (P = 0.05), but attained a plateau (14.7%) by 16 months on feed in the Angus steers. These results confirm that Wagyu cattle must be raised to greater physiological maturity before they differ from Angus cattle in *M. longissimus thoracis* i.m. lipid concentration. Subcutaneous adipose tissue concentrations of oleic (18:1n-9) was greater in Wagyu steers than in Angus steers (P = 0.05). All monounsaturated fatty acids (MUFA) increased between the U.S. and Japanese endpoint, whereas slip points of lipids in s.c.

adipose tissue were 10°C lower in Japanese endpoint steers than in U.S. endpoint steers ($P = 0.01$). Angus adipose tissue exhibited peak SCD enzyme activity at 16 months (corn-based diet) but activity in Wagyu adipose tissue was greatest at 20 months (hay-based diet) (breed x diet x endpoint, $P = 0.08$). However, SCD gene expression in Angus adipose tissue was maximal at 12 months (hay diet), whereas Wagyu adipose tissue had peak expression at 16 months (corn diet) ($P < 0.03$).

Trans-10, cis-12 CLA has been reported as a potent inhibitor of adipocyte differentiation. CLA (40 μM) strongly decreased SCD and PPARγ expression in bovine adipocytes, even in the presence of 5 mM arginine. It can be concluded that arginine up-regulates bovine preadipocyte differentiation, and CLA antagonizes this effect.

DEDICATION

This Dissertation is dedicated to my wife, Ji Young, and daughter, Eunice, who changed my life.

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I would like to express my sincere appreciation to Dr. Stephen B. Smith for his patience, guidance, and encouragement during my Ph.D program. He provided a great example of not only a sincere advisor in the school but also a family guidance role model. I greatly appreciate the graduate committee members who gave me their patience, knowledge, and support. Dr. David Lunt encouraged me to understand critically practical science and supported me in interpreting practical research data. Dr. Jason Sawyer challenged me to think critically about practical research. I would like to thank Dr. Lee Cartwright for his guidance and expertise as a member of my committee. I also would like to thank Dr. Chang B. Choi who laid the groundwork for my development as a researcher. I also want to thank Dr. Hiroyuki Kawachi for his assistance and technical guidance.

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Most importantly, I would like to thank my wife, Ji Young and my daughter, Eunice for their sacrifice, patience, and love. Finally, I want to thanks God for giving me the strength and health to finish.

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CHAPTER I

INTRODUCTION

Beef is one of the major food sources in modern countries and this could have a major impact on the health of consumers in these countries. Bovine adiposity is not only a component of carcass composition but also a determinant of carcass quality and economic value. The MUFA:saturated fatty acid (SFA) ratio of bovine adipose tissues is an indicator of fat softness and an important aspect of meat quality in some countries. Adiposity and fatty acid composition differ by breed type and diet (Zembayashi et al., 1995). Japanese Black (Wagyu) cattle, known to be genetically superior in intramuscular fat (i.m., marbling) accumulation, need a long-term finishing period (Lunt et al., 1993). Long feeding periods cause significant alterations in fatty acid composition in several regional adipose tissues (May et al., 1994; 1995). It has been determined that various systems differentially regulate regional adipose tissue accretion. Enzyme systems like phosphatidic phosphohydrolase-mediated diacylglycerol and triacylglycerol biosynthesis are dissimilar between i.m. and subcutaneous (s.c.) adipose tissues (Smith et al., 1998). Furthermore, the activities of fatty acid synthetase and glucose-6-phosphate dehydrogenase are different in s.c. and i.m. adipose tissue (May et al., 1994). Dietary linoleic acid and α-linolenic acid (from corn and hay) regulates fatty acid metabolism.

Martin et al. (1999) found that increases in SCD mRNA concentration preceded an increase in lipogenesis and lipid accumulation in bovine s.c. adipose tissue.

This dissertation follows the style and format of the Journal of Animal Science.

Oleic acid, the primary product of SCD activity, also stimulated differentiation of porcine preadipocyte in culture (Ding and Mersmann, 2001). Furthermore, alteration of SCD activity may regulate de novo fatty acid biosynthesis in adipose tissue and thus may regulate fattening of beef cattle.

The primary objective of this study is to describe the effects of diets and endpoint of production on adiposity, metabolism, and fatty acid composition of adipose tissues of Angus and Wagyu steers. This study demonstrated that SCD gene expression and enzyme activity is regulated by dietary and time on feed differences, which may in turn regulate lipid accumulation in adipose tissue.

CHAPTER II

REVIEW OF LITERATURE

Adipocyte Proliferation

Fat tissue within animals consists of approximately one-third adipocytes. The remaining two-thirds are a combination of small blood vessels, nerve tissue, fibroblasts, and preadipocytes in various stages of development (Geloen et al., 1989). The distinction between preadipocyte and fibroblasts is difficult to make, and the inability to align preadipocytes at similar developmental stages confounds detailed in vivo studies. To some extent, preadipocyte primary culture has been used. The use of primary culture suffers several faults. First, it is difficult to isolate preadipocytes from other fibroblast-like cells, because factors such as temperature, features of lipids, and environment reduce yields. Second, large amounts of adipose tissue are required because preadipocytes constitute only a small percentage of total adipose tissue. In addition, primary cultures have a limited life span. However, primary culture has been used as a primary means study for characteristics and functions of animal adipocytes in vitro.

Preadipocyte differentiation therefore has been studied primarily by using in vitro models of adipogenesis; much of the knowledge of adipocyte differentiation has been based on the validity of these tissue culture models. There are advantages and disadvantages to using a cell line to study preadipocyte differentiation. A cell line derived from cloning is a homogenous population of cells that are all at the same stage of differentiation. This allows for a definitive response to treatments. In addition, these

cells can be passaged indefinitely, which provides a consistent source of preadipocytes for study.

Adipocyte precursor cell lines can be segregated into two classes, i.e., pluripotent fibloblasts and unipotent preadipocytes. The pluripotent fibroblasts have the ability to be converted into several cell types. Unipotent preadipocytes have undergone determination and can either remain as preadipocytes or undergo conversion to adipose tissue (Ntambi and Kim, 2000). They are ideal for studying the molecular events responsible for the conversion of preadipocytes into adipocytes. The 3T3-L1 and 3T3-F422A culture lines, derived from disaggregated Swiss 3T3 mouse embryos, are the most widely used culture models (Green and Kehinde, 1974).

There is some variation in the differentiation requirements of each cell line. It is believed that these differences represent variations in the developmental stage at which cells were arrested when derived (Cornelius et al., 1994; Smas and Sul, 1995). The identification of specific developmental markers will allow for the alignment of the developmental programs of the various cell lines.

Adipocyte Differentiation

The 3T3-L1 cell line is one of the most well-characterized and reliable models for studying the conversion of preadipocytes into adipocytes. When injected into mice, 3T3-L1 preadipocytes differentiate and form fat pads that are indistinguishable from normal adipose tissue. In culture, differentiated 3T3-L1 preadipocytes possess most of the structural characteristics of adipocytes from animal tissue. The formation and

appearance of developing fat droplets also mimic live adipose tissue (Green and Kehinde, 1974).

Confluent 3T3-L1 preadipocytes can be differentiated synchronously by a defined adipogenic component. Maximal differentiation is achieved upon treatment with the combination of insulin, a glucocorticoid, an agent that elevates intracellular cAMP levels, and fetal bovine serum (Cornelius et al., 1994). Insulin is known to act through the insulin-like growth factor 1(IGF-1) receptor. IGF-1 can be substituted for insulin in the adipogenic cocktail. Dexamethasone, a synthetic glucocorticoid agonist, is traditionally used to stimulate the glucocorticoid receptor pathway. Methylisobutylxanthine, a cAMP-phosphodiesterase inhibitor, is traditionally used to stimulate the cAMP-dependent protein kinase pathway. These adipogenic components are commonly abbreviated MDI (Methylisobutylxanthine, Dexamethasone, Insulin).

Approximately 24 h after induction by MDI, differentiating preadipocytes undergo a postconfluent mitosis and subsequent growth arrest (Bernlohr et al., 1985). The cells undergo at least one round of DNA replication and cell division. By d 2 of differentiation, the cells complete the postconfluent mitosis and enter into an unusual growth arrest called G_D (Scott et al., 1982). This terminal mitosis is believed necessary to unwind DNA, allowing transcription factors access to regulatory response elements present in genes involved in modulating the mature adipocyte phenotype (Cornelius et al., 1994). After the growth arrest, cells are committed to becoming adipocytes. The growth arrest is required for subsequent differentiation. Growth-arrested cells begin to express late markers of differentiation at d 3. These late markers consist of lipogenic and

lipolytic enzymes, as well as other proteins responsible for modulating the mature adipocyte phenotype. The cells then round up, accumulate fat droplets and become terminally differentiated adipocytes by d 5–7.

Function of Adipocytes in Body Metabolism

Secretion of glycerol and fatty acids from the adipocyte plays an important role in hepatic and peripheral glucose metabolism. Moreover, adipose tissue as well as heart and skeletal muscle are the only known tissues to express and regulate the insulin-dependant glucose transporter, GLUT4, which facilitates the entry of glucose into these cells and out of circulation postprandially. Emerging data suggest that the adipocyte also plays an important role in numerous processes through its secretary products and endocrine functions. In this regard, leptin widely regulates biological activities, independent of satiety, including effects on fertility, reproduction, and hematopoiesis, in addition to cytokines and compliment factors whose various functions are linked inseparably to the adipocyte as a source for their production (Gregoire et al., 1998).

Although the adipocyte is important to energy homeostasis, adipose tissue may also play a central role in many of the pathologies associated with obesity and its related disorders. Genetic mutations that alter the release of leptin from the mature adipocyte or suppress its interaction with receptors in the hypothalamus are well-known mechanisms of obesity in mice. Cytokines and lipids released from adipose tissue may lead to a decrease in glucose utilization in skeletal muscle and enhance glucose production by the liver, both of which contribute to high levels of glucose in the peripheral circulation, a reason for noninsulin-dependent diabetes mellitus (Morris and Farmer, 2000).

Characteristics of Adipose Tissues from Wagyu and Angus Steers

Wagyu steers deposit more marbling, and their adipose tissues deposit more MUFA, than is observed in Angus steers when cattle are fed for long periods (Sturdivant et al., 1992; Lunt et al., 1993; May et al., 1993). This occurs even when the cattle are fed the same diet for the same amount of time (550 d) and are slaughtered at the same physiological maturity. This indicates a genetic predisposition for Wagyu cattle to deposit marbling and MUFA.

In spite of greater amounts of marbling within the longissimus muscle in Wagyu steers, Wagyu marbling adipocytes are smaller and exhibit twice the rate of DNA synthesis as marbling adipocytes from Angus steers (May et al., 1994). The same is true for s.c. adipose tissue. This suggests greater rates of preadipocyte proliferation in Wagyu than in Angus adipose tissue depots. This certainly seems to be the case for i.m. adipocytes; Angus i.m. adipose tissue has significantly greater activities of fatty acid synthetase and glucose-6-phosphate dehydrogenase than Wagyu marbling adipose tissue (May et al., 1994), and therefore may be more differentiated than Wagyu i.m. adipose tissue. To date, no one has demonstrated the effects of disparate diets on preadipocytes from Wagyu and Angus breed types.

Lipogenesis

Miller et al. (1991) used explant cultures to compare the difference in incorporation rates of acetate into lipids in s.c. and i.m. adipose tissue from Santa Gertrudis steers. Over a 2-d period, s.c. adipose tissue showed a decrease in the incorporation of acetate into lipids, whereas i.m. adipose tissue activity increased. The

increase in activity was the consequence of an increased conversion of acetate into lipids in existing adipocytes and the proliferation and subsequent filling of new adipocytes. If the latter of the theories is correct, then the stromal vascular cells within i.m. adipose tissue may be quite proliferative.

Research also has determined that the primary substrate for de novo fatty acid synthesis in the bovine species is acetate. Smith and Crouse (1984) assessed the contribution of acetate, lactate, and glucose to lipogenesis in Angus steers. One group was fed a corn silage diet and the other group was fed a ground corn diet. Animals in the high-energy diet were found to have high ATP-citrate lyase and NADP-malate dehydrogenase activities. Their data also indicated that different regulatory mechanisms were present in s.c. and i.m. adipose tissues. For example, acetate contributed 70-80% of the acetyl units for in vitro lipogenesis in s.c. adipose tissue, but only 10-25% in i.m. adipose tissue. Likewise, glucose contributed 1-10% of the acetyl units in s.c. adipose tissue and 50-75% in i.m. adipose tissue. Lactate contributed 15-30% in both s.c. and i.m. adipose tissues.

Diet also affects lipogenesis. Prior and Scott (1980) found that a high-energy intake resulted in higher lipogenic enzyme activity in bovine s.c. adipose tissue. After feeding corn or alfalfa diets, they found that incorporation rates of ^{14}C-acetate and lactate were similar despite the fact that the enzyme activities for acetyl CoA carboxylase, fatty acid synthetase, ATP-citrate lyase, NADP-malate deyhydrogenase, and hexokinase were greater in the corn-fed steers. The corn-fed steers had a faster rate of gain and slightly higher metabolically energy intakes.

Stearoyl-CoA Desaturase

This laboratory previous demonstrated stearoyl-CoA desaturase (SCD) activity in bovine adipose tissue, liver, intestinal mucosa, and longissimus muscle (St. John et al., 1991; Chang et al., 1992). A clearly regulated step in the biosynthesis of MUFA is the introduction of the double bond into acyl-CoA at the carbon 9 position. SCD is a microsomal enzyme that catalyzes the NADH- and O_2-dependent desaturation of SFA. Stearoyl-CoA desaturase is an endoplasmic reticulum-anchored enzyme that converts palmitoyl-CoA and stearoyl-CoA to palmitoleoyl-CoA and oleoyl-CoA, respectively (Miyazaki and Ntambi, 2003). MUFA are used as major precursors for the synthesis of various lipid forms, including triaylglycerol (TAG), phospholipids, cholesterol ester (CE), and wax esters. Oleic acid, a major MUFA in animal adipose tissue synthesized by SCD, is the active precursor for acyl-CoA cholesterol acyltransferase (ACAT) in CE biosynthesis and diacylglycerol acyltransferase (DGAT) in TAG synthesis. Oleic acid also has been reported to regulate cell development and differentiation through control of membrane fluidity and signal transduction (Ntambi, 1999; Miyazaki et al., 2001). Therefore, SCD activity affects not only the fatty acid composition in plasma membranes but also lipid metabolism in adipose tissue. Three isoforms of the SCD gene have been reported in rodents. The SCD1 and SCD2 isoforms are expressed in most organs of the mouse, except brain and skin (Ntambi. 1988). The SCD3 isoform is expressed in skin (Zheng et al., 2001). The regulation of bovine SCD activity has not yet been established. Cattle have only one isoform of SCD (Campbell et al., 2001). Breed and diet differences might affect activity and gene expression of SCD in beef cattle.

Oleic acid stimulated lipid filling in porcine preadipocytes in vitro (Ding and Mersmann, 2001). Also, expression of the SCD gene preceded lipid filling in cattle (Martin et al., 1999) and pigs (Smith et al., 1999). Therefore, depression of SCD activity may cause not only a reduction of MUFA in the regional fat depots but also reduction of i.m. lipids in the longissimus marbling.

Many hormonal, dietary, and environmental factors controlling SCD expression have been studied in in vivo and in vitro models. High-carbohydrate diets stimulate SCD expression through the sterol regulatory element-binding protein (SREBP-1) (Kersten, 2001). Insulin, glucose, fructose, cholesterol, and retinoic acid increase SCD gene expression in several tissues (Waters and Ntambi, 1994; Jones et al., 1998; Repa et al., 2000). However, n-6 and n-3 polyunsaturated fatty acids (PUFA), *trans*-10, *cis*-12 CLA, tumor necrosis factor-alpha (TNF-α), and cAMP decrease SCD gene expression and activity in rodents (Weiner et al., 1991; Ntambi. 1992). Whole cottonseed contains the cyclopropene fatty acid, sterculic acid, which is a potent inhibitor of SCD (Raju and Reiser, 1972). Consumption of whole cottonseed has been shown to increase stearic acid in adipose tissue of Australian carcasses (Smith et al., 1998), by depressing SCD activity (Yang et al., 1999).

Metabolic syndrome is accompanied by impaired glucose regulation/insulin resistance and abdominal obesity (Moller and Kaufman. 2005). Vidal et al. (2001) reported that lipoprotein lipase, glycogen synthase, leptin, PPARγ, and GLUT4 gene expression, and insulin sensitivity are greater in human s.c adipose tissue than in omental adipose tissue. However, tumor necrosis factor alpha (TNFα), which induces

dedifferentiation and apoptosis of human adipocytes and decreases the expression of PPARγ, is expressed at greater levels in omental adipose tissue than in s.c. adipose tissue. Cattle exhibit several aspects of metabolic syndrome (McCann and Reimers, 1986; Matsuzaki et al., 1997), and have distinct metabolic differences across adipose tissue depots (May et al., 1994; Landis et al., 2002). We predicted that bovine preadipocytes may serve as a useful model for the study of metabolic syndrome.

Dietary arginine reduces serum glucose and increases insulin sensitivity in rats (Wu et al., 1999). Nitric oxide (NO) is synthesized by a family of NO synthases (NOS) that use arginine as a substrate in almost all mammalian cells (Kapur et al., 2000). Fu et al. (2005) reported that dietary arginine supplementation not only reduced adiposity in zuker diabetic fat rats but also increased the expression of several genes that would depress adiposity in rat epididymal adipose tissue.

CLA, naturally synthesized by the rumen microbes, reduces adiposity of rats, mice, and chickens, and increases lean body mass (Park et al., 1997). CLA depresses preadipocyte proliferation and [^{3}H]thymidine incorporation into DNA in 3T3-L1 preadipocytes (Satory and Smith, 1999) and porcine adipose tissue explants (Adams et al., 2005). Moreover, t10, c12 CLA prevents lipid filling of preadipocytes by decreasing PPARγ gene expression (Granlund et al., 2003; Kang et al., 2003). The c9,t11 and t10,c12 CLA isomers are structurally similar to oleic acid, and both depress NO synthesis and iNOS gene expression in endothelial cells (Eder et al., 2003). One objective was to test the interaction between media arginine and CLA isomers is bovine preadipocytes.

CHAPTER III

PRODUCTION CHARACTERISTICS AND CARCASS QUALITY OF ANGUS AND WAGYU STEERS FED TO U.S. AND JAPANESE ENDPOINTS

Overview

We hypothesized that carcass and fatty acid composition of Angus and Japanese Black (Wagyu) steers would not differ if the steers were fed to a typical U.S. final weight, but that Wagyu steers fed to a typical Japanese endpoint body weight would have greater quality grades and softer fat than Angus steers. Sixteen Angus and 16 Wagyu 8-month-old, weaned steers were assigned to a corn-based diet for 8 or 16 months (n = 4 per breed type and time) or hay-based diet for 12 or 20 months (n = 4 per breed type and time) in a 2 x 2 x 2 factorial arrangement. Average daily gain was greater in Angus steers than in Wagyu steers, due primarily to the initially high ADG in corn-fed Angus steers. Marbling scores and USDA quality grades were not different between breed types ($P = 0.31$), but were higher in corn-fed steers than in hay-fed steers. USDA yield grade was greater at the Japanese endpoint than at the U.S. endpoint, but only in Angus steers (breed x endpoint, $P = 0.03$). There also was a significant ($P = 0.05$) breed x endpoint interaction for 12th rib *M. longissimus thoracis* i.m. lipid concentration; i.m. lipid continued to increase to over 20% in the Wagyu steers, but attained a plateau (14.7%) by 16 months on feed in the Angus steers. These results confirm that Wagyu cattle must be raised to greater physiological maturity before they differ from Angus cattle in *M. longissimus thoracis* i.m. lipid concentration.

Introduction

Zembayashi et al. (1995) demonstrated totally trimmed *M. longissimus lumborum* of Japanese Black (Wagyu) cattle fed in Japan may contain as much as 20% extractable lipid. A subsequent report from this laboratory showed that the *M. longissimus dorsi* from Wagyu steers fed a corn and barley-based diet for 552 days contained nearly 19% extractable lipid, even though the steers were a mixture of 3/4 or 7/8 crossbred Wagyu steers (Lunt et al., 1993). In the same study, *M. longissimus dorsi* of Angus steers fed the same diet for the same period of time contained 14.5% extractable lipid. Although USDA quality grades were similar among the Angus and Wagyu steers in that study (Prime^{19} vs Prime^{48}, respectively) the Wagyu steers had greater beef marbling score (7.30 vs 4.50) and quality grade (4.40 vs 3.40) than the Angus steers, based on the Japanese grading system. Marbling is assessed at the 12th rib under the USDA grading system and at the 6th rib for the Japanese grading system (Harris et al., 1994).

Although Japanese Black and American Wagyu steers produce carcasses with higher quality grades than Angus steers when fed to a typical Japanese endpoint (650 kg), it is less clear whether Japanese cattle will produce higher quality carcasses if fed to a typical U.S. live weight endpoint. Japanese Black cattle fed in Japan typically are fed diets low in concentrate and high in fiber, but the concentration of i.m. lipid in the *M. longissimus thoracis* appears to increase throughout their extended feeding periods (Zembayashi et al., 1995). It is not known if Angus steers can deposit intramuscular lipid throughout extended feeding or if they can produce high quality carcasses if fed high

roughage diets for extended periods. Therefore, we compared Angus and Wagyu cattle fed a corn-based finishing diet or a hay-based diet to 525 kg or 650 kg. We predicted that Angus steers would produce carcasses of equal quality to Wagyu steers when fed either the corn-based or hay-based diet to 525 kg, but that Wagyu steers would produce greater quality carcasses if fed high-roughage diets to 650 kg.

Materials and Methods

Animals and Experimental Procedures

Sixteen Japanese Wagyu and 16 Angus steers were purchased as calves at weaning (approximately 8 mo of age). Coastal bermuda grass hay containing 9.5% crude protein was fed free choice for 8 d after the steers were transported to the Texas A&M University Agricultural Research Center, McGregor. Eight steers of each breed type were assigned to a high-energy, corn-based diet (Table 3.1). The diet was formulated to achieve an average gain of 1.36 kg/d and was fed free choice for 8 mo or 16 mo after weaning (n = 4 per breed type and time on feed). The remaining 8 steers of each breed type continued on the coastal bermuda grass hay diet, supplemented with the corn-based diet to achieve a targeted rate of gain of 0.9 kg day. The hay-fed steers were fed for 12 or 20 mo after weaning (n = 4 per breed type and time on feed; Figure 3.1). The average initial weights for Wagyu and Angus steers were 169 and 211 kg, respectively. Targeted final body weights were 525 kg for steers fed for 8 mo or 12 mo the corn- or hay-based diets, respectively and were 650 kg for steers fed for 16 or 20 mo the corn- or hay-based diets, respectively. One Angus steer from the 8-mo, corn-fed group escaped the holding pen before slaughter and had to be removed from the

investigation.

Table 3.1 Ingredients and chemical composition of the high-corn diet at each time on feed interval

	Diets at each time on feed interval			
Item	1 mo	2 mo	3 mo	4 mo to end
Ground milo	20.00	20.00	20.00	20.00
Ground corn	21.80	40.55	47.55	48.05
Cottonseed meal	10.00	8.00	6.50	6.00
Cottonseed hulls	35.00	20.00	15.00	15.00
Molasses	10.00	8.00	7.50	7.50
Limestone	0.96	0.96	0.96	0.96
Trace mineralized salt[a]	0.56	0.56	0.56	0.56
Dicalcium phosphate	0.23	0.23	0.23	0.23
Potassium chloride	0.16	0.16	0.16	0.16
Zinc oxide	0.01	0.01	0.01	0.01
Ammonium sulphate	0.00	0.25	0.25	0.25
Vitamin premix[b]	0.08	0.08	0.08	0.08
R-1500[c]	1.20	1.20	1.20	1.20
Total percentage	100.00	100.00	100.00	100.00
Nutritional compositiond[d]				
Dry matter, %	88.80	89.08	89.13	89.13
Crude protein, %	11.41	11.58	11.34	11.16
NEm (Mcal/kg)	1.48	1.72	1.81	1.81
NEg (Mcal/kg)	0.88	1.11	1.19	1.19
Acid detergent fiber, %	27.04	17.50	14.19	14.12
Calcium, %	0.58	0.54	0.52	0.52
Phosphorous, %	0.34	0.36	0.36	0.36

[a] Trace mineralized salt: NaCl, 98%; Zn, 0.35%; Mn. 0.28%; Fe, 0.175%; Cu, 0.035%; I, 0.007%; Co, 0.0007%

[b] Vitamin premix: vitamin A, 2,200,000 IU/kg; vitamin D, 1,100,000 IU/kg; vitamin E, 2,200 IU/kg.

[c] R-1500: 1.65 g monensin sodium (Rumensin^{TM}) per kg.

[d] Calculated values based on NRC (1996).

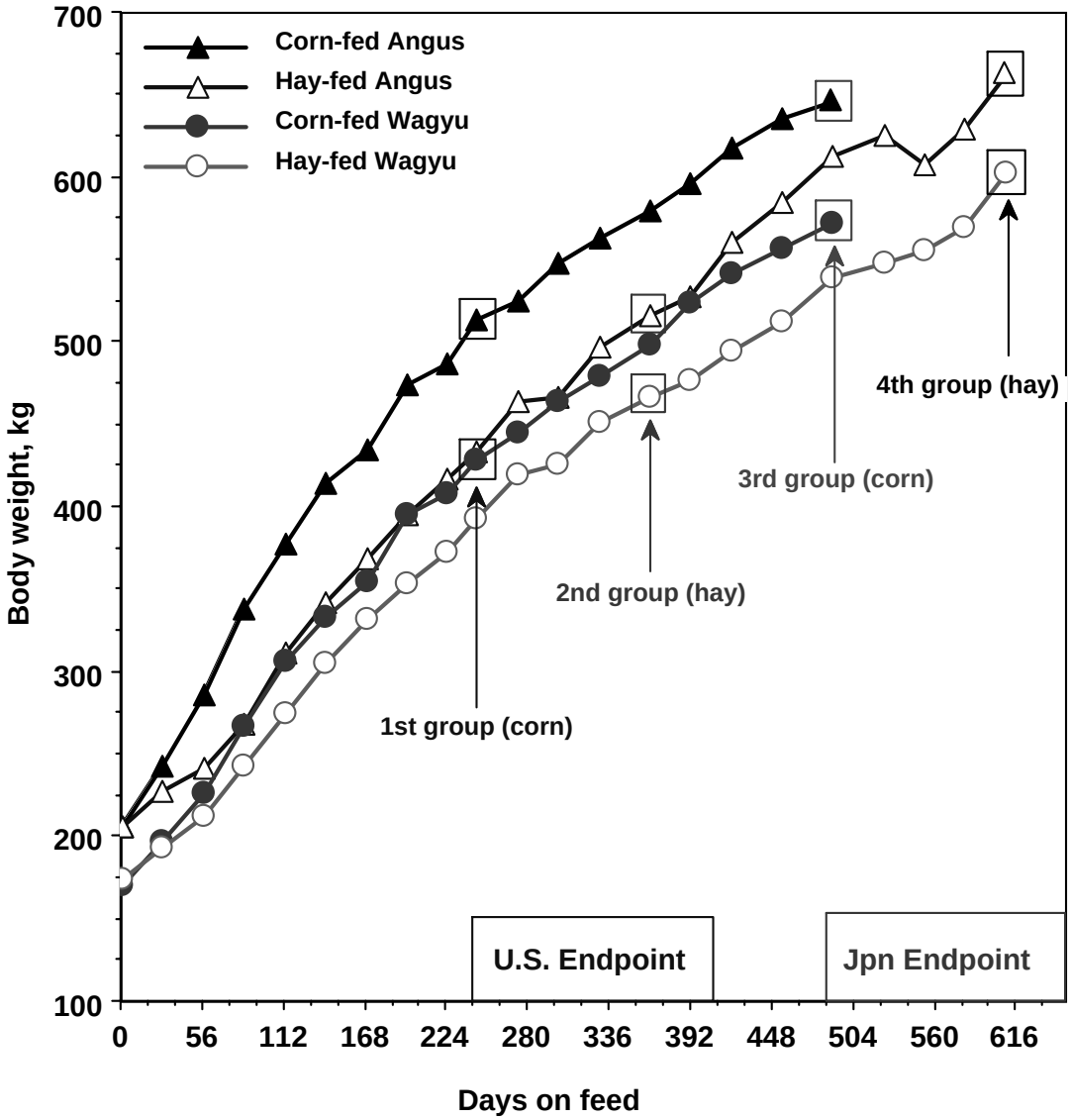


Figure 3.1 Changes in body weight over days on feed in Angus and Wagyu steers fed either a corn-based diet or a hay-based diet. Arrows indicate the times at which steers were slaughtered and sampled and boxes indicate which groups were sampled. First and 2nd groups were slaughtered at the U.S. weight endpoint, whereas the 3rd and 4th groups were slaughtered at the Japanese weight endpoint. Average daily gain was greater ($P = 0.03$) for the Angus steers, primarily because the corn-fed Angus steers grew so rapidly during the first 220 d on feed. Angus steers were approximately 40 kg heavier at weaning than the Wagyu steers at the beginning of feeding at 8 mo of age, which is commonly observed for these breed types.

After being fed for their respective time periods, the steers in each time-on-feed group were slaughtered on two consecutive days. Immediately following removal of the hide, a section of the *M. longissimus thoracis* and overlying s.c. adipose tissue was removed from the carcass. Samples of s.c. adipose tissue were snap-frozen in liquid nitrogen and stored at –94 °C. Blood samples were collected at exsanguination in tubes containing 15% K EDTA, centrifuged at 1,800 x g at 5°C for 30 min and plasma were taken and stored at –20°C until analyzed for fatty acid composition. After evisceration, an incision was made distal to the pyloric valve and duodenal contents (approximately 50 g) were collected and stored at –20°C for analysis of fatty acid composition.

Carcass Characteristics

Carcasses were chilled at 4 °C for 48 h and quality and yield grade factors were evaluated by trained personnel (Barker et al., 1995). USDA quality grade factors include overall maturity score and marbling score, whereas USDA yield grade was calculated based on adjusted fat thickness, *M. longissimus dorsi* cross-sectional (ribeye) area, carcass weight and percentage of kidney, pelvic and heart fat.

Fats and Moistures

A 100-g portion of the *M. longissimus thoracis*, completely trimmed of s.c. adipose tissue, was homogenized in a Virtis homogenizer (The Virtis Company, Inc., Gardiner, N.Y., USA). Fat and moisture content were determined by standard methods (AOAC, 1990).

Statistical Analysis

All statistical analyses were performed by using SPSS version 11 (SPSS Inc.,

Chicago, IL, USA.). Data were compared by ANOVA as three-factor designs that independently compared main effects (breed type, diet and end point) and all possible interactions. The $P < 0.05$ probability level was established for statistical significance.

Results

Production and Carcass Characteristics

Initial body weight was greater ($P = 0.01$) for weaned Angus steers than for weaned Wagyu steers (Table 3.2). We previously documented that Wagyu calves have lesser weaning weights than Angus calves (Smith et al., 1992). Final body weight and carcass weights were also greater for Angus steers than for Wagyu steers across diets and all times-on-feed ($P = 0.002$; Figure 3.1). The corn-based diet was formulated to provide the same average daily gain of 1.36 kg d (approximately 325 kg over the 8-mo period) for both breed types. This targeted gain was nearly achieved by the Angus steers in the U.S. endpoint weight group, as they gained an average of 316 kg over the duration of the feeding trial, but the corn-fed Wagyu steers had lower rates of gain, accumulating only 259 kg over the same period. The hay diet was designed to provide similar live weights at slaughter as the corn diet, i.e., 325 kg of gain over a 12-mo period. Hay-fed Angus steers approached this objective; gaining an average of 320 kg whereas hay-fed Wagyu steers gained 304 kg, so that, even on the hay diet, the Angus steers had greater rates of gain.

A similar breed effect was observed in the Japanese endpoint weight group of cattle. Cattle of both breed types in both diet treatment groups were anticipated to achieve a total gain of 540 kg. Neither breed achieved the targeted gain. Angus steers

were heavier than Wagyu after either 16 mo on the corn-based diet or 20 mo on the hay-based diet. However, the difference in average final weight between the breeds was only 16 kg in the long-fed groups as opposed to a difference of 41 kg in the U.S. endpoint comparison.

The goal of the study was to slaughter the cattle at the same physiological maturity at each slaughter interval. Over all time periods, no breed effect was apparent. Angus steers, however, tended to mature at a more rapid rate than the Wagyu steers ($P < 0.08$; Table 3.3). There was a time-on-feed effect on maturity scores ($P < 0.001$), because the hay-fed steers were by design 4 months older than the corn-fed steers in both endpoint groups. All maturity values were within "A" maturity, the most youthful classification in the U.S. grading system.

Diet and endpoint affected marbling scores and carcass quality grade (Table 3.3), but there was no difference between breed types for either measure. Marbling scores and quality grades were numerically greater for Angus steers at the U.S. endpoint and greater for Wagyu steers at the Japanese endpoint, but the breed x endpoint interaction was not significant ($P = 0.21$).

Chemically extractable lipid may be a more appropriate means to quantify differences in marbling scores in long-fed cattle. Although no overall breed effect was observed, the breed x endpoint interaction was significant ($P < 0.03$); *M. longissimus thoracis* from the hay-fed Wagyu carcasses at the Japanese endpoint contained more than 20% lipid as compared to 12% for the Angus carcasses at the same endpoint. Intramuscular lipid increased in Angus steers until 16 months on feed, but declined

Table 3.2 Production and carcass characteristics of Angus and Wagyu steers fed to U.S. and Japanese endpoints

	Months on feed/diet												
	U.S. endpoint				Japanese endpoint								
	8 mo/corn		12 mo/hay		16 mo/corn		20 mo/hay			*P*-value			
Item	Angus	Wagyu	Angus	Wagyu	Angus	Wagyu	Angus	Wagyu	SE	Breed	Diet	Endpt[a]	BxE[a]
Initial BW[a], kg	208.7	169.1	207.5	175.1	218.6	174.3	205.5	175.3	6.9	0.01	0.89	0.81	0.96
Final BW, kg	525.0	427.9	528.4	479.4	662.8	573.3	663.1	603.4	16.8	0.002	0.32	0.001	0.97
ADG[b], kg	1.25	1.03	0.88	0.84	0.93	0.84	0.78	0.73	0.03	0.03	0.01	0.01	0.48

[a]Endpt = live body weight endpoint; BxE = breed x endpoint interaction. BW = live body weight.
[b]Cummulative average daily gain for each slaughter interval.

Table 3.3 Carcass characteristics of Angus and Wagyu steers fed to U.S. and Japanese endpoints

	Months on feed/diet												
	U.S. endpoint				Japanese endpoint								
	8 mo/corn		12 mo/hay		16 mo/corn		20 mo/hay			*P*-value			
Item	Angus	Wagyu	Angus	Wagyu	Angus	Wagyu	Angus	Wagyu	SE	Breed	Diet	Endpt[a]	BxE[a]
Carcass wt, kg	323.4	252.3	307.7	283.0	407.8	357.2	403	353.1	11.2	0.001	0.89	0.001	0.92
Skeletal maturity[b]	133.3	140.0	165.0	140.0	167.5	172.5	185.0	185.0	3.9	0.42	0.001	0.001	0.16[e]
Lean maturity[b]	160.0	147.5	160.0	150.0	170.0	160.0	170.0	177.5	2.6	0.17	0.27	0.002	0.27
Overall maturity[b]	145.6	142.5	162.5	146.2	168.7	165.0	178.7	181.2	2.8	0.08	0.001	0.001	0.12
Marbling score[c]	673.3	612.5	580.0	572.5	802.5	897.5	672.5	762.5	29.2	0.55	0.05	0.01	0.21
Quality grade[d]	483.3	462.5	443.7	468.7	531.2	562.5	487.3	518.7	9.8	0.31	0.07	0.001	0.37
No. steers grading USDA Prime	2/3	0/4	0/4	1/4	3/4	4/4	1/4	3/4					
Adjusted fat thickness, cm	1.44	0.95	1.30	1.05	2.51	1.53	1.90	1.30	0.11	0.002	0.19	0.001	0.20
Ribeye area, cm^2	78.3	68.4	71.8	68.9	76.0	87.3	85.2	82.6	1.9	0.75	0.91	0.002	0.10
KPH, %	3.00	2.88	2.63	3.13	2.75	3.00	2.50	3.25	0.09	0.07	0.86	0.86	0.41
Yield grade	3.33	2.75	3.33	3.08	5.17	3.27	4.04	3.29	0.16	0.001	0.33	0.001	0.03
Lipid, %	9.3	6.1	8.3	7.8	14.7	14.1	12.0	20.4	1.03	0.48	0.44	0.001	0.05[e]

[a]Endpt = live body weight endpoint; BxE = breed x endpoint interaction.
[b]Maturity: A = 100; B = 200; C = 300; D = 400; E = 500.
[c]Marbling score: Modest = 500; Moderate = 600; Slightly Abundant = 700; Moderately Abundant = 800; Abundant = 900.
[d]Quality grade: Choice = 400; Prime = 500.
[e]There also was a significant breed x diet interaction ($P < 0.05$)

thereafter. In contrast, i.m. lipid continued to increase in the Wagyu cattle until the end of the study. There was sufficiently greater s.c. fat thickness in the Angus steers (P = 0.001) to cause a significant difference in yield grade (P = 0.01; Table 3.3). Time-on-feed also had a significant effect on ribeye area, adjusted fat thickness and USDA yield grade (P = 0.01).

Discussion

The results of the current study support the hypothesis that Wagyu cattle perform better on a hay-based diet than on a higher concentrate corn-based diet. In a previous study, Lunt et al. (1993) demonstrated that Angus steers fed a moderately high-roughage diet had greater rates of gain than Wagyu steers fed the same diet. Although a tendency for a breed effect on average daily gain was noted ($P < 0.08$), almost all of the difference was observed in the first 8 months the cattle were on feed.

Wagyu cattle are characterized by a greater ability to accumulate marbling adipocytes than other breed types within the *M. longissimus thoracis* and *M. longissimus dorsi* (Lunt et al., 1993; Zembayashi et al., 1995; Oka et al., 2002). Previously, these comparisons were made in steers fed to typical Japanese market endpoints, with steers fed in excess of 500 d (to B maturity). In the A maturity steers of the current study, marbling scores and USDA quality grades were not different between breed types (P = 0.31; Table 3.3). It should be noted, however, that most of the carcasses in the 16 months and 20 months groups (14/16) were in the USDA prime grade. At this high level of marbling, under the USDA grading system it is difficult to discern differences between such highly marbled carcasses.

In a previous investigation, Zembayashi et al., (1995) demonstrated that i.m. lipid (i.e., marbling) in the *M. longissimus thoracis* of Japanese Black cattle increased indefinitely with age (up to 900 d of age), whereas in Charolais x Japanese Black/Holstein crossbred cattle, the accumulation of i.m. lipid ceased after approximately 500 d of age. In another study, 126 Angus steers fed to 680 kg live weight (Cameron et al., 1993) had marbling scores that were similar to those achieved by the Angus steers in the present study, but were lower in marbling score than Wagyu steers in these present study (Lunt et al., 1993; Cameron et al., 1993; USDA. 1997). As predicted, corn-fed Angus steers had marbling scores and percentage i.m. lipid that exceeded those of corn-fed Wagyu steers at the U.S. endpoint. However, marbling score and i.m. lipid content were greater in the hay-fed Wagyu steers fed to the Japanese endpoint. These findings provide a strong rationale for the long-term, high roughage feeding of Wagyu cattle in Japan.

The USDA yield grade is calculated based on carcass weight, ribeye area, adjusted s.c. fat thickness at the 12th thoracic rib and percentage KPH (Barker et al., 1995). Numerically higher yield grade of Angus steers was due to their markedly greater s.c. fat thickness compared to Wagyu steers, especially at the Japanese endpoint. Others have demonstrated that Wagyu cattle have the unique ability to accumulate large amounts of marbling without excessive outside fat (Zembayashi. 1994)

Implications

Previous results, combined with the data of the present study, indicate that differences in marbling between Wagyu cattle and British or Continental breed types may not become evident until the cattle are fed to a greater physiological maturity. We further conclude that Wagyu cattle should be fed a high roughage diet for a relatively lengthy feeding period in order to reach their genetic potential to deposit maximum levels of marbling.

CHAPTER IV

LIPID CHARACTERISTICS OF SUBCUTANEOUS ADIPOSE TISSUE AND *M. longissimus thoracis* OF ANGUS AND WAGYU STEERS FED TO U.S. AND JAPANESE ENDPOINTS

Overview

We hypothesized that the concentrations of monounsaturated fatty acids and cholesterol of adipose tissue and *M. longissimus thoracis* would not differ between Angus and American Wagyu steers when fed to a typical U.S. live weight, but would diverge when fed to a Japanese live weight. To test this, 8 steers of each breed type were assigned to a high-energy, corn-based diet, and another 8 steers of each breed type were fed coastal bermuda grass hay diet, supplemented with the corn-based diet to achieve a daily gain of 0.9 kg/d. Targeted final body weights were 525 kg for steers fed for 8 or 12 mo the corn- or hay-based diets, respectively, and were 650 kg for steers fed for 16 or 20 mo the corn- or hay-based diets. Digesta concentrations of stearic (18:0) and *trans*-vaccenic acid decreased, whereas linoleic acid (18:2n-6) increased between the U.S. and Japanese endpoints (all $P \leq 0.03$). α-Linolenic acid (18:3n-3) increased in digesta only in the hay-fed steers during this time. Plasma concentrations of palmitic (16:0) and palmitoleic acid (16:1n-7), and the 16:1:18:0 ratio, were higher in Angus steers than in Wagyu steers. Also, the plasma 16:1:18:0 ratio was decreased by hay feeding in Angus steers, but increased in Wagyu steers, when fed to the Japanese endpoint. Concentrations of oleic (18:1n-9), linoleic, α–linolenic, and 18:2*trans*-10,*cis*-12 CLA all were higher in Wagyu than in Angus s.c. adipose tissue, whereas myristic

(14:0) and palmitic acid were higher in Angus s.c. adipose tissue ($P \leq 0.05$). All MUFA increased, and SFAs decreased, between the U.S. and Japanese endpoints. Slip points of lipids in s.c. adipose tissue were over 10°C lower ($P = 0.01$) in Japanese endpoint steers than in U.S. endpoint steers, consistent with the overall increase in MUFA with time on feed. The concentration of cholesterol in the *M. longissimus thoracis* increased with time, which may have been related to the increase in oleic acid. Because the breed x endpoint interaction was not significant for cholesterol or any of the adipose tissue fatty acids, we conclude that our original hypothesis was incorrect. Of the three factors tested (breed type, diet, and slaughter age endpoint), endpoint had the greatest effect on adipose tissue lipid composition.

Introduction

Monounsaturated fatty acids (MUFA) in meat have been shown to influence beef palatability (Dryden and Marchello, 1970; Westerling and Hedrick, 1979) and fat softness (Perry et al., 1998; Smith et al., 1998). The balance between stearic acid (18:0) and oleic acid (18:1n-9) is primarily responsible for differences in fat softness (Smith et al., 1998), and the concentrations of these fatty acids in s.c. adipose tissue are affected by breed, sex, age, and nutrition (Clemens et al., 1973; Eichhorn et al., 1986; Huerta-Leidenz et al., 1993; Mandell et al., 1998). Japanese Black cattle produce carcasses that have adipose tissues with higher percentages of MUFA than Holstein, Japanese Brown, Charolais, or Angus steers (Sturdivant et al., 1992; Zembayashi et al., 1995; Oka et al., 2002). Early studies from this laboratory were with American Wagyu cattle that had been derived from the two Japanese Black and two Japanese Brown bulls originally

imported into the U.S. (Lunt et al., 1993). The Japanese bulls were crossed on Angus and Hereford x Angus cows, and subsequent generations were mated back to the imported bulls to produce a synthetic breed. As seen for purebred Japanese Black cattle, these American Wagyu steers produced carcasses higher in MUFA than Angus steers when fed high-roughage diets to a typical Japanese endpoint (650 kg; May et al., 1993).

We hypothesized that adipose tissue MUFA concentrations in Angus steers would equal those of Wagyu steers if steers were fed a corn-based diet to a typical U.S. carcass weight (525 kg). Therefore, this study was designed to document the interaction between diet (corn vs. a hay-based diet) and slaughter weight (525 or 650 kg) on fatty acid composition of digesta, plasma, and s.c. adipose tissue, and melting points (estimated by slip points) of s.c. adipose tissue lipids. We also measured cholesterol concentrations of lipids from *M. longissimus thoracis* from the same animals to document their relationship to i.m. lipid and oleic acid concentrations in cattle raised to the Japanese endpoint.

Materials and Methods

Animals and Diets

Sixteen American Wagyu and 16 Angus steers were purchased as calves at weaning (approximately 8 mo of age). The American Wagyu steers were from the same foundation herd that provided steers for our earlier study (Lunt et al., 1993). Coastal bermuda grass hay containing 9.5% crude protein was fed free choice for 8 d after the steers were transported to the Texas A&M University Research Center, McGregor. Eight steers of each breed type were assigned to a high-energy, corn-based diet

containing 48% ground corn, 20% ground sorghum, 15% cottonseed hulls, 7.5% molasses, 0.96% limestone, 0.56% trace mineral salt, and 0.08% vitamin premix. The diet was designed to achieve an average gain of 1.36 kg/d, and was fed free choice for 8 mo or 16 mo after weaning (n = 4 per breed type and time on feed). The remaining 8 steers of each breed type continued on the coastal bermuda grass hay diet, supplemented with the corn-based diet to achieve a targeted rate of gain of 0.9 kg/d. The hay-fed steers were fed for 12 or 20 mo after weaning (n = 4 per breed type and time on feed).

Production characteristics of these cattle were reported previously (Lunt et al., 2005). The average initial weights for Wagyu and Angus steers were 169 and 211 kg, respectively. Targeted final body weights were 525 kg, considered as a U.S. endpoint for steers fed the corn- or hay-based diets for 8 mo or 12 mo, respectively, and were 650 kg, considered as a typical Japanese endpoint for steers fed mo the corn- or hay-based diets for 16 or 20, respectively. The Angus steers achieved the targeted body weights (average of 526.7 and 663.0 kg for the U.S. and Japanese endpoints, respectively; Lunt et al., 2005). The Wagyu steers averaged 453.7 and 588.4 kg for the U.S. and Japanese endpoints, respectively. Part of the difference in final body weights was caused by the 42-kg lesser initial body weights of the Wagyu calves; however, the Wagyu calves also had lower average daily gains (0.86 vs 0.96 kg/d for the Angus calves), due primarily to slower rates of gain of Wagyu calves on the corn-based diets (Lunt et al., 2005).

After being fed for their respective time periods, the steers in each time-on-feed group were slaughtered on two consecutive days. Immediately following removal of the hide, a section of the *M. longissimus thoracis* and overlying s.c. adipose tissue was

removed from the carcass. Samples of s.c. adipose tissue were snap-frozen in liquid nitrogen and stored at –94 °C. Blood samples were collected at exsanguination in tubes containing 15% K_3EDTA, and centrifuged at 1,800 x g at 5°C for 30 min; plasma was stored at –20°C until analyzed for fatty acid composition. After evisceration, an incision was made distal to the pyloric valve and duodenal contents (approximately 50 g) were collected and stored at –20°C for analysis of fatty acid composition.

Total Lipid Extraction

Total lipid was extracted by a modification of the method of Folch et al., (1957). Approximately 5 g of digesta, 1 mL of plasma, and 1 g of s.c. adipose tissue were homogenized with 5.0 mL of chloroform:methanol (2:1, vol/vol) and held with shaking at room temperature (approximately 20°C) for 48 h to extract lipid. The homogenate was filtered through Whatman GF/C filters (Whatman Ltd., Maidstone, England) and rinsed with an additional 10 mL of chloroform:methanol. The extracted lipid was combined with 8 mL of 0.74% KCl and vortexed for 1 min. Once the phases were separated, the aqueous layer was removed and discarded. The lipid layer was transferred to 20-mL scintillation vials and the solvents were evaporated by heating at 60°C under nitrogen.

Fatty Acid Analysis

Approximately 80 mg of each lipid extract was converted to its fatty acid methyl esters (FAME) as described (Morrison and Smith, 1964). FAME analyzed with a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 autosampler, Varian Inc., Walnut Creek, CA), equipped with a fused silica capillary column CP-Sil88 [100 m x

0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands), with helium as the carrier gas (flow rate = 1.2 mL/min) (Smith et al., 2002). After 32 min at 180°C, oven temperature was increased at 20°C/min to 225°C and held for 13.75 min. Total run time was 48 min. Injector and detector temperatures were at 270 and 300 °C, respectively. Individual FAME were quantified as g fatty acid/100 g of total FAME identified. Identitites of FAME were established by comparison to authentic standards (GLC 96; Nu-Chek Prep, Inc, Elysian, MN, U.S.A.). Conjugated linoleic acid isomers were identified by comparison to a commercial preparation (CLA-60; Natural of Hovdebygda, Norway).

Slip Points

Melting points of the s.c. adipose tissue lipids were approximated by determining slip points (Smith et al., 1998). After heating to approximately 45°C, the lipids were drawn 1 cm into glass capillary tubes. Duplicate tubes were collected for each sample and frozen at –20°C. After freezing, the capillary tubes were suspended vertically in a chilled water bath with the portion of the tube containing lipid submerged in the water. The water bath was heated at 2°C/min with constant stirring. Temperature of the water was monitored with a Type K thermocouple (model KTSS-HH, Omega Engineering, Inc., Stamford, CT, U.S.A.) attached to a digital thermometer (model 91100-50, Cole-Parmer Instrument Co., Vernon Hills, IL, U.S.A.). Slip point is defined as the temperature at which the lipid moved up the capillary tube and reported as the means between duplicates for each sample.

Cholesterol Concentration

Cholesterol concentration of the *M. longissimus thoracis* was analyzed as described (Rule et al., 1997; Rule et al., 2002) using gas chromatography. Briefly, 100 g of *M. longissimus thoracis* was freeze-dried and homogenized in a home-style electric grinder. Cholesterol was extracted with 3 mL of ethanol to 100 mg of dried tissue. The lipids were saponified by the addition of 1 mL of 33% (wt/vol) KOH and heating for 60 min in an 80 – 90°C water bath. Cholesterol was isolated on an SPB-1, fused capillary column [30 mm x 0.53mm (i.d.)](Suppelco, Bellefonte, PA, U.S.A.) with column temperature at 250°C and detector and injector temperatures at 300°C. Helium was the carrier gas with a 1:3 split ratio. Stigmasterol was used as the internal standard to quantify the total cholesterol.

Statistical Analysis

All statistical analyses were performed by using SPSS version 11 (SPSS Inc., Chicago, IL, U.S.A.). Fatty acid composition of plasma and s.c. adipose tissue, slip points, and cholesterol concentrations were compared by ANOVA as three-factor designs with main effects (breed type, diet, and endpoint) and all possible interactions. The $P < 0.05$ probability level was established for statistical significance.

Results

Digesta Fatty Acid Composition

It was not possible to obtain a representative sample of the hay-based diet for total fatty acid composition, because the steers had free access to the Bermuda grass hay and were supplemented with the corn concentrate diet to achieve their desired rate of

gain. Therefore, digesta was sampled for each animal at each slaughter interval and analyzed for fatty acid composition. Digesta of the corn-fed steers was higher in palmitic (16:0) ($P < 0.01$) and palmitoleic acid (16:1n-7) ($P \leq 0.02$) than digesta from hay-fed steers, whereas digesta of hay-fed was higher in α-linolenic acid (18:3n-3; $P < 0.01$; Table 4.1). Differences in myristoleic (14:1n-5) ($P \leq 0.05$), *trans*-vaccenic ($P < 0.01$), and α–linolenic acid ($P < 0.01$) were detected between breeds types; these fatty acids were greater in Wagyu digesta than in Angus digesta. There was a breed x diet x endpoint interaction for digesta *trans*-vaccenic acid; this fatty acid was greater in digesta of corn-fed Wagyu steers than in corn-fed Angus steers at the U.S. endpoint, but was not different between breed types under any other conditions.

There were significant diet x endpoint interactions for digesta palmitic ($P < 0.01$), stearic (18:0) ($P \leq 0.02$), and *trans*-vaccenic acid ($P < 0.01$), total SFA ($P \leq 0.04$), linoleic (18:2n-6) ($P < 0.01$), and α-linolenic acid ($P < 0.01$), the 16:1:18:0 ratio ($P \leq 0.01$), and total PUFA ($P < 0.01$) (Table 4.1). The concentrations of palmitic and linoleic acid, the 16:1:18:0 ratio, and total PUFA were greater in digesta of corn-fed steers fed to the Japanese endpoint than in corn-fed steers raised to the U.S. endpoint. Conversely, the concentrations of stearic and *trans*-vaccenic acid and total SFA were less in digesta of corn-fed steers fed to the Japanese endpoint than in corn-fed steers fed to the U.S. endpoint. These effects were not observed in hay-fed steers. Digesta concentrations of α-linolenic acid declined in hay-fed steers between the U.S. and Japanese endpoints.

Table 4.1 Fatty acid concentrations (g/100 g total fatty acids) in digesta of Wagyu and Angus steers fed to U.S. and Japanese endpoints

	Months on feed/diet													
	U.S. endpoint				Japanese endpoint									
	8 mo/corn		12 mo/hay		16 mo/corn		20 mo/hay			*P*-values				
Item	Angus	Wagyu	Angus	Wagyu	Angus	Wagyu	Angus	Wagyu	SE	Breed	Diet	Endpoint	DxE[a]	BxDxE[b]
14:0	0.63	0.61	1.02	1.00	0.50	1.04	0.64	0.71	0.08	0.39	0.38	0.57	0.15	0.48
14:1n-5[c]	0.34	0.95	0.94	0.76	0.29	0.83	0.35	0.49	0.07	0.05	0.81	0.06	0.19	0.48
16:0	19.2	16.8	25.2	26.1	25.4	22.5	23.6	22.3	0.68	0.13	<0.01	0.08	<0.01	0.65
16:1n-7	0.69	0.42	1.18	1.27	0.97	0.72	0.95	0.90	0.08	0.46	0.02	0.98	0.07	0.81
18:0	57.1	48.1	46.0	37.0	27.6	38.5	44.3	40.1	2.22	0.48	0.81	0.03	0.02	0.35
18:1*t*11[c]	2.46^{y}	7.88^{x}	1.81^{yz}	1.25^{yz}	0.46^{z}	1.47^{yz}	1.49^{yz}	2.81^{y}	0.50	0.01	0.08	0.01	<0.01	0.03
18:1n-9	14.8	18.6	12.7	17.4	16.9	17.8	15.4	18.9	0.95	0.12	0.65	0.51	0.72	0.86
18:2n-6	4.76	6.30	10.0	14.1	27.9	16.8	12.9	13.2	1.56	0.58	0.60	<0.01	<0.01	0.36
18:3n-3	0.0	0.25	0.83	1.01	0.0	0.23	0.24	0.48	0.08	<0.01	<0.01	<0.01	<0.01	0.91
16:1:18:0	0.013	0.010	0.031	0.037	0.038	0.021	0.021	0.022	0.003	0.55	0.19	0.64	0.01	0.71
SFA[d]	79.4	73.5	74.1	65.4	54.0	63.6	70.1	66.0	2.03	0.54	0.73	0.02	0.04	0.46
MUFA[d]	15.9	20.0	14.8	19.5	18.1	19.4	16.7	20.3	0.98	0.12	0.81	0.60	0.90	0.84
PUFA[d]	4.76	6.56	11.1	15.1	27.9	17.0	13.2	13.7	1.56	0.65	0.75	<0.01	<0.01	0.36
MUFA:SFA	0.20	0.28	0.22	0.32	0.34	0.34	0.24	0.31	0.02	0.20	0.68	0.26	0.29	0.76

[a]Diet x endpoint interaction. All breed x endpoint and breed x endpoint interactions $P > 0.10$.
[b]Breed x diet x endpoint interaction. [xyz]Means with different superscripts are different.
[c]There was a significant breed x diet interaction for 14:1n-5 ($P = 0.03$) and 18:1*trans*-11 ($P = 0.05$).
[d]SFA = total SFA (14:0 + 16:0 + 17:0 + 18:0 + 18:1*t*11). MUFA = total monounsaturated fatty acids (14:1n-5 + 16:1n-7 + 18:1n-9). PUFA = total polyunsaturated fatty acids (18:2n-6 + 18:3n-3).

There also were significant breed x diet interactions for myristoleic ($P = 0.03$) and *trans*-vaccenic acid ($P = 0.05$). These fatty acids were higher in digesta of corn-fed Wagyu steers in digest of hay-fed Wagyu steers, but did not differ between corn- and hay-fed Angus steers. This was the only significant breed x diet interaction observed in this study.

Plasma Fatty Acid Composition

Angus steers had higher plasma concentrations of palmitic ($P = 0.05$) and palmitoleic acid ($P = 0.03$) and a higher plasma 16:1:18:0 ratio than Wagyu steers (Table 4.2). Plasma from steers fed the corn-based diet contained higher concentrations of myristoleic and palmitoleic acid ($P \leq 0.02$), but lesser plasma stearic acid than hay-fed steers ($P = 0.01$). Consequently, plasma from the corn-fed steers had a 16:1:18:0 ratio greater than plasma from hay-fed steers, more MUFA, and a higher MUFA:SFA ratio (all $P \leq 0.03$).

Plasma from steers raised to the U.S. endpoint had a higher concentration of palmitoleic acid ($P = 0.01$), less stearic acid ($P = 0.02$), and a higher 16:1:18:0 ratio ($P = 0.05$) than steers raised to the Japanese endpoint. Plasma α-linolenic acid tended to be higher ($P = 0.06$) in steers raised to the Japanese endpoint.

The diet x endpoint interaction was significant for myristic and myristoleic acid (both $P < 0.01$) and the MUFA:SFA ratio ($P = 0.04$), all of which increased with time on feed in the corn-fed steers but decreased in the hay-fed steers. The three-way interaction of breed, diet, and endpoint was significant for palmitoleic acid and, therefore, the 16:1:18:0 ratio (Table 4.2). Relative to corn-fed steers, hay feeding decreased plasma

Table 4.2 Fatty acid concentrations (g/100 g total fatty acids) in plasma of Wagyu and Angus steers fed to U.S. and Japanese endpoints

	Months on feed/diet													
	U.S. endpoint				Japanese endpoint									
	8 mo/corn		12 mo/hay		16 mo/corn		20 mo/hay			*P*-values				
Item	Angus	Wagyu	Angus	Wagyu	Angus	Wagyu	Angus	Wagyu	SE	Breed	Diet	Endpoint	DxE[a]	BxDxE[b]
14:0	0.15	0.24	0.96	0.50	0.84	0.58	0.0	0.0	0.11	0.43	0.67	0.59	<0.01	0.31
14:1n-5	0.25	0.42	0.50	0.28	0.61	0.89	0.0	0.0	0.08	0.68	0.02	0.91	<0.01	0.84
16:0	16.7	13.9	15.8	14.6	17.4	13.4	12.3	11.9	0.57	0.05	0.11	0.17	0.13	0.63
16:1n-7	1.31^{x}	1.40^{x}	1.18^{x}	0.25^{z}	1.33^{x}	0.50^{y}	0.18^{z}	0.59^{y}	0.11	0.03	<0.01	0.01	0.69	<0.01
18:0	22.7	20.4	22.2	21.9	18.0	19.1	21.8	21.7	0.42	0.59	0.01	0.02	0.07	0.27
18:1n-9	14.6	12.0	14.2	12.4	16.3	14.9	11.5	10.9	0.68	0.25	0.11	0.94	0.11	0.98
18:2n-6	43.9	51.1	44.7	49.0	45.4	50.4	53.9	54.8	1.34	0.11	0.28	0.15	0.19	0.90
18:3n-3	0.35	0.47	0.48	0.88	0.0	0.22	0.30	0.15	0.09	0.44	0.32	0.06	0.68	0.38
16:1:18:0	0.058^{x}	0.070^{x}	0.053^{x}	0.012^{z}	0.075^{x}	0.026^{y}	0.008^{z}	0.027^{y}	0.006	0.05	<0.01	0.05	0.94	<0.01
SFA[c]	39.6	34.5	39.0	37.1	36.3	33.0	34.1	33.6	0.81	0.11	0.96	0.05	0.59	0.96
MUFA[c]	16.1	13.8	15.9	13.0	18.3	16.4	11.7	11.5	0.73	0.19	0.03	0.85	0.07	0.68
PUFA[c]	44.3	51.6	45.2	49.9	45.4	50.6	54.2	54.9	1.34	0.10	0.25	0.19	0.20	0.86
MUFA:SFA	0.40	0.40	0.41	0.35	0.51	0.50	0.34	0.34	0.02	0.53	0.01	0.39	0.04	0.61

[a]Diet x endpoint interaction. All breed x diet and breed x endpoint interactions $P > 0.20$.
[b]Breed x diet x endpoint interaction. [xyz]Means with different superscripts are different.
[c]SFA = total SFA (14:0 + 16:0 + 17:0 + 18:0). MUFA = total monounsaturated fatty acids (14:1n-5 + 16:1n-7 + 18:1n-9). PUFA = total polyunsaturated fatty acids (18:2n-6 + 18:3n-3).

palmitoleic acid in Wagyu steers raised to the U.S. endpoint, but had no effect in Wagyu steers raised to the Japanese endpoint. The plasma concentration of palmitoleic acid was reduced in Angus steers only in response to hay feeding to the Japanese endpoint.

Subcutaneous Adipose Tissue Fatty Acid Composition

Wagyu s.c. adipose tissue contained less myristic and palmitic acid (both $P = 0.01$) than Angus s.c. adipose tissue, whereas oleic, linoleic, α-linolenic, and 18:2*trans*-10,*cis*-12 CLA (18:2t10,*c*12), and total PUFA were greater ($P \leq 0.04$) in Wagyu s.c. adipose tissue (Table 4.3). Wagyu s.c. adipose tissue also tended ($P = 0.08$) to have a greater MUFA:SFA ratio than Angus adipose tissue.

Adipose tissue of corn-fed steers contained higher concentrations of palmitoleic, *trans*-vaccenic, and linoleic acid, more total MUFA and PUFA, and higher 16:1:18:0 and MUFA:SFA ratios than adipose tissue of hay-fed steers, whereas adipose tissue of hay-fed steers contained more stearic and α-linolenic acid and more total SFA (all $P \leq 0.05$; Table 4.3). Oleic acid also tended to be higher ($P = 0.08$) in adipose tissue of corn-fed steers. Feeding cattle to the Japanese endpoint increased the adipose tissue concentrations of all MUFA and the 16:1:18:0 and MUFA:SFA ratios (all $P \leq 0.01$). Thus, all indices of monounsaturation of fatty acids increased in cattle fed to the Japanese endpoint, relative to the U.S. endpoint. Conversely, palmitic ($P = 0.07$) and stearic acid ($P < 0.01$), and total SFA ($P < 0.01$), decreased in adipose tissue of cattle raised to the Japanese endpoint.

Table 4.3 Fatty acid concentrations (g/100 g total fatty acids) in subcutaneous adipose tissue of Wagyu and Angus steers fed to U.S. and Japanese endpoints

	Months on feed/diet													
	U.S. endpoint				Japanese endpoint									
	8 mo/corn		12 mo/hay		16 mo/corn		20 mo/hay			*P*-values				
Item	Angus	Wagyu	Angus	Wagyu	Angus	Wagyu	Angus	Wagyu	SE	Breed	Diet	Endpoint	DxE[a]	BxDxE[b]
14:0	3.88	3.07	3.72	3.11	3.50	3.62	4.06	2.84	0.12	0.01	0.72	0.79	0.91	0.10
14:1n-5	1.14	1.38	0.88	1.08	2.48	2.34	2.30	1.75	0.14	0.77	0.13	0.01	0.80	0.67
16:0	29.2	28.0	30.7	28.5	28.3	27.6	29.1	26.7	0.34	0.01	0.45	0.07	0.43	0.76
16:1n-7	3.13	4.34	2.49	2.63	7.15	7.07	6.38	5.46	0.41	0.87	0.04	<0.01	0.99	0.92
18:0	17.1	15.1	22.0	20.9	7.89	7.67	9.90	9.11	1.26	0.56	0.05	<0.01	0.30	0.83
18:1*t*11	2.12	1.66	2.63	2.64	1.05	1.27	1.49	1.54	0.13	0.80	<0.01	<0.01	0.29	0.37
18:1n-9	39.8	42.3	34.6	37.3	45.5	46.0	43.3	48.6	1.00	0.05	0.08	<0.01	0.06	0.40
18:2n-6	2.73	2.68	1.68	2.24	2.63	2.92	1.87	2.08	0.09	0.04	<0.01	0.73	0.83	0.16
18:3n-3	0.0	0.10	0.25	0.32	0.06	0.21	0.15	0.20	0.03	0.04	<0.01	0.72	0.03	0.68
18:2*c*9,*t*11	0.30	0.45	0.41	0.42	0.55	0.33	0.52	0.64	0.03	0.81	0.16	0.07	0.40	0.06
18:2*t*10,*c*12	0.04	0.26	0.13	0.27	0.32	0.28	0.26	0.46	0.03	0.02	0.32	<0.01	0.90	0.15
16:1:18:0	0.19	0.44	0.11	0.19	1.01	1.04	0.66	0.60	0.08	0.52	0.02	<0.01	0.33	0.83
SFA[c]	52.3	47.9	59.1	55.2	40.7	40.2	44.6	40.2	1.51	0.09	0.03	<0.01	0.19	0.56
MUFA[c]	44.4	48.5	38.4	41.5	55.7	55.8	52.5	56.5	1.45	0.13	0.04	<0.01	0.16	0.51
PUFA[c]	2.74	2.78	1.93	2.56	2.69	3.13	2.01	2.56	0.09	0.01	<0.01	0.84	0.33	0.14
MUFA:SFA	0.85	1.05	0.65	0.80	1.39	1.40	1.18	1.41	0.06	0.08	0.05	<0.01	0.44	0.39

[a]Diet x endpoint interaction. All breed x diet and breed x endpoint interactions $P > 0.20$.
[b]Breed x diet x endpoint interaction.
[c]SFA = total SFA (14:0 + 16:0 + 17:0 + 18:0 + 18:1*t*11). MUFA = total monounsaturated fatty acids (14:1n-5 + 16:1n-7 + 18:1n-9 + 18:2*c*9,*t*11). PUFA = total polyunsaturated fatty acids (18:2n-6 + 18:3n-3).

Adipose tissue concentrations of 18:2cis-9,*trans*-11 CLA tended ($P = 0.07$) to be greater in adipose tissue of cattle raised to the Japanese endpoint, and 18:2*trans*-10,*cis*-12 CLA was significantly greater ($P < 0.01$) in cattle raised to the Japanese endpoint (Table 4.3). The breed x diet x endpoint interaction also tended ($P = 0.06$) to be significant for 18:2cis-9,*trans*-11 CLA, in that this CLA isomer increased only in adipose tissue of hay-fed Wagyu steers fed to the Japanese endpoint. Hay feeding depressed the s.c. adipose tissue concentration of oleic acid at the U.S. endpoint, but had no effect on oleic acid by the time the steers reached the Japanese endpoint ($P = 0.06$).

There was a high, negative correlation ($R^2 = 0.925$) between concentrations of stearic and palmitoleic acid (Figure 4.1). Palmitoleic acid was highest in corn-fed steers and, with the exception of one corn-fed, Wagyu steer, was lowest in hay-fed steers. The very highest concentration of palmitoleic acid was observed in adipose tissue of steers fed corn to the Japanese endpoint.

Slip Points

Neither breed type nor diet affected slip points of lipids extracted from s.c. adipose tissue ($P \geq 0.14$; Table 4.4). Slip points decreased by more than 10°C (38.7 versus 28.4°C) between the U.S. and Japanese endpoints. There was a strong, positive correlation ($R^2 = 0.917$) between the concentration of stearic acid and slip points (Figure 4.2). Adipose tissue lipids from cattle of both breed types fed to the Japanese endpoint had the lowest concentrations of stearic acid and consequently the lowest slip points.

Table 4.4 Slip points of lipids from subcutaneous adipose tissue and cholesterol concentrations of *M. longissimus thoracis* of Wagyu and Angus steers fed to U.S. and Japanese endpoints

	Months on feed/diet													
	U.S. endpoint				Japanese endpoint									
	8 mo/corn		12 mo/hay		16 mo/corn		20 mo/hay			*P*-values				
Item	Angus	Wagyu	Angus	Wagyu	Angus	Wagyu	Angus	Wagyu	SE	Breed	Diet	Endpoint	DxE[a]	BxDxE[b]
Slip point,														
°C	37.9	35.3	42.8	38.9	27.8	27.9	31.3	26.7	1.29	0.14	0.15	0.01	0.39	0.64
Cholesterol,														
mg/100 g	72.1	70.8	71.5	68.3	78.4	83.9	78.9	89.3	1.98	0.43	0.85	0.01	0.53	0.64

[a]Diet x endpoint interaction.
[b]Breed x diet x endpoint interaction.

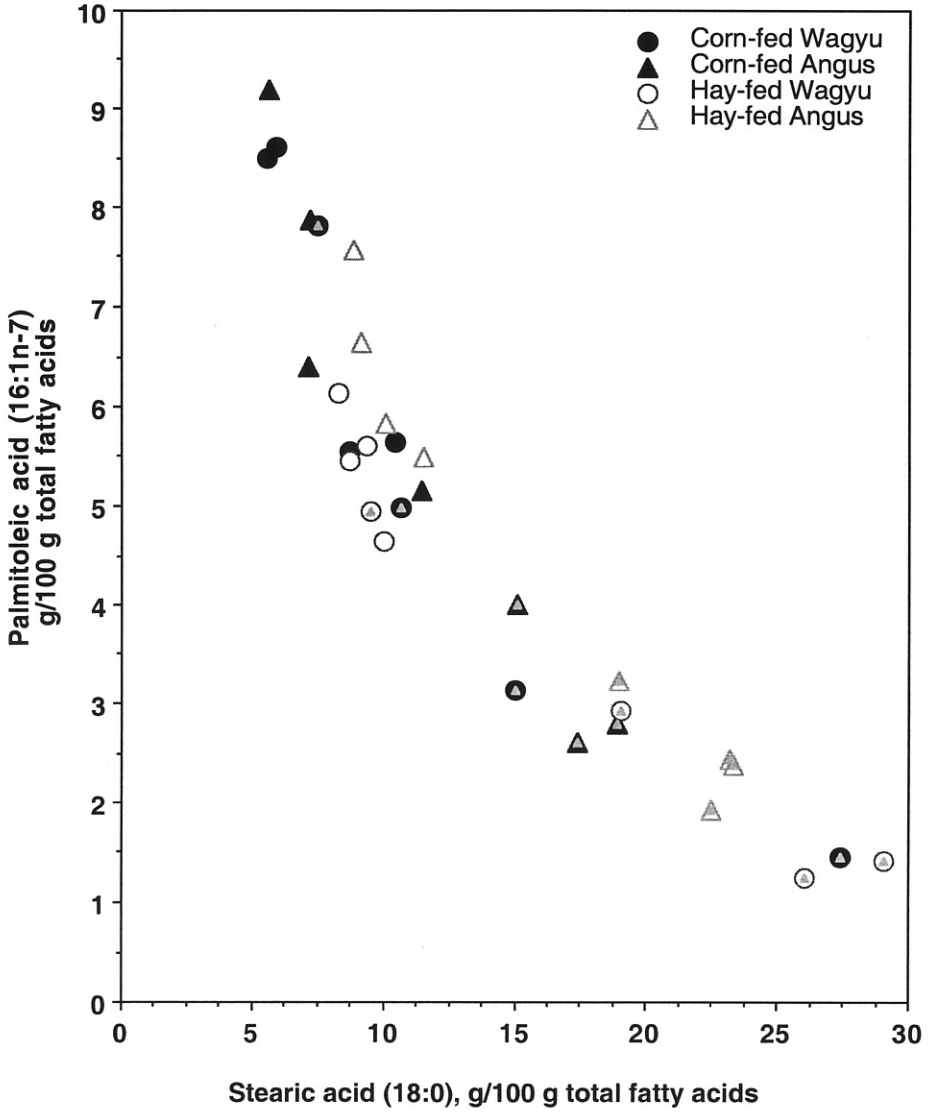


Figure 4.1 Palmitoleic acid (16:1n-7) as a function of stearic acid (18:0) in subcutaneous adipose tissue of Wagyu and Angus steers fed corn-based or hay-based diets to U.S. or Japanese endpoints. Closed symbols, corn-fed steers; open symbols, hay-fed steers; circles, Wagyu steers; triangles, Angus steers. Symbols for the cattle raised to the U.S. endpoint contain shaded triangles. $y = 0.015x^2 - 7.96x + 12.32$; $R^2 = 0.925$; $P < 0.001$).

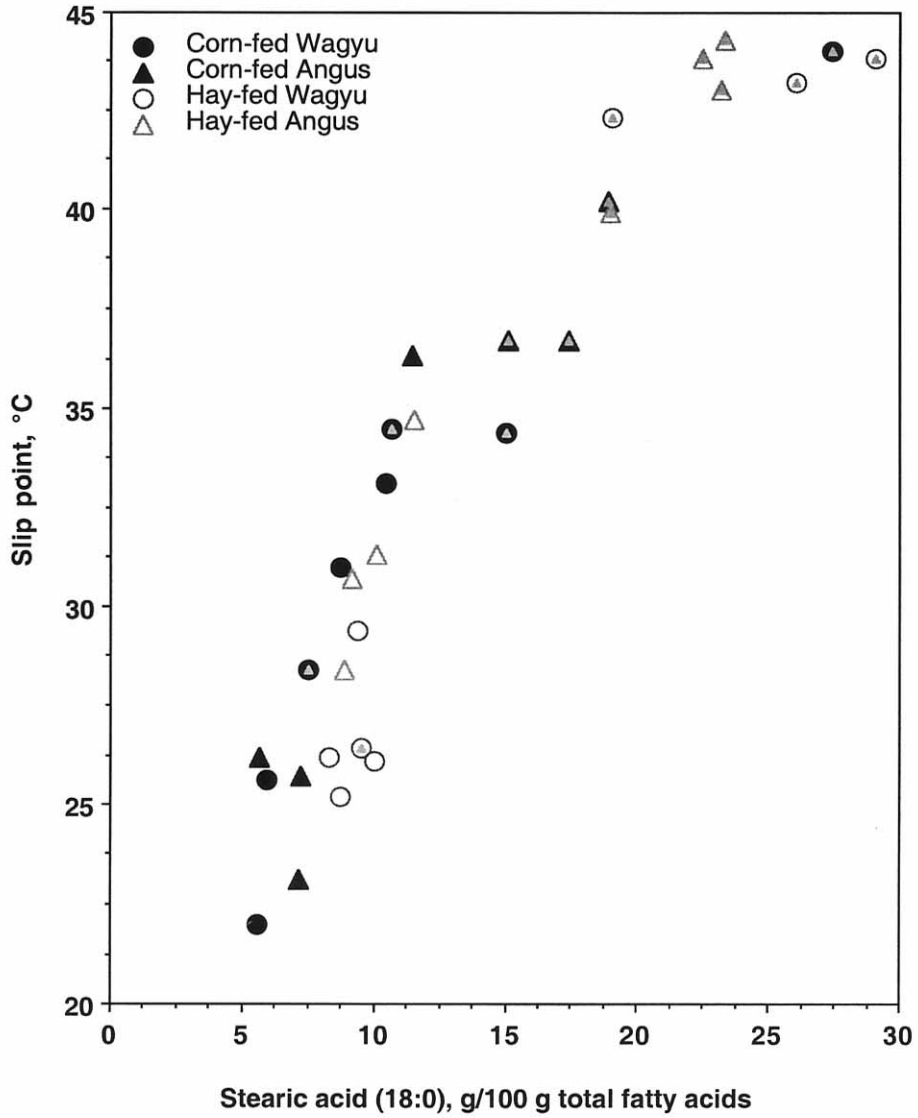


Figure 4.2 Slip points as a function of the concentration of stearic acid (18:0) in subcutaneous adipose tissue of Angus and Wagyu steers fed corn-based or hay-based diets to U.S. or Japanese endpoints. Closed symbols, corn-fed steers; open symbols, hay-fed steers; circles, Wagyu steers; triangles, Angus steers. Symbols for the cattle raised to the U.S. endpoint contain shaded triangles. $y = -0.039x^2 + 2.23x + 12.07$; $R^2 = 0.917$; $P < 0.001$).

M. Longissimus Thoracis Cholesterol

The concentration of cholesterol in the *M. longissimus thoracis* increased (P = 0.01) in steers fed to the Japanese endpoint, relative to those fed to the U.S. endpoint (Table 4.4). There were no effects of breed type (P = 0.43) or diet (P = 0.85) on *M. longissimus thoracis* cholesterol. There was a significant (P = 0.03), though weak (R^2 = 0.154) relationship between percentage i.m. lipid and cholesterol (Figure 4.3). There was a stronger (P = 0.006; R^2 = 0.231) relationship between s.c. oleic acid and *M. longissimus thoracis* cholesterol (Figure 4.4).

Discussion

We have reported production and carcass characteristics for the cattle used in this study in a separate article (Lunt et al., 2005), and selected results are reported herein. Breed type affected neither overall marbling scores nor USDA quality grades for the Angus (Moderate^{82}; Choice^{86}) and Wagyu steers (Slightly Abundant^{11}; Prime^{03}). Corn-fed steers had significantly greater marbling scores (Slightly Abundant^{51}) than hay-fed steers (Moderate^{46}). Also, steers raised to the Japanese endpoint had higher marbling scores and USDA quality grades (Slightly Abundant^{84}; Prime^{25}) than steers raised to the U.S. endpoint (Moderate^{05}; Choice^{63}).

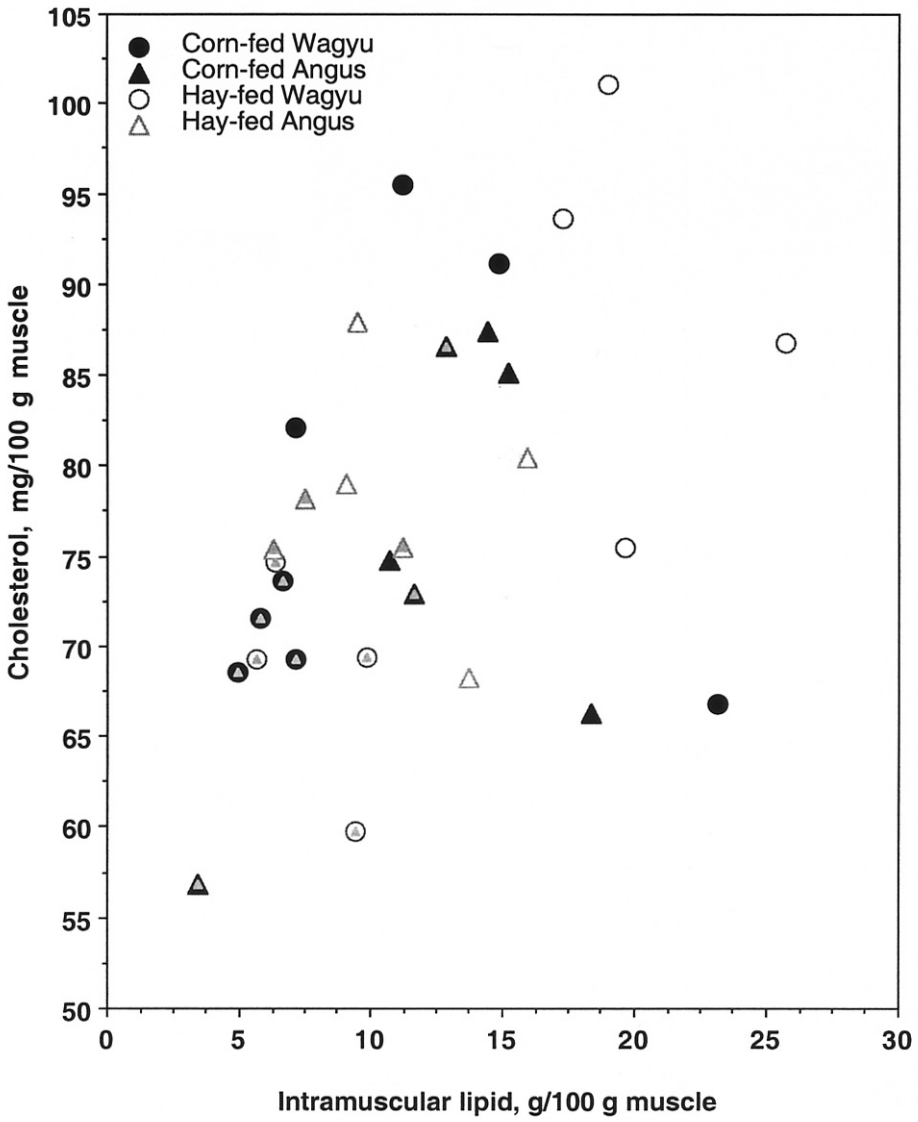


Figure 4.3 Relationship between intramuscular cholesterol and intramuscular lipid in *M. longissimus thoracis* of Wagyu and Angus steers fed corn-based or hay-based diets to U.S. or Japanese endpoints. Closed symbols, corn-fed steers; open symbols, hay-fed steers; circles, Wagyu steers; triangles, Angus steers. Symbols for the cattle raised to the U.S. endpoint contain shaded triangles. $y = 0.734x + 68.77$; $R^2 = 0.154$; $P = 0.03$). Data for intramuscular lipid were taken from Lunt et al. (2005).

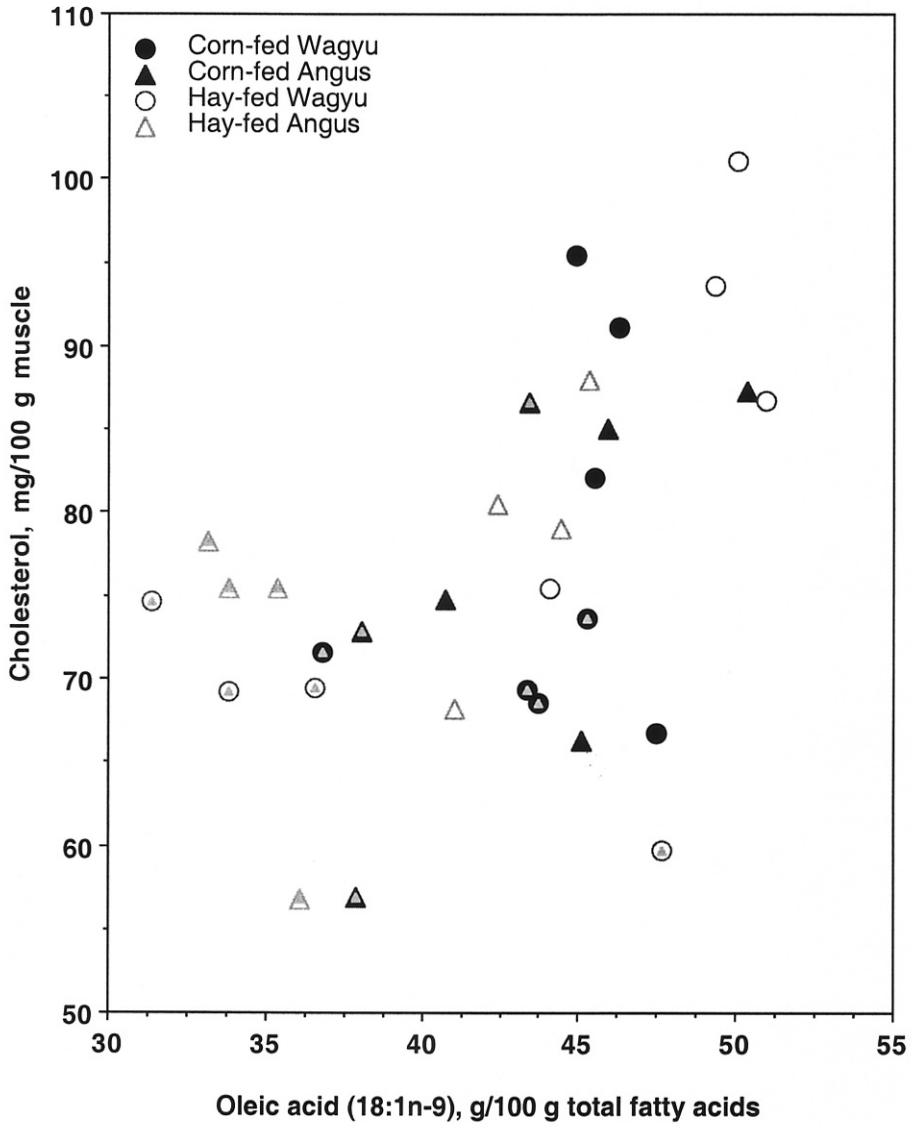


Figure 4.4 Relationship between intramuscular cholesterol in *M. longissimus thoracis* and oleic acid (18:1n-9) in s.c. adipose tissue of Wagyu and Angus steers fed corn-based or hay-based diets to U.S. or Japanese endpoints. Closed symbols, corn-fed steers; open symbols, hay-fed steers; circles, Wagyu steers; triangles, Angus steers. Symbols for the cattle raised to the U.S. endpoint contain shaded triangles. $y = 0.955x + 36.39$; $R^2 = 0.231$; $P = 0.006$).

There was a significant breed x endpoint interaction for percentage lipid in the *M. longissimus thoracis*; muscle from Wagyu steers raised to the Japanese endpoint had more extractable lipid (17.2%) than muscle from Angus steers raised to the same endpoint (13.3%) (Lunt et al., 2005). These results were essentially identical to those of our early comparison of forage-fed, Angus and Wagyu steers (Lunt et al., 1993).
In an earlier study, the percentages of oleic acid were 45.2 and 50.2% of total fatty acids in s.c. adipose tissue in Angus and Wagyu steers, respectively, fed a high-roughage diet for 550 d (May et al., 1993). These results are similar to those of the current study for hay-fed Angus and Wagyu steers raised to the Japanese endpoint. Others previously had demonstrated that oleic acid increased with age in feedlot cattle (Waldman et al., 1968; Huerta-Leidenz et al., 1996; Rule et al., 1997), and the results of the current study are consistent with these earlier findings.

It is well documented that Japanese Black and American Wagyu cattle deposit higher concentrations of MUFA in their muscle and adipose tissues than other breed types. Tanaka (1985) reported a higher percentage of oleic acid and a lower percentage of palmitic acid in adipose tissue of Japanese Black steers than in Japanese Shorthorn or Holstein steers. Japanese Black and American Wagyu steers exhibit higher percentages of oleic acid in s.c. and i.m. lipids than Holstein or Angus steers (Sturdivant et al., 1992; May et al., 1993; Zembayashi et al., 1995). The current results are novel in that they indicate that the previously documented differences in oleic acid between Wagyu and Angus steers are expressed regardless of diet or endpoint. Thus, although feeding Wagyu and Angus steers hay-based diets reduced oleic acid, and feeding to the Japanese

endpoint increased oleic acid, Wagyu adipose tissue contained a higher concentration of this fatty acid than Angus steers overall.

Alterations in digesta fatty acids may have contributed to the increased unsaturation of s.c. adipose tissue in corn-fed steers raised to the Japanese endpoint. Digesta stearic acid decreased substantially between the U.S. and Japanese endpoints, but only in the corn-fed steers, and a similar tendency was observed for plasma stearic acid. The digesta concentration of *trans*-vaccenic acid also decreased, and linoleic acid increased between the U.S. and Japanese endpoints, again only in corn-fed steers. These observations indicate a depression in the ruminal conversion of linoleic to *trans*-vaccenic acid and ultimately to stearic acid in long-fed steers consuming the corn-based diet. This suggests a reduction with time on feed in those microflora responsible for the isomerization and hydrogenation of linoleic acid in steers consuming the corn-based diet. The lower digesta concentration of stearic acid in corn-fed, Japanese endpoint steers was not expected, and warrants further investigation.

We did not expect to find breed differences for any of the digesta fatty acids, yet myristoleic, *trans*-vaccenic, and α-linolenic acid were higher in digesta of Wagyu steers than in Angus steers. The Angus and Wagyu weaned steers were purchased from different suppliers, so initial differences in digesta fatty acid composition at weaning would have been expected. However, after a minimum of 8 mo of feeding at the Texas A&M University Research Center at McGregor, initial differences between breed types in ruminal composition and microflora should have been eliminated. The higher concentration of α-linolenic acid in Wagyu digesta ultimately was reflected in the

adipose tissue; Wagyu s.c. adipose tissue had a higher concentration of α -linolenic acid than Angus s.c. adipose tissue.

There was a strong, negative correlation between palmitoleic acid and stearic acid for the lipid samples analyzed by Smith et al. (1998). This relationship was shown graphically in a recent review (Smith et al., 2004), and is confirmed by the results of the current study (Figure 4.2). It is remarkable that such a strong relationship occurs between palmitoleic acid and stearic acid, regardless of breed type, age, or diet. Because palmitoleic acid occurs at low levels in the diet, its concentration in adipose tissue is dictated primarily by the activity of SCD (or Δ^9 desaturase). The high, negative correlation between palmitoleic acid and stearic acid further suggests that the concentration of stearic acid also is dictated by the activity of SCD; adipose tissues with high desaturase activity exhibit concomitantly high concentrations of palmitoleic acid and low concentrations of stearic acid. Similar results were reported by Mitsuhashi et al. (1988), who first demonstrated that the proportion of MUFA increased with age in adipose tissues of Japanese Black steers.

In a recent review, Wood et al. (2004) demonstrated the positive correlation lipid melting points and percentage of stearic acid in lamb s.c. adipose tissue. The current study provides additional support for a strong relationship between stearic acid and slip points in bovine adipose tissue. Variation in fatty acid saturation dictates fat firmness, which in turn affects the economics of meat processing and consumer acceptance of meat (Perry et al., 1998). Multivariate analysis that includes melting point in the model (instead of sire breed) indicates that fatty acid composition is related to melting point

and environment (Perry et al., 1998). Similarly, Smith et al. (1998) demonstrated large increases in melting points (estimated as slip points) as the percentage of stearic acid increased in s.c. adipose tissue lipids in cattle raised in Japan and Australia. Adipose tissue lipids from Japanese Black cattle raised in Japan contained less than 8% stearic acid, with an average slip point of 22.8°C. Mitsuhashi et al. (1988) previously reported that, in Japanese Black cattle, the melting point of adipose tissue lipids decreased from 35.5°C in 14-mo-old steers to 21.2°C in 28-mo-old steers; they suggested that melting point may be controlled by Δ^9 desaturase. Adipose tissue lipids of other breed types raised in Australia contained over 25% stearic acid, with an average slip point of 45.1°C (Smith et al., 1998). The Australian cattle had been fed a grain-based diet (which did not include corn), supplemented with 10% whole cottonseed; We suggested that sterculic acid contained in the whole cottonseed depressed Δ^9 desaturase activity (Smith et al., 1998; Yang et al., 1999). However, we cannot rule out the possibility that forages unique to the Australian production environment also contributed to the high stearic acid and slip points. The current study is unique in that the major factors that affect fat firmness, breed type, age, and diet, were controlled within the same study.

We did not anticipate that s.c. adipose lipids from Angus steers would have 16:1:18:0 ratios as high, nor slips points as low, as those from Wagyu steers. However, corn-fed Angus steers raised to the Japanese endpoint accumulated adipose tissues lipids that were remarkably unsaturated. These results confirm the impact of both time on feed and grain feeding in producing more unsaturated, softer fat. It also was unexpected that lipids from one of the corn-fed, U.S. endpoint Wagyu steers would have such a high slip

point and low 16:1:18:0 ratio. Otherwise, the highest slip points and lowest 16:1:18:0 ratios were observed in hay-fed, U.S. endpoint steers.

The cholesterol concentrations of bovine muscle and adipose tissue have proven resistant to nutritional (Miller et al., 1991; Eichhorn et al., 1986) and breed effects (Eichhorn et al., 1986; Wheeler et al., 1987), but are directly related to marbling score (i.e., amount of i lipid). Rhee et al., (1982) demonstrated that as U.S. marbling scores increased from Practically Devoid (2.73 g lipid/100 g muscle) to Moderately Abundant (12.08 g lipid/100 g muscle), i.m. cholesterol increased from 51.77 to 64.74 mg/100 g of muscle [cholesterol = 0.87*(i.m. lipid %) + 54.73]. The regression equation derived from the current study contained a lesser slope (0.734 mg cholesterol/g i.m. lipid) and a higher intercept (68.77 mg cholesterol; Figure 4.3). The reduction in slope was caused primarily by unusually low cholesterol values for some of the muscle samples from steers fed corn to the Japanese endpoint.

As reported in Lunt et al. (2005), the highest concentration of *M. longissimus thoracis* intramuscular lipid (20.4 g/100 g muscle) was observed in Wagyu steers fed to the Japanese endpoint, and it was muscle from these steers that contained the greatest concentration of cholesterol (89.3 mg/100 g muscle; Figure 4.3). Based on the regression equation of Rhee et al. (1982), we would have predicted an average of 72.5 mg cholesterol/100 g of muscle, far below the observed cholesterol concentration. The greater than predicted accumulation of cholesterol in *M. longissimus thoracis* of hay-fed, Japanese-endpoint steers may have been related to the concentration of oleic acid in the muscle. Fatty acid composition of muscle lipids was not measured in this study.

However, we previously reported that s.c. and marbling adipose tissue of 1/2 – 7/8 American Wagyu steers contained 47.1 and 46.4% oleic acid, respectively, whereas in purebred Japanese Black cattle, s.c. and marbling adipose tissues contained 55.2 and 54.1% oleic acid, respectively (Sturdivant et al., 1992). Thus, concentrations of oleic acid are similar between these two depots.

As indicated in Figure 4.4, there was a highly significant and positive relationship between the concentration of s.c. adipose tissue oleic acid and *M. longissimus thoracis* cholesterol. The relationship was only apparent in cattle raised to the Japanese endpoint, in which the highest concentrations of oleic acid and cholesterol were observed. In liver (Goodman et al., 1964) and intestinal mucosa of rodents (Haugen and Norum, 1976), oleic acid is the preferred substrate for the synthesis of cholesterol esters via acyl-CoA: cholesterol acyl transferase. Although this has not been documented for adipose tissue from any species, oleic acid may similarly promote cholesterol ester synthesis and accumulation in marbling adipose tissue. If this is true, then any production practice that promotes an increase in the concentration of oleic acid (grain feeding or feeding to heavier weights) also would elevate cholesterol beyond that predicted by the increase in i.m. lipid.

Implications

The results obtained in the current study reject our hypothesis that fatty acid composition of Wagyu and Angus adipose tissue lipids would be similar when the cattle were fed to the U.S. endpoint. Instead, Wagyu adipose tissue consistently contained higher concentrations of oleic acid and other MUFAs, regardless of diet or endpoint. The reduction in digesta stearic acid in corn-fed steers raised to the Japanese endpoint may have contributed to the lesser amounts of stearic acid, hence lower slip points, observed in the adipose tissue of these animals. Of the three factors tested in this study (breed type, diet, and slaughter endpoint), endpoint has the strongest and most consistent effect on fatty acids of s.c. adipose tissue, and on cholesterol concentrations and melting points of lipids from *M. longissimus thoracis*.

CHAPTER V

STEAROYL-COENZYME A DESATURASE GENE EXPRESSION AND ENZYME ACTIVITY IN ADIPOSE TISSUE OF ANGUS AND WAGYU STEERS RAISED TO U.S. AND JAPANESE WEIGHT ENDPOINTS

Overview

We predicted that s.c. adipose tissue of Wagyu steers would exhibit greater SCD gene expression and enzyme activity raised to either U.S. or Japanese endpoints than Angus steers. Angus (n = 8) or Wagyu (n = 8) steers were fed a corn-based diet for either 8 or 16 mo, whereas another group of either Angus (n = 8) or Wagyu (n = 8) steers were fed a hay-based diet for 12 or 20 mo. Fatty acid biosynthesis in vitro was measured in s.c. and i.m. adipose tissues from Angus and Wagyu steers slaughtered at targeted constant weights of 525 and 650 kg. Subcutaneous adipose tissues of Wagyu steers fed the corn diet for 8 mo had the greatest lipogenic acitivity ($P = 0.05$), and ^{14}C-acetate incorporation into lipids was higher in s.c. than i.m. adipose tissue of both breed types. Angus s.c. adipose tissue had peak SCD enzyme activity at 16 mo (corn-based diet) but activity in Wagyu adipose tissue was greatest at 20 mo (hay-based diet) (breed x diet x endpoint interaction $P = 0.08$). Desaturase gene expression in Angus adipose tissue either declined (hay diet) or did not change (corn diet) between the U.S. and Japanese endpoints. Conversely, SCD gene expression increased over time in both the hay-fed and corn-fed Wagyu steers (breed x endpoint interaction $P = 0.01$). Thus, breed type strongly influenced SCD gene expression, and interacted with time on feed in such a manner as to

explain the higher concentration of MUFAs in American Wagyu cattle raised to the Japanese endpoint.

Introduction

Stearoyl-CoA desaturase (SCD) is responsible for converting MUFA to SFA (Jeffcoat et al., 1977). The MUFA:SFA ratio of lipids from bovine adipose tissues is an indicator of fat softness and an important aspect of meat quality in some countries (Smith et al., 1998). Japanese Black (and American Wagyu) cattle, known to be genetically superior not only in i.m. fat (marbling) accumulation but also MUFA composition, require a long-term finishing period (Lunt et al., 1993). Long feeding periods cause significant increases of MUFA in several regional adipose tissues (Huerta-Leidenz et al.,1996; May et al., 1994, 1995).

An earlier investigation (Cameron et al., 1994) indicated no difference in SCD enzyme activity or gene expression in s.c. adipose tissue between Angus and American Wagyu cattle fed to a typical Japanese endpoint, in spite of significantly greater MUFA in Wagyu adipose tissue (May et al., 1993). In this study, it was hypothesized that differences in fatty acid composition between Wagyu and Angus cattle may be due to greater SCD activity earlier in production, which would result in increased MUFA deposition at some point before slaughter.

Therefore, the primary objective of this study was to document the effects of production endpoint and diet on metabolism and SCD gene expression and activity in s.c. adipose tissue of Angus and Wagyu steers. This study demonstrated that SCD gene expression and enzyme activity were regulated by breed type, diet, and production

endpoint, which in turn may have influenced the extent of MUFA accumulation in adipose tissue.

Materials and Methods

Animals and Diets

Production characterizations for the cattle used in this study were reported previously (Lunt et al., 2005), and are summarized briefly here. Sixteen Angus and 16 American Wagyu steers were purchased as weanling calves (approximately 8 mo of age) and assigned to one of two dietary treatments: a high-energy, corn-based finishing diet designed to provide 1.36 kg/d ADG; and a medium-energy, coastal burmuda grass hay-based diet that provided 0.9 kg/d ADG. Corn-fed steers were fed 8 or 16 mo, and the hay-fed steers were fed for 12 or 20 mo after weaning (n = 4 per breed and time on feed). The average initial weights for Wagyu and Angus steers were 174 kg and 210 kg, respectively, and the diets were designed so that hay-fed and corn-fed steers within a breed type achieved the same body weight. At the U.S. endpoint, Angus and Wagyu steers weighed 527 and 454 kg, respectively. At the Japanese endpoint, Angus and Wagyu steers weighed 663 and 588 kg, respectively (Lunt et al., 2005). After being fed for their respective time periods, the steers in each group were slaughtered on two consecutive days.

All steers were slaughtered at the Rosenthal Meat Science and Technology Center on the Texas A&M University Campus in accordance with the Humane Methods of the Slaughter Act of 1978 (TAMU protocol number 3-513). Immediately post-exsanguination, a sample of longissimus muscle from the 5th to 8th thoracic region (with

adhering s.c. adipose tissue) was removed for sampling of both s.c. and i.m. adipose tissue for lipogenesis (s.c. and i.m.), or SCD enzyme activity and gene expression (s.c. only). One Angus steer from the 8-mo, corn-fed group escaped the holding pen before slaughter, and had to be removed from the investigation.

In Vitro Lipogenesis

Two-hour *in vitro* incubations were performed on s.c. and i.m. adipose tissue immediately after slaughter as described previously (May et al., 1994). Acetate incorporation into neutral lipids was measured by incubating adipose tissue (50 to 100 mg) in Krebs-Henseleit bicarbonate buffer (pH 7.4), 5 m*M* sodium acetate, 5 m*M* glucose, 10 m*M* hepes buffer, and 1 μCi [U-^{14}C]acetate (Amersham, Arlington Heights, IL) in 3 mL total volume. The vials were then gassed for 1 min with 95% O_2:5% CO_2, capped, and incubated in a shaking water bath (37°C) for 2 h. The reactions were terminated by adding 3 mL of 5% trichloroacetic acid to each vial. Lipids were extracted according to published procedures (Folch et al., 1957; May et al., 1994). Lipids were evaporated to dryness with at Multi-Vap evaporator (Associates Inc., South Berlin, MA) in a 40°C water bath. The samples were resuspended with 10 mL of Econo-Safe scintillation fluid (Research Products International Corp., Mount Prospect, IL) and counted on a Beckman LS-3800 scintillation counter (Beckman Instruments, Palo Alto, CA). The incorporation rate of acetate into neutral lipid is expressed as nmol•2h^{-1}•10^5 cells^{-1}.

Cellularity

The procedure of Etherton et al. (1977) as modified by Prior (1983) was used to estimate number of adipocytes per gram of adipose tissue. Frozen s.c. and i.m. adipose tissue was sliced into 1-mm thick sections, fixed with 3% osmium tetraoxide, and digested with 8 M urea. The fixed cells were filtered through 240-µm, 64-µm, and 20-µm nylon mesh screens with 0.01% Triton in 0.154 *M* NaCl. Cell fractions collected from the 64-µm and 20-µm mesh screens were used to determine cells per gram of adipose tissue with a Coulter Counter, Model ZM equipped with a channelizer, Model Z56 (Coulter Electronics, Hialeah, GA).

Microsome Extraction and SCD Enzyme Activity

Subcutaneous adipose tissue was homogenized in three volumes of buffer (wt/vol) with a Polytron homogenizer (The Virtis Company, Inc., Gardiner, N.Y.) for 60 sec. The buffer (pH = 7.4) was composed of 0.25 *M* sucrose, 0.01 *M* potassium phosphate, 1 m*M* EDTA, and 1 m*M* dithioerythritol. The homogenate was centrifuged at 5,000 x *g* for 15 min. The supernate was decanted into another tube and the pellet and fat cake were discarded. The supernate was centrifuged at 17,300 x *g* for 30 min and decanted into another tube. The tube was centrifuged at 104,000 x *g*. The supernate was discarded and the pellet was retained. The microsomes were collected and resuspended in 100 m*M* Tris-HCL buffer (pH = 7.4), fast frozen with liquid nitrogen, and stored at -80^{o}C until further analysis.

Desaturase enzyme activity was determined as described by St. John et al. (1991) modified as described by Archibeque et al. (2005). The assay system was composed of

100 m*M* Tris-HCl (pH 7.4), 2 m*M* NADPH, 25 μ*M* palmitoyl-CoA and 0.025 μCi [1-^{14}C]palmitoyl-CoA in 1.5 mL total volume. The resulting solutions were started with 0.1 mg protein and incubated in a 37^{o}C water bath for 7 min. The reaction was stopped by adding 1 mL of 12% KOH in ethanol, followed by heating for 1 h at 80^{o}C. After acidification by addition of 9 mL of 3 *N* HCl, fatty acids were washed with 9 mL of n-pentane. The pentane phases were evaporated under nitrogen and methylated with addition of 14% BF_3 in methanol. Methyl esters were separated by thin layer chromatography on 10% $AgNO_3$ impregnated silica gel plate in a petroleum ether:diethylether solvent system (97:3). After separation, plates were sprayed with 0.2% dichlorofluoroscein in ethanol. Spots containing palmitate methyl ester and palmitoleate methyl ester were scraped and counted using a liquid scintillation spectrometer. The ratio of palmitate methyl ester: palmitoleate methyl ester was used to quantify nmol palmitic acid converted to palmitoleic acid per 7 min per mg microsomal protein.

Northern Blot Analysis

The cDNA fragment of bovine the SCD gene (662 bp, GenBank accession number AB075020) was cloned into the vector of pCRII-TOPO (Invitrogen) using RT-PCR and conventional DNA techniques. The primers used for RT-PCR were forward primer, 5'-CCTGTGGAGTCACCGAACC -3' and reverse primer, 5'-CCTTGGATACTTTCTTCCGGTC-3'. The cDNA insert was confirmed by sequencing. The digoxigenin (DIG)-labeled antisense RNA probe was generated with DIG RNA Labelling Kit (SP6/T7) (Roche Diagnostics, Mannheim, Germany).

Total RNA samples (2 μg) were separated in 1% agalose gels containing 6.7% formaldehyde and transferred to a Hybond N+ membrane (Amersham Biosciences, NJ, USA). The membrane was hybridized with the DIG-labeled RNA probes. The bands corresponding to SCD mRNA were detected using DIG Luminescent Detection Kit for Nucleic Acids (Roche Diagnostics).

Northern blotting was analyzed by ImageQuant, version 5.2 software (Molecular Dynamics). The SCD bands vs 28S ribosomal RNA were analyzed for qualifying and quantifying the detected bands.

Statistical Analyses

Data were analyzed using the GLM as a three-factor design with the SAS version 8.1 (SAS Inst. Inc., Cary, NC). Means for lipogenesis, SCD activities, and gene expression from Wagyu (n = 16) and Angus (n = 15) steers were compared by three-factor ANOVA. Main effects were breed type, diet, and endpoint (U.S. or Japanese), and the model tested all 2- and 3- way interactions against their appropriate error terms. Interaction means were separated using the probability statement of GLM in the significant difference ($P < 0.05$).

Results

Fatty Acid Biosynthesis

There were significant ($P = 0.01$) diet x endpoint interactions for lipogenesis in i.m. adipose tissue (Figure 5.1) s.c. adipose tissue (Figure 5.2). Both breed types exhibited the highest lipogenic activity at 8 mo in i.m. and s.c. adipose tissues ($P \leq 0.05$) and then markedly declined after 8 mo in cattle fed the corn-based diet. However,

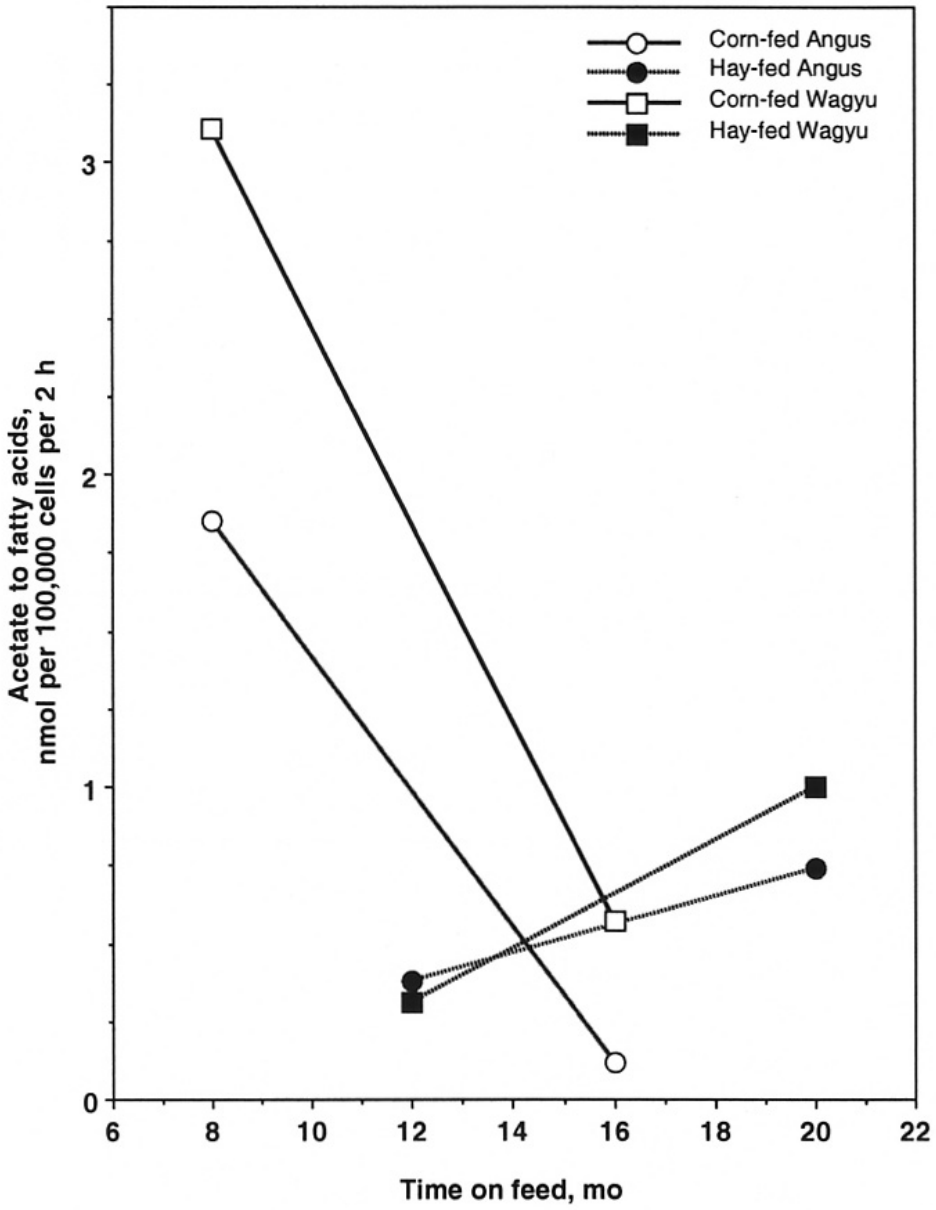


Figure 5.1 Changes in lipogenesis with time on feed in intramuscular adipose tissue of Angus and Wagyu steers fed either a corn-based diet or a hay-based diet. There was a significant diet x endpoint ($P = 0.01$) effect for lipogenesis, and there tended to be significant diet ($P = 0.08$) and endpoint ($P = 0.08$) effects. Lipogenesis in i.m. adipose tissue decreased in corn-based steers but was unchanged in hay-based steers between the U.S. and Japanese endpoint.

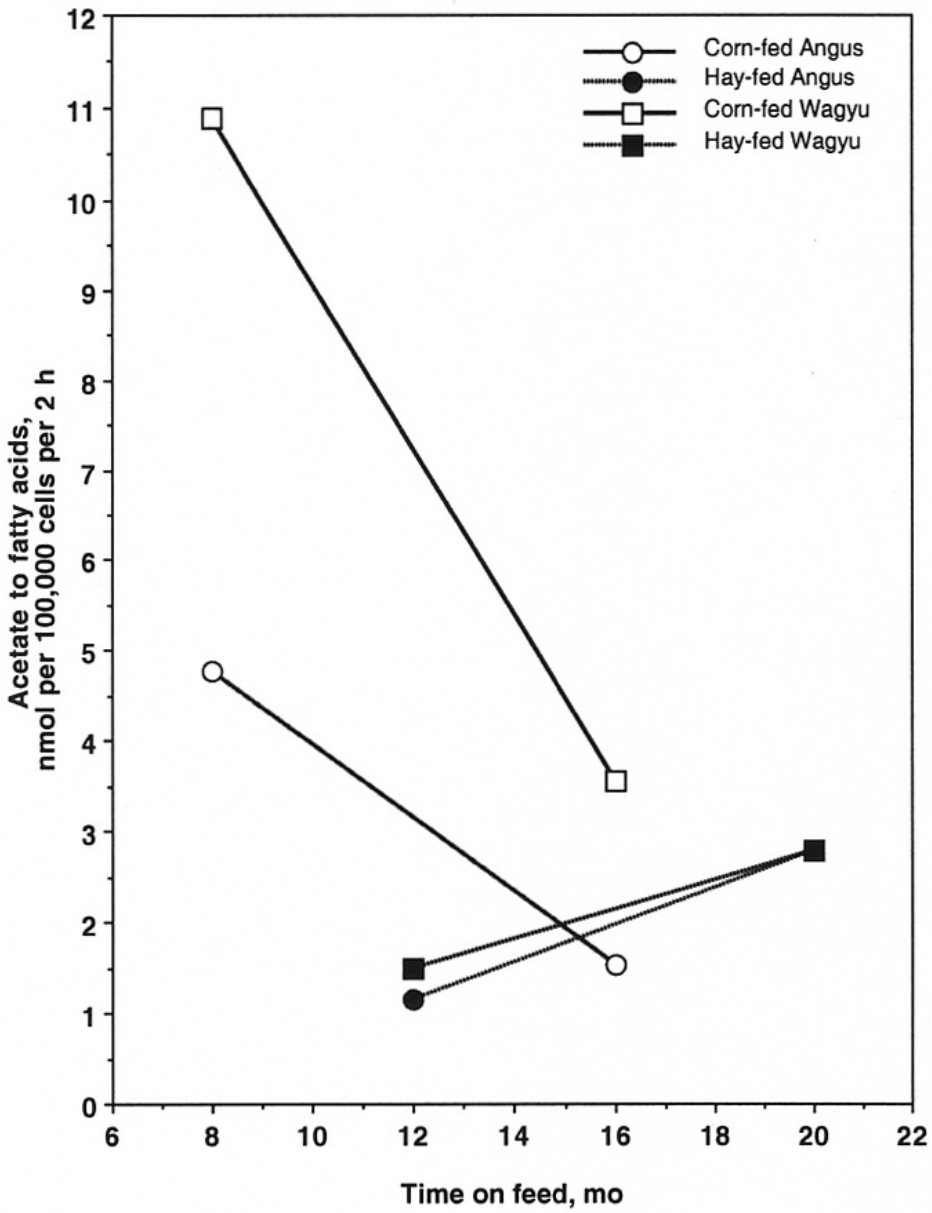


Figure 5.2 Changes in lipogenesis with time on feed in subcutaneous adipose tissue of Angus and Wagyu steers fed either a corn-based diet or a hay-based diet. There were significant diet x endpoint ($P = 0.01$), diet ($P = 0.01$), and breed ($P = 0.05$) effects, and there tended to be significant breed x diet ($P = 0.06$) and endpoint ($P = 0.06$) effects. Lipogenesis in s.c. adipose tissue decreased in corn-fed steers but was unchanged in hay-based steers between the U.S. and Japanese endpoint.

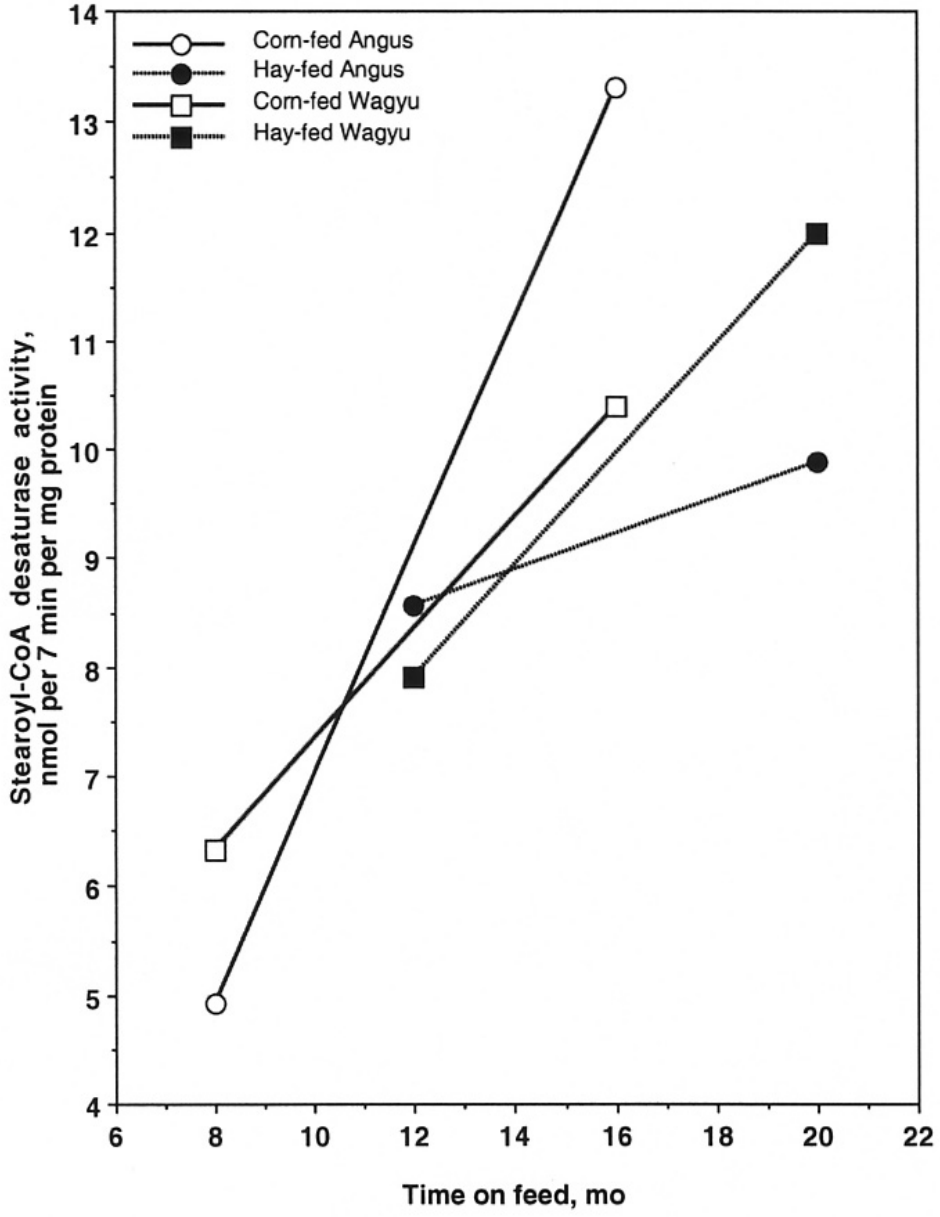


Figure 5.3 Changes in stearoyl-CoA desaturase enzyme activity with time on feed in s.c. adipose tissue of Angus and Wagyu steers fed either a corn-based diet or a hay-based diet. There was a significant endpoint ($P = 0.01$) effect for desaturase activity, and there tended to be significant diet x endpoint ($P = 0.08$) and breed x diet x endpoint ($P = 0.08$) effects. Desaturase activity increased between the U.S. and Japanese endpoint, but not in the hay-fed Angus steers.

lipogenesis in i.m. and s.c. adipose tissues did not change significantly between 12 and 20 mo in steers fed the hay-based diet. Lipogenesis in Wagyu s.c. adipose tissue was greater than in Angus s.c. adipose tissue ($P = 0.05$), due primarily to the greater activity in samples from the corn-fed wagyu steers.

Stearoyl-Coenzyme A Desaturase Enzyme Activity

There was no effect of breed type on overall SCD enzyme activity ($P = 1.0$; Figure 5.3). There was a significant difference in activity between U.S. and Japanese endpoints ($P = 0.01$), and there tended to be significant diet x endpoint ($P = 0.08$) and breed x diet x endpoint ($P = 0.08$) interactions. The three-way interaction indicated that SCD activity increased over time in corn-fed Angus steers, but not in hay-fed Angus steers.

Northern Blot Analysis

The SCD mRNA was approximately 4.9 kb in size (Figure 5.4). There tended to be significant diet ($P = 0.06$) and endpoint ($P = 0.07$) effects, and there were significant diet by endpoint ($P = 0.05$) and breed x endpoint ($P = 0.01$) effects (Figure 5.4). However, FAS gene expression could not explain difference of breeds and age (Figure 5.5). Desaturase gene expression was greater in hay-fed than in corn-fed steers and gene expression increased between the U.S. and Japanese endpoints only in Wagyu s.c. adipoce tissue.

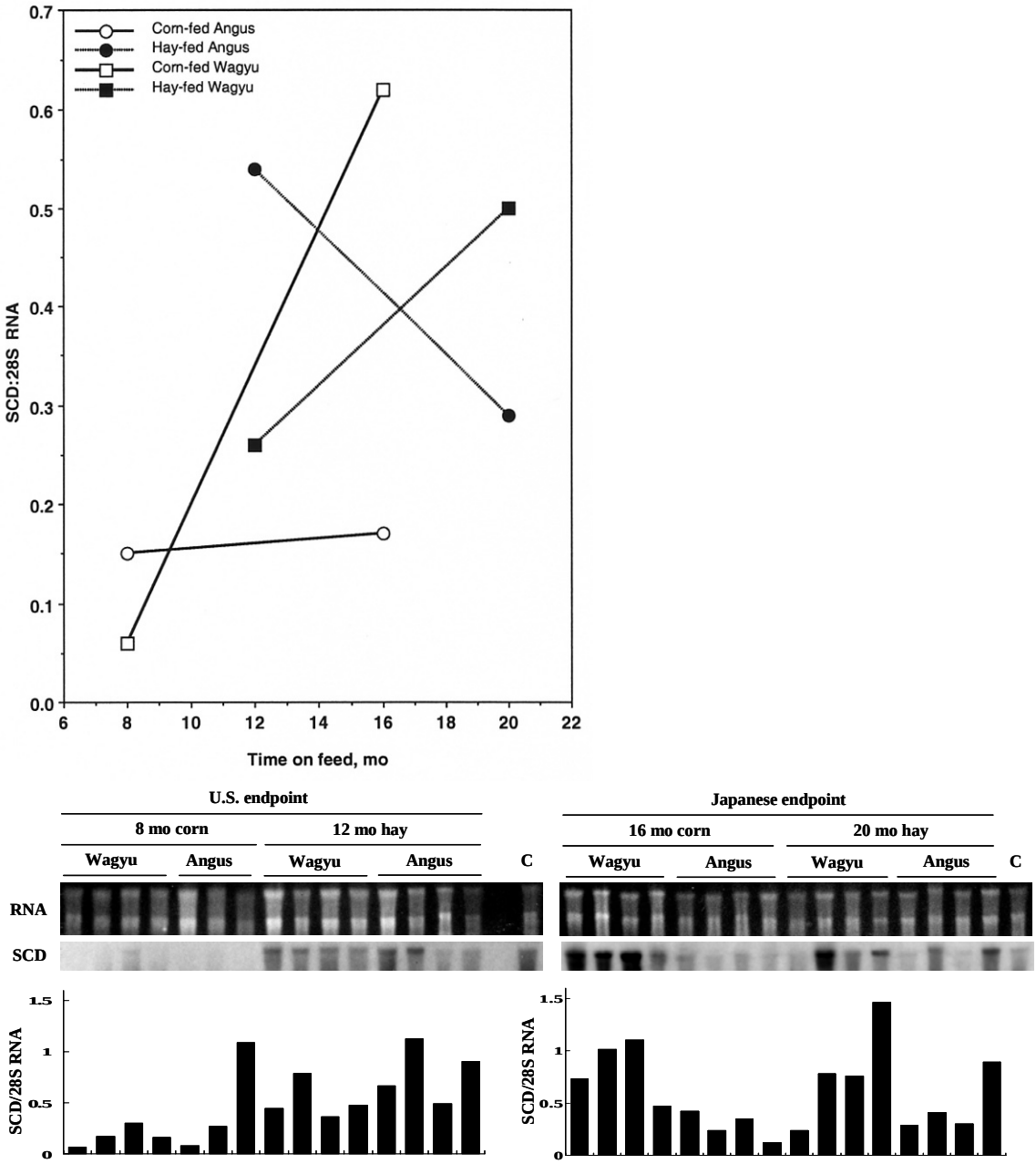


Figure 5.4 Changes in stearoyl-CoA desaturase (SCD) gene expression with time on feed in s.c. adipose tissue of Angus and Wagyu steers fed either a corn-based diet or a hay-based diet. There tended to be significant diet ($P = 0.06$) and endpoint ($P = 0.07$) effects, and there were significant diet by endpoint ($P = 0.05$) and breed x endpoint ($P = 0.01$) effects. The increase in gene expression was greater in corn-fed steers, and desaturase gene expression increased over time only in Wagyu steers.

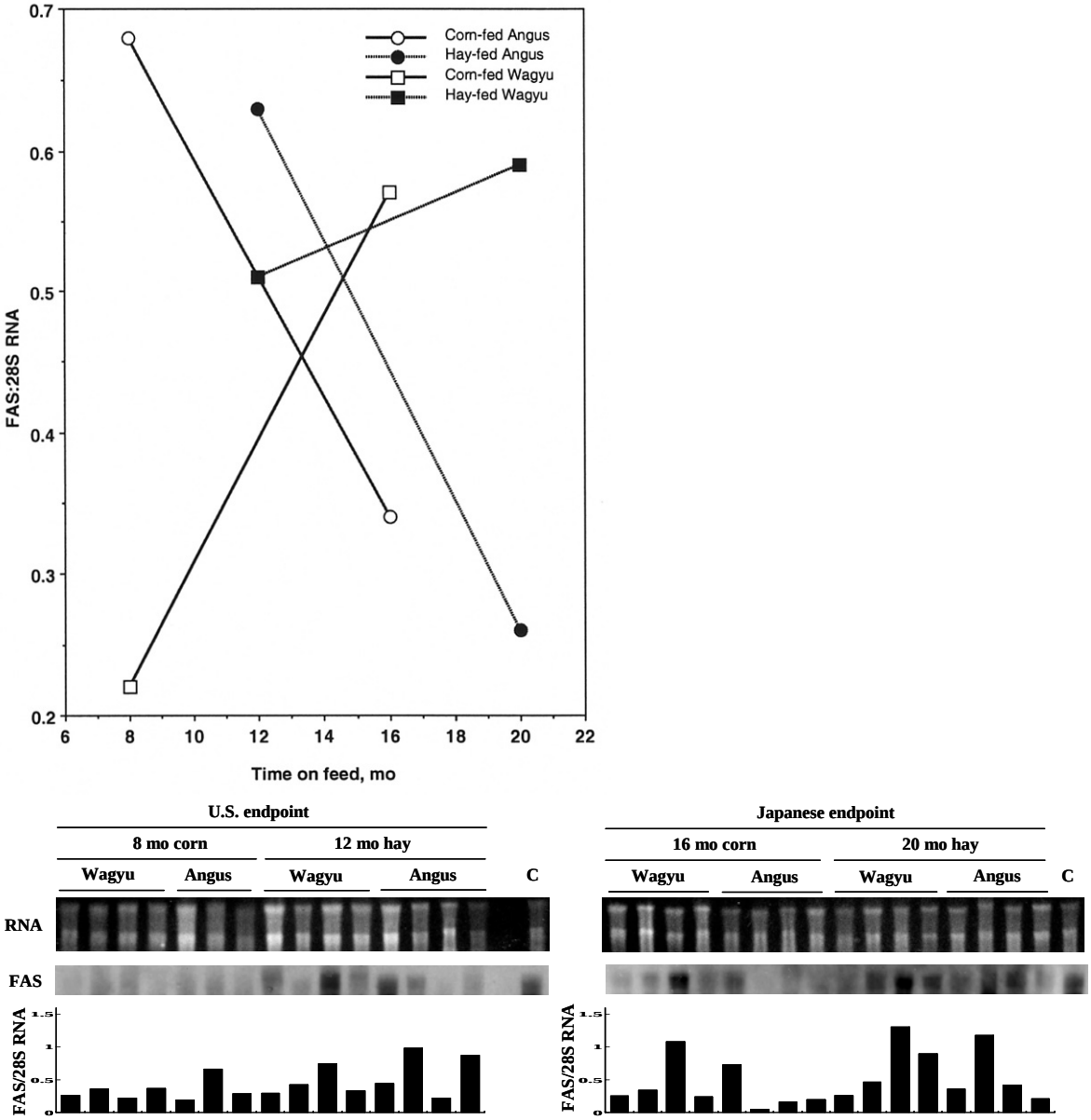


Figure 5.5 Changes in fatty acid syntathase (FAS) gene expression with time on feed in s.c. adipose tissue of Angus and Wagyu steers fed either a corn-based diet or a hay-based diet. The decrease in gene expression was greater in Angus steers, and FAS gene expression increased over time in Wagyu steers.

Discussion

Production data for this study have been reported previously (Lunt et al., 2005). This study was designed for the same end-point weights between diet groups; the high-energy diet (corn) for rapid growth rate and medium-energy diet (hay) for a slow growth rate. Four months between the corn (8 mo and 16 mo) and hay (12 mo and 20 mo) groups were predicted to produce similar end-points (525 kg and 650 kg). However, Wagyu steers had smaller weaning weights (169 vs 208 kg) and lower ADG than Angus steers for both diets (Lunt et al., 2005).

In spite of the differences in slaughter weight between breed types at each endpoint, the pattern of change in lipogenesis over time was not different between breed types. May et al. (1994) reported that there were no significant differences in lipogenesis in s.c. or i.m. adipose tissues between Angus and Wagyu steers. The current data show that time on feed and diet were major determinants for lipogenic activity in s.c. and i.m. adipose tissues from both breed types. It is apparent that, although 16-mo corn-fed steers exhibited a strong depression in lipogenic activity in s.c. and i.m. adipose tissues, long-term feeding did not affect lipogenic activity in adipose tissues from hay-fed steers. Smith et al. (1984) showed that, although not affected by diet, ^{14}C-acetate incorporation into fatty acids was constant until 8 mo on a corn-based finishing diet, and then markedly decreased with additional time on feed. Smith et al. (1984) also demonstrated that activities of lipogenic enzymes such as acetyl-CoA carboxylase, fatty acid synthetase, ATP-citrate lyase, and NADP-malate dehydrogenase declined after 8 mo on feed. The current data are similar in that, in both breed types, lipogenesis

significantly decreased after 8 mo on feed. Smith and Crouse (1984) reported that acetate provided 70-80% of the acetyl units to in vitro lipogenesis in s.c. adipose tissue, but only 10-25% in i.m. adipose tissue. This is consistent with the higher ^{14}C-acetate incorporation into lipids in s.c. adipose tissue than in i.m. adipose tissue for both diet groups.

Sturdivant et al. (1992) reported unusually high MUFA muscle and adipose tissues in Japanese Black cattle and suggested greater SCD enzyme activity in J. Black than in Angus cattle. However, Cameron et al. (1994) demonstrated that SCD mRNA and enzyme activity were not significantly different in s.c. adipose tissue between Angus and American Wagyu steers. This study observed similar results overall, but the breed x diet x endpoint interaction was significant. Unlike the results of Cameron et al. (1994), s.c. adipose tissue from hay-fed Wagyu steers had greater enzyme activity and gene expression by the Japanese endpoint. Desaturase gene expression generally increased over time in Wagyu adipose tissue, but decreased over time (hay-fed) or did not change (corn-fed) in Angus adipose tissue. Thus, at the time of peak SCD enzyme activity in Angus adipose tissue, SCD gene expression was on the wane. Similarly, desaturase activity did not increase over time in hay-fed Angus steers, in which there was a strong depression in SCD gene expression by the Japanese endpoint. In Wagyu steers, desaturase activity and gene expression both were elevated at the Japanese endpoint, suggesting that MUFA would increase indefinitely in this breed type.

Martin et al. (1999) found that increases in SCD mRNA concentration preceded an increase in lipogenesis and lipid accumulation in Angus s.c. adipose tissue. Alteration of SCD activity may regulate de novo fatty acid biosynthesis in adipose tissue and thus may regulate fattening of beef cattle. We previously published data for percentage i.m. lipid (Lunt et al., 2005) and fatty acid composition of s.c. adipose tissue (Chung et al., 2006). There is a strong, positive correlation between i.m. lipid and oleic acid (Figure 5.6), suggesting that lipid accumulation and SCD gene expression may be regulated in concert. Oleic acid is the major precursor for the synthesis of various lipid forms, including triaylglycerol (TAG), phospholipids, cholesterol ester, and wax esters. Oleic acid, the major MUFA in animal adipose tissue synthesized by SCD, is the primary precursor for acyl-CoA:cholesterol acyltransferase in cholesterol ester biosynthesis and diacylglycerol acyltransferase in TAG synthesis (Miyazaki and Ntambi, 2003). Therefore, SCD gene expression may affect not only the fatty acid composition in tissues but also lipid metabolism in adipose tissue.

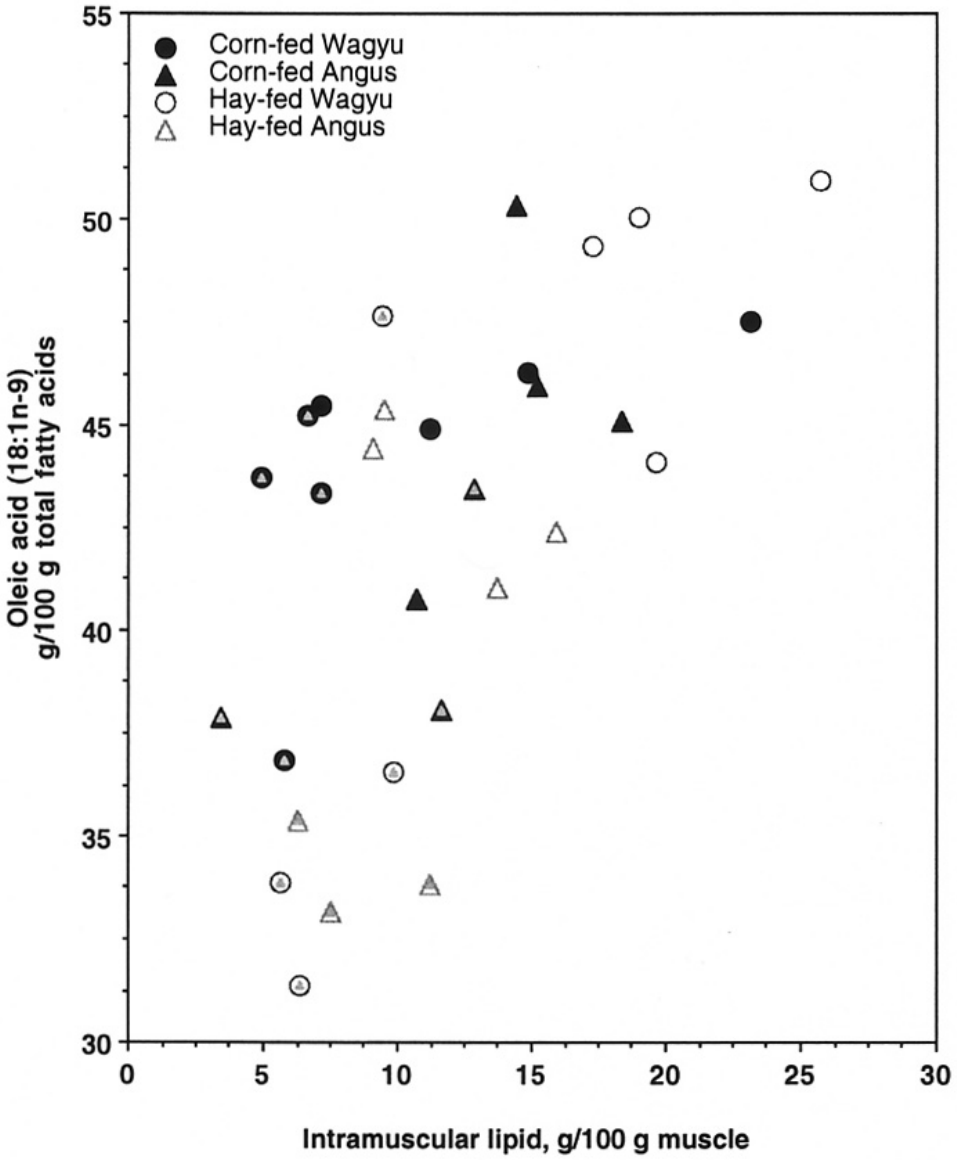


Figure 5.6 Relationship between oleic acid (18:1n-9) in s.c. adipose tissue and percentage intramuscular lipid in longissimus muscle from Wagyu and Angus steers fed corn-based or hay-based diets to U.S. or Japanese endpoints. Closed symbols, corn-fed steers; open symbols, hay-fed steers; circles, Wagyu steers; triangles, Angus steers. Symbols for the cattle raised to the U.S. endpoint contain shaded triangles. Overall: $y = 0.602x + 35.34$; $R^2 = 0.378$; $P < 0.01$.

Implications

This investigation provides additional evidence that not only difference of SCD genetic and enzyme activity were exist between Wagyu and Angus steers but also those tends to regulated by diet or time differences. It also seems that MUFA composition of adipose tissue from these breeds was controlled by time differences in enzyme activity. The greater rate of SCD gene expression and SCD enzyme activity of adipose tissue from 20 mo hay-fed Wagyu may explain the great ability of these steers to accumulate oleic acid in their adipose tissue.

CHAPTER VI

TRANS-10, CIS-12 CONJUGATED LINOLEIC ACID DOWN-REGULATES ARGININE-PROMOTED DIFFERENTIATION OF BOVINE PREADIPOCYTE

Overview

Trans-10, *cis*-12 CLA, synthesized by rumen microbes of ruminants, has been reported as a potent inhibitor of adipocyte differentiation in many species. Objectives of this study were to establish an in vitro culture system for preadipocyte derived from bovine adipose tissue and to document the interaction between arginine and CLA in the regulation of adipocyte differentiation. We hypothesized that arginine would up-regulate differentiation of bovine preadipocyte via PPARγ, and CLA would antagonize this effect. Bovine stromal-vascular cells were collagenase-liberated from perirenal adipose tissue of 16-mo corn-fed Angus steers. Extracted RNA from the cells was hybridized to antisense RNA probes. PPARγ, SCD, LPL, TNFα and Pref-1, but not C/EBPβ, exhibited greater expression in 5 mM arginine-treated than in non-treated, differentiated preadipocytes. CLA (40 μM) strongly decreased SCD and PPARγ expression, even in the presence of 5 mM arginine. It can be concluded that arginine up-regulates bovine preadipocyte differentiation, and CLA antagonizes this effect.

Introduction

Metabolic syndrome, highly correlated with adiposity of animal models, is accompanied by impaired glucose regulation/insulin resistance and abdominal obesity (Moller et al., 2005). Vidal et al. (2001) reported that lipoprotein lipase, glycogen synthase, leptin, PPARγ, and GLUT4 gene expression, and insulin sensitivity are greater

in human s.c adipose tissue than omental in adipose tissue. However, tumor necrosis factor alpha (TNFα**)**, which induces dedifferentiation and apoptosis of human adipocytes and decreases the expression of PPARγ, is expressed at greater levels in omental adipose tissue than in s.c. adipose tissue. Cattle exhibit several aspects of metabolic syndrome (McCann and Reimers, 1986; Matsuzaki et al., 1997), and have distinct metabolic differences across adipose tissue depots (May et al., 1994; Landis et al., 2002). We predicted that bovine preadipocytes may serve as a useful model for the study of metabolic syndrome.

Dietary arginine reduces serum glucose and increases insulin sensitivity in rats (Wu et al., 1999). Nitric oxide (NO) is synthesized by a family of NO synthases (NOS) that use L-arginine as a substrate in almost all mammalian cells (Kapur et al., 2000). Fu et al. (2005) reported that dietary arginine supplementation not only reduced adiposity in zuker diabetic fat rats (ZDF) but also increased the expression of several genes that would depress adiposity in rat epididymal adipose tissue.

CLA, naturally synthesized by the rumen microbes, reduces adiposity of rats, mice, and chickens, and increases lean body mass (Park et al., 1997). CLA depresses preadipocyte proliferation and [^{3}H]thymidine incorporation into DNA in 3T3-L1 preadipocytes (Satory and Smith, 1999) and porcine adipose tissue explants (Adams et al., 2005). Moreover, t10,c12 CLA prevents lipid filling of preadipocytes by decreasing PPARγ gene expression (Granlund et al., 2003; Kang et al., 2003). The c9,t11 and t10,c12 CLA isomers are structurally similar to oleic acid, and both depress NO synthesis and iNOS gene expression in endothelial cells (Eder et al., 2003). This study

was demonstrated that arginine stimulates and t10,c12 CLA antagonizes gene expression in bovine perirenal preadipocytes. This is the first demonstration of the interaction between arginine and CLA for any cell line.

Materials and Methods

Primary Cell Culture

Perirenal adipose tissue was collected from 16-mo-old corn-fed Angus steers. The collagenase-digested adipose tissue was filtered through a 250-μm nylon membrane, and the cell suspension centrifuged at 2000 x g. After discarding the supernate, the pellet was washed three times in Dulbecco's modified Eagle's Medium (DMEM). The pellet, containing stromal cascular (s.v.) cells, was resuspended and maintained in growth media composed of DMEM, 5% fetal bovine serum, and antibiotics, 95:5 O_2:CO_2 at 37^oC under a humidified atmosphere of 95% O_2 and 5% CO_2. Upon reaching confluence, the growth medium was replaced by a differentiation medium composed of DMEM and 10 μg/mL insulin, 10 μg/mL holo-transferrin, 1.0 μg/mL dexamethasone, 5 μM pioglitazone, and antibiotics (Suryawan and Hu, 1997; Ohyama et al., 1998). Preadipocytes were treated with combinations of t10, c12 CLA (5-100 μM) and arginine (5 mM) during differentiation.

In Vitro Lipogenesis

Lipogenesis was measured in primary cells after 72 h incubation with combinations of CLA and arginine. The cells were trypsinized with 0.25% trypsin-EDTA then transfer to 50-mL glass vials. Acetate incorporation into neutral lipids was measured by incubating adipocytes in Krebs-Henseleit bicarbonate buffer (pH 7.4), 5

mM sodium acetate, 5 mM glucose, 6 mM hepes buffer, and 1 μCi [U-^{14}C]acetate (Amersham, Arlington Heights, IL). The vials were gassed for 1 min with 95:5 O_2:CO_2 and capped and incubated in a shaking water bath (37°C) for 2 h. The reactions were terminated by adding 3 mL of 5% trichloroacetic acid to each vial. Lipids were extracted according to published procedures (Folch et al., 1957; May et al., 1994). The samples were then resuspended with 10 mL of scintillation cocktail and counted on a Beckman LS-3800 scintillation counter (Beckman Instruments, Palo Alto, CA).

Fatty Acid Composition

Fatty acid methyl esters (FAME) were produced from adipose tissue lipids as described (Folch et al., 1957; Morrison et al., 1964). FAME was analyzed with a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 autosampler, Varian Inc., Walnut Creek, CA). Separation of FAME was accomplished on a fused silica capillary column CP-Sil88 [100 m x 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands), with helium as the carrier gas (flow rate 2.1 mL/min). Total run time was 48 min. Injector and detector temperatures were at 270 and 300°C, respectively. Individual FAME were quantified as a percentage of total FAME identified.

Northern Blot Analysis

cDNA fragments of bovine SCD1, PPARγ, C/EBPβ, LPL, TNFα, Pref-1 and FAS gene were cloned into the vector of pCRII-TOPO (Invitrogen) using RT-PCR and conventional DNA techniques. The primers used for RT-PCR are shown in Table 6.1. The cDNA inserts were confirmed by sequencing. The digoxigenin (DIG)-labeled antisense RNA probes were generated with DIG RNA Labelling Kit (SP6/T7) (Roche

Diagnostics, Mannheim, Germany). Total RNA samples (2 μg) were separated in 1% agarose gels containing 6.7% formaldehyde and transferred to a Hybond N.+ membrane (Amersham Biosciences, NJ, USA). The membrane was hybridized with the DIG-labeled RNA probes. The bands corresponding to all genes mRNA was detected using DIG Luminescent Detection Kit for Nucleic Acids (Roche Diagnostics). Northern blotting was analyzed by ImageQuant, version 5.2 software (Molecular Dynamics). The gene expression bands vs 28S ribosomal RNA were analyzed for qualifying and quantifying the detected bands.

Results

CLA, Arginine, and Gene Expression.

Pilot studies determined the optimum combinations of CLA (40 μM) and arginine (5 mM) to demonstrate their effects on differentiation and gene expression. The rate of ^{14}C-acetate incorporation into total lipids in differentiated preadipocytes was significantly decreased by t10,c12 CLA, but was unaffected by c9,t11CLA (Figure 6.1A). The monounsaturated:saturated fatty acid ratio also was significantly depressed in t10,c12 CLA-treated adipocytes but not in c9,t11 CLA-treated adipocytes (Figure 6.1B). Neither SCD nor fatty acid synthase (FAS) gene expression was measurable in confluent preadipocytes prior to differentiation (Figure 6.2). Upon addition of the PPARγ agonist pioglitazone and insulin, expression of these genes increased markedly.

c9,t11 CLA depressed SCD and FAS gene expression only at 100 μM. However, 20, 40, and 100 μM, 10,c12 CLA measurably depressed SCD and FAS gene expression.

The expression levels of SCD, PPARγ, and lipoprotein lipase (LPL) were two-fold greater in preadipocytes incubated with 5 mM arginine than in control cultures (Figure 6.3A). Relatively smaller effects were seen for TNFα and Pref-1, and no effect of arginine was seen C/EBPβ. SCD gene expression was very high in control samples and may not have been stimulated by arginine in this experiment (Figure 6.3B). However, 40 μM t10,c12 CLA virtually ablated SCD gene expression, and this effect was not overcome by co-incubating with arginine. CLA had no effect on PPARγ gene expression, but it eliminated the arginine-induced stimulation of PPARγ gene expression elicited by arginine. Morphological differences were not observed between control and arginine-treated adipocytes. However, CLA-treated adipocytes contained only relatively small numbers of lipid vacuoles and smaller diameters of lipid vacuoles, even in the presence of arginine (Figure 6.4).

Table 6.1 Primers used in Northern analysis

Primer name (Bovine)	Primer size	Primer sequences	Accession Number
C/EBPβ	1047 bp	Forward: 5'- CTCCGACCTCTTCTCCGACGA-3' Reverse: 5'- GCTGTGCTTGTCCACCGTCT-3'	NM_176788
FAS	1190 bp	Forward: 5'- CAGCACAGCCTACTACGCGC-3' Reverse: 5'- GACGCTTCTCACATATGCGC-3'	AF285607
LPL	717 bp	Forward: 5'- CATTCCTGGAGTGACCGAATC-3' Reverse: 5'- TCTTTGGAATTGCACCGGTA-3'	M16966
PPARγ	913 bp	Forward: 5'-CAGCATTTCCACTCCGCACTA-3' Reverse: 5'- GAATCCTTGGCCCTCGGATAT-3'	NM_181024
Pref1	427 bp	Forward: 5'- GAAAATGGATTCTGCGACGAT-3' Reverse: 5'- ATGCAGCTGTTGGTCACGATC-3'	AB009278
SCD1	662 bp	Forward: 5'-CCTGTGGAGTCACCGAACC-3' Reverse: 5'-CCTTGGATACTTTCTTCCGGTC-3'	AB075020
TNFα	673 bp	Forward: 5'- CACCAAAAGCATGATCCGG-3' Reverse: 5'- CTGCCCAGACTCGGCATA-3'	AF348421

C/EBPβ, CCAAT/enhancer binding protein β; FAS, fatty acid synthetase; LPL, lipoprotein lipase; PPARγ, peroxisome proliferator activated receptor γ; Pref1, preadipose factor 1; SCD1, stearoyl coenzyme A desaturase 1; TNFα, tumor necrosis factor α.

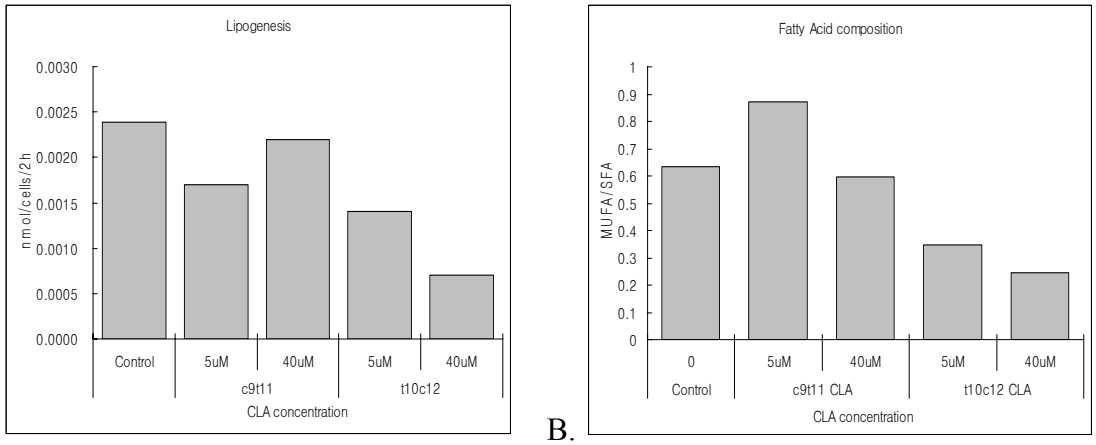


Figure 6.1 Lipogenesis and fatty acid composition of CLA-treated bovine adipocytes. (A) ^{14}C-acetate incorporation into preadipocytes treated with c9,t11 or t10,c12 CLA (5 or 40 μM). (B) monounsaturated:saturated fatty acid (MUFA/SFA) ratio of lipids from bovine adipocytes treated with c9,t11 or t10,c12 CLA (5 or 40 μM)

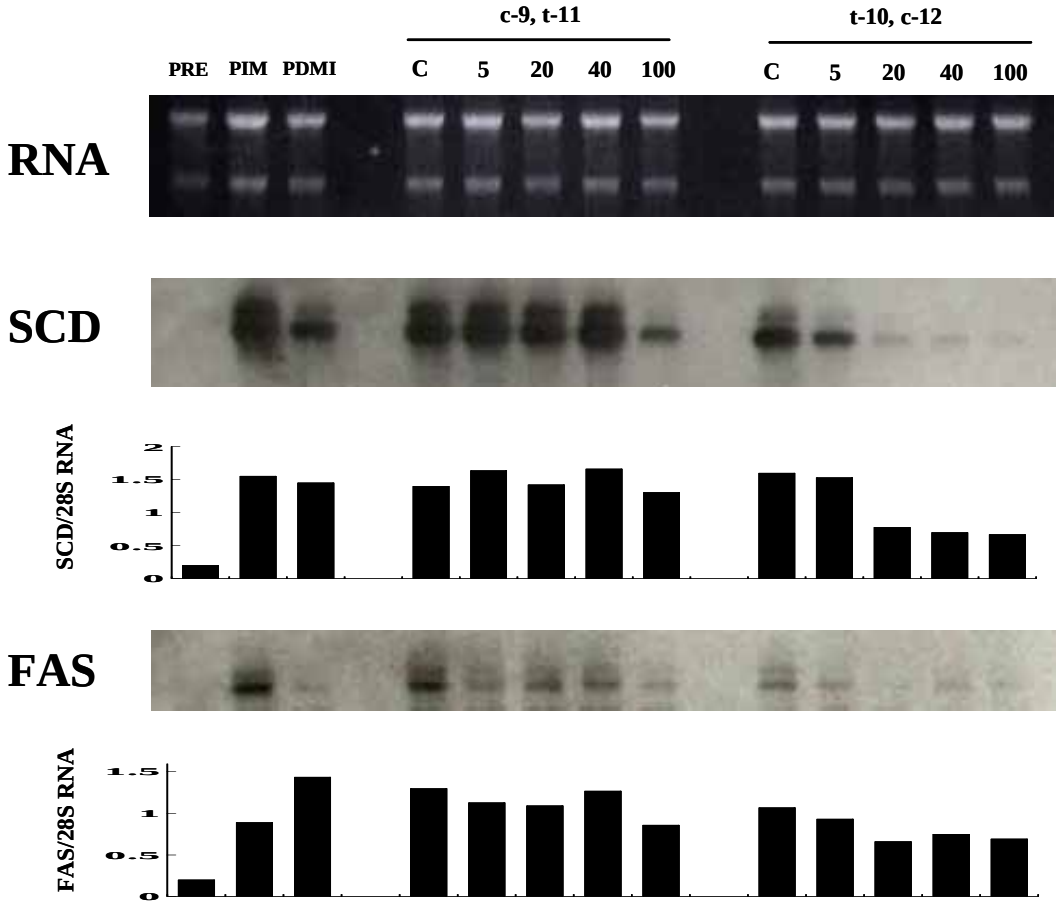
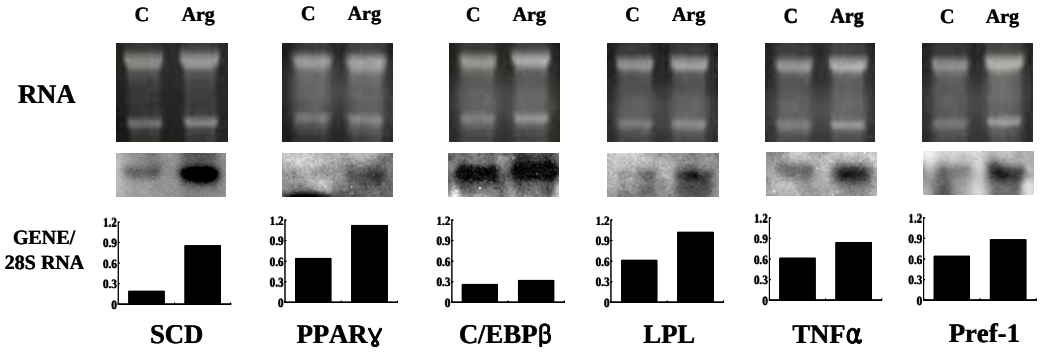


Figure 6.2 SCD and FAS gene expression at different concentrations of cis-9, trans-11 (c-9, t-11) and trans-10, cis-12 (t-10, c-12) CLA. C = no added CLA. RNA from differentiated bovine preadipocytes was used as the template for northern blotting

A.



B.

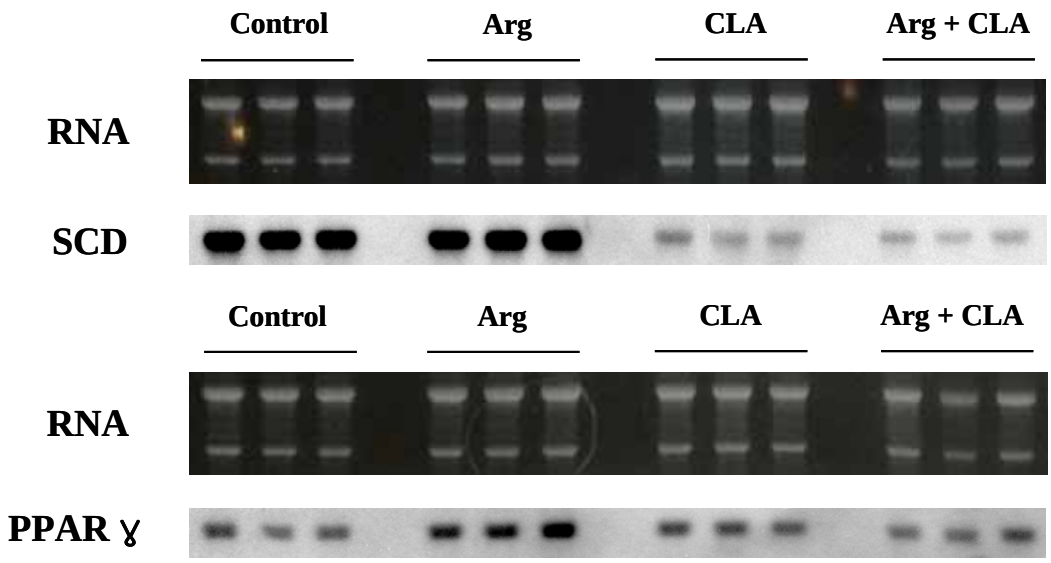


Figure 6.3 Expression of adipogenic and transcriptional factors in CLA and arginine-treated bovine adipocytes. (A) Northern analysis of bovine preadipocytes treated with arginine during differentiation. (B) SCD and PPARγ gene expression of bovine preadipocytes treated with combinations of 5 mM arginine and 40 μM trans-10, cis-12 CLA

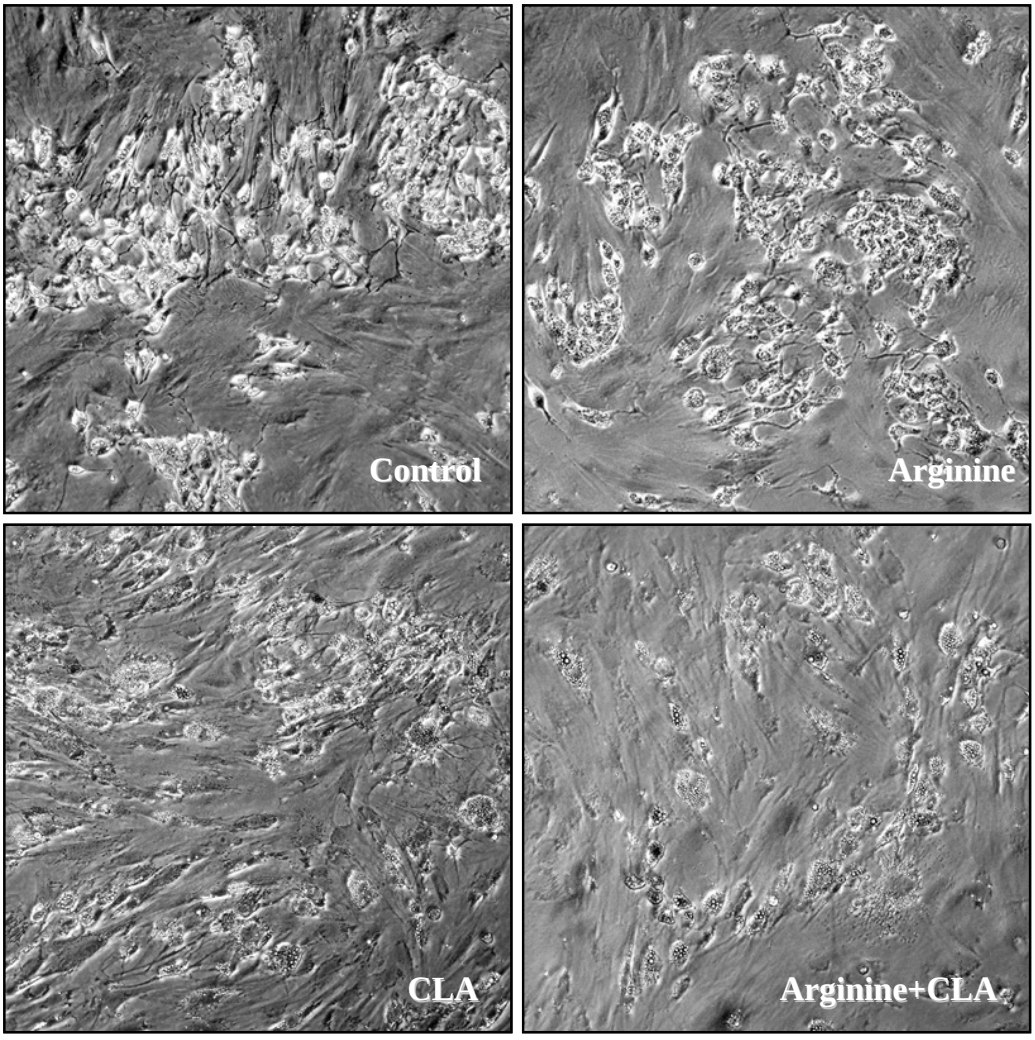


Figure 6.4 Morphological differences among preadipocytes treated with 5 mM arginine, 40 μM trans-10, cis-12 CLA, or the combination of arginine plus CLA

Discussion

After confirming that t10,c12 CLA depressed adipogenic gene expression in bovine s.v. cells, we determined the effects of including arginine during differentiation. We had anticipated that arginine would depress the expression of adipogenic gene based on the in vivo study by Fu et al. (2005). However, our data confirm the findings of Yan

et al. (2002), who demonstrated a similar effect in 3T3-L1 preadipocytes. These results indicate clearly that arginine stimulates bovine preadipocyte differentiation, at least in part by its marked effects on PPARγ. These data suggest that t10,c12 CLA decreases NO production from arginine in vitro, thereby eliminating the effects of arginine on bovine preadipocyte differentiation. Other studies have shown that t10,c12 CLA depresses PPARγ gene expression in preadipocytes (Granlund et al., 2003; Kang et al.,2003; Brown et al., 2003). It remains to be demonstrated whether or not dietary arginine and/or an endogenous increase in NO production in bovine adipose tissue would increase adiposity. These results would be opposite to the effects observed in rats (Fu et al., 2005; Khedara et al., 1999). It has been proposed that, with the onset of obesity, adipose tissue may become anoxic, leading to the production of cytokines (Moller et al., 2005). As fat mass increases with age, limited circulation within fat depots, coupled with marked increases in adipocyte size, potentially leads to anoxia of the adipocytes. This promotes the production of cytokines such as TNFα, which may in turn induce iNOS gene expression and NO production. As our preadipocyte incubations with arginine suggest, this would stimulate adipogenic gene expression. In this case, we suggest that NO exacerbates the obese state, leading to further increases in cytokines. Thus, t10,c12 CLA may be especially effective in reducing adiposity in individuals with metabolic syndrome.

CHAPTER VII

CONCLUSION

This investigation demonstrated that increasing glucose uptake by feeding a corn-based diet would increase marbling score relative to a hay-based diet in both breeds, although differences between breeds may not become evident until the cattle are fed to a greater physiological maturity. We further conclude that Wagyu steers should be fed a hay-based diet for a relatively lengthy feeding period in order to reach their genetic potential to deposit maximum levels of marbling. The results obtained in the current study also reject our hypothesis that fatty acid composition of Wagyu and Angus adipose tissue lipids would be similar when the cattle were fed to the U.S. endpoint. However, Wagyu adipose tissue consistently contained higher concentrations of oleic acid and other MUFAs, regardless of diet or endpoint. The reduction in digesta stearic acid in corn-fed steers raised to the Japanese endpoint may have contributed to the lesser amounts of stearic acid, hence lower slip points, observed in the adipose tissue of these animals. There is wide variation across contries in the fatty acid composition of adipose tissue from grain-fed cattle, which influences the hardness of the fat in their feed. In the U.S., adipose tissue accumulates monounsaturase fatty acids, which coincides with an increase in stearoyl-CoA desaturase gene expression and catalytic activity. Although trans-10, cis-12 CLA strongly depresses preadipocyte differentiation in primary cell cultures, its concentration in bovine adipose tissue probably is too low to depresses adiposity during the fattening period.

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