METABOLIC REGULATION OF CATTLE ADIPOSITY IN DIFFERENT BREED TYPES USING TWO DISPARATE DIETS

A Thesis

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

MASTER OF SCIENCE

August 2004

Major Subject: Nutrition

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Approved as to style and content by:

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ABSTRACT

Metabolic Regulation of Cattle Adiposity in Different Breed Types

Using Two Disparate Diets. (August 2004)

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Fifteen steers were used to evaluate the difference of diets (corn-based for 8 mo or hay-based for 12 mo) and breeds (Angus; n = 7 or Wagyu; n = 8) in a completely randomized design with 2 x 2 factorial arrangement of treatments to test the hypothesis that there are differences in fatty acid metabolism and cellularity in subcutaneous (s.c.) and intramuscular (i.m.) adipose tissue between these breeds types. Fat thickness, carcass weight, overall maturity, and yield grade of Angus steers were higher than those of Wagyu steers fed either corn (34%, 22%, 3%, and 8% higher, respectively) or hay diets (20%, 8%, 10%, and 8% higher, respectively) ($P < 0.03$). Moreover, marbling scores tended (P = 0.70) to be greater in Angus steers than in Wagyu steers fed either diet. Lipogenesis from acetate in both s.c and i.m. adipose tissue was higher in Wagyu steers (212.82 and 86.23 nmol/(10^5 cells per 2 h)) than in the Angus steers (86.23 and 29.66 nmol/(10^5 cells per 2 h)). Also, acetate incorporation into fatty acids was greater in s.c. adipose tissue than in i.m. adipose tissue ($P < 0.05$). Subcutaneous adipose tissue stearoyl-CoA desaturase (SCD) activity was significantly greater in corn-fed steers and than in hay-fed steers ($P < 0.05$), but there was no difference in SCD activity between Angus and Wagyu steers ($P > 0.05$). Adipocyte cellularity data demonstrated that both

breeds have more cells per gram adipose tissue and smaller cell volumes in i.m. adipose tissue than in s.c. adipose tissue. In s.c. adipose tissue, saturated fatty acids tended to be lower in corn-fed Angus and Wagyu steers than in hay-fed steers ($P < 0.06$). Similarly, monounsaturated fatty acids were higher in corn-fed Wagyu and Angus steers than in hay-fed Wagyu and Angus steers ($P < 0.01$). Slip point was positively correlated with percentage stearic acid in corn-fed and hay-fed steers, and there was a negative correlation between slip point and the SCD index. These data demonstrated that corn-based diets provide not only increased contents of monounsaturated fatty acid in Angus and Wagyu adipose tissue but also increased lipogenic activity.

DEDICATION

This Thesis is dedicated to my daughter, Eunice, who changed my life.

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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 monounsaturated fatty acid (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 and conjugated linolenic acid (CLA) composition in adipose tissue. CLA was shown to inhibit overall lipid development and

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deposition and enhance lean development in mice and pigs (Dugan et al., 1997). The *trans*-10, *cis*-12 isomer of CLA reduced lipoprotein lipase activity and triacylglycerol concentration in 3T3-L1 preadipocytes (Park et al., 1999). The stearoyl coenzyme-A desaturase (SCD) gene is a marker of the terminal differentiation of preadipocytes, which is mediated by a cAMP response element and is down-regulated by CLA. CLA may modify plasma membrane fatty acid composition by inhibiting SCD activity (Casimir et al., 1996; Lee et al., 1998; Smith et al., 2002). 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. 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 high and low energy diets on adiposity, metabolism, and fatty acid composition of adipose tissues of Angus and Wagyu steers. This study demonstrated that SCD activity is regulated by dietary differences, which may in turn regulate lipid accumulation in adipose tissue.

CHAPTER II

REVIEW OF LITERATURE

Adipose Tissue Development

After an oocyte has been fertilized, the cytoplasm of one cell is divided into smaller cells known as blastomeres. During the process, the cells become the ectoderm, endoderm, and mesoderm. These primary germ layers give rise to all organ systems. The ectoderm gives rise to hair, skin, nervous system, ears, and eyes. The endoderm gives rise to the respiratory, digestive and urinary tracts. The mesoderm gives rise to connective tissue, bones, muscle, blood and blood vessels, lymphatics, and adipose tissue (Campbell, 1993). The mesodermal cells differentiate into fibroblasts, some of which may eventually differentiate into connective tissue or adipose tissue. The preadipocytes closest to the blood vessels are filled first and deposition extends outward (Cornelius et al., 1994). Hausman et al. (1980) maintains that this pattern of lipid filling is a matter of physiological convenience. That is, the transport of lipoprotein lipase is shortest to those cells situated next to blood vessels. In addition to these findings, lipid deposition has been found to occur as cell morphology changes. Napolitano and Gagne (1963) reported an electron microscope study on lipid-depleted white adipose cells of fat pads from newborn to 9-d-old rats. They found that lipid accumulation occurred as the number of mitochondria increased and the number of endoplasmic reticuli and Golgi apparati decreased. As adipose cell development continues, more lipid-accumulating cells are recruited from a pool of fibroblasts that are not necessarily destined to become any particular type of cell. Prenatal adipose tissue growth and development is based on

concomitant lipid accretion and cell morphological changes and recruitment of new lobules. The factors that affect recruitment and differentiation of new lobules are an area of aggressively pursued research. It has been determined that adipocyte proliferation and differentiation originates from the stromal vascular fraction of adipose tissue (Van, 1985). However, very little is known about that factors that determine the physiologic destiny of a fibroblast.

In humans, preadipocytes begin to differentiate into adipose tissue during late embryonic development, with a majority of the differentiation occurring shortly after birth (Burdi et al., 1985). This enables the newborn to cope more efficiently with intervals between nutrient intake (MacDougald and Lane, 1995). Rat and mouse preadipocytes do not begin conversion into adipose tissue until after birth (Ailhoud et al., 1992). All species have the ability to differentiate preadipocytes throughout their life spans in response to body fat storage demands.

There are many markers expressed during preadipocyte differentiation, such as glycerol-3-phosphate dehydrogenase, acetyl coenzyme A carboxylase, fatty acid synthatase, phospholipase A_2, adipocyte-lipid binding protein, phosphoenolpyruvate carboxylase, and adipsin (Spiegelman et al., 1983; Butterwith, 1994). Many of the factors involved in preadipocyte proliferation and differentiation have been identified. However, the factors involved in recruitment of fibroblasts into the adipocyte lineage remain to be elucidated.

Postnatal Adipose Tissue Development and Deposition

The difference in characteristics and distribution of adipose tissue among anatomical locations is important in meat animal development (Anderson et al., 1972; Allen, 1976). Buttery et al. (1986) described the fat deposition pattern and cellular characteristics in selected depots of Fresian cattle. From approximately 200 to 600 d of age, perirenal adipose tissue had the highest cell volume and fewest adipocytes per gram in comparison to s.c. and intermuscular depots. The increase in adipose tissue mass at the perirenal depot over the 400-d period was caused by cell hypertrophy. These data also indicated that the perirenal depot was one of the earlier depots that became increasingly more inactive as lipids were deposited elsewhere.

Many researchers have studied cell size in regional adipose tissues. Smith and Crouse (1984) reported that s.c. adipose tissue has fewer cells per gram, larger mean cell diameter, and higher mean cell volume than i.m. adipose tissue. Some researchers (Anderson et al., 1972; Hood and Allen, 1973) have suggested that adipocyte number in meat animals is fixed. However, Bergen (1974) proposed that fat accretion continued to occur after protein accretion attained a plateau. In fact, the fat accretion curve climbed steadily. If the adipocyte number was fixed, the fat accretion curve should have reached a plateau. Allen (1976) concluded that a large number of preadipocytes are formed initially, and that hyperplasia can occur at any time.

There is other research that compares breed differences in fat distribution. It was found that Friesian cattle had less s.c. fat than Angus cattle. Charles and Johnson (1976) evaluated steer carcasses of Hereford, Angus, Friesian, and Charolais crossbred cattle.

Hereford carcasses had the highest amount of s.c. fat, followed by Angus, Friesian, and Charolais crossbreds, respectively. However, Charolais crossbreds had the highest percentage of i.m. fat, followed by Friesian, Angus, and Hereford breeds, respectively.

Age is another factor influencing fat deposition and development. There was a substantial increase in cell number and little increase in cell volume within the first 100 d of life in Fresian cattle (Buttery et al., 1986). Conversely, there was a substantial increase in cell volume and minimal increase in cell number between 200 and 400 d of age. Cianzio et al. (1985) found that from 11 to 19 mo of age, fat tissue developed primarily by hypertrophy. However, hyperplasia did occur in the i.m. fat depot from 11 to 15 mo. Hyperplasia also occurred in the brisket depot from 15 mo onward.

Hood and Allen (1973) noted that i.m. adipose tissue had a relatively high number of small adipocytes when compared to s.c. and perirenal adipose tissue. They concluded that the i.m. adipose depot was still maturing, and that hyperplasia was ongoing throughout their study. It is worth mentioning that bimodal distributions for cell diameter also have been noted in obese animals. Mersmann et al. (1975) compared adipose characteristics between fat and lean pigs. A bimodal distribution of cell diameter occurred in the fat pigs, but there was a unimodal distribution of cell diameter in the lean pigs. These findings support the conclusions that hyperplasia can occur at any time.

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.

Adipocyte Proliferation

In vivo study of preadipocyte differentiation is difficult. 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 in culture. 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 monounsaturated fatty acids (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.

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 saturated fatty acids (SFA). SCD 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 conjugated linoleic acid (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).

CHAPTER III

FATTY ACID COMPOSITION AND STEAROYL-CO A DESATURASE ACTIVITIES IN SUBCUTANEOUS ADIPOSE TISSUE ARE DIFFERENT BETWEEN WAGYU AND ANGUS STEERS FED A CORN-BASED FINISHING DIET OR A HAY-BASED FINISHING DIET

Overview

We hypothesized that there would be an interaction between a corn-based and a hay-based diet on the metabolism and deposition of s.c. adipose tissue of Wagyu and Angus steers. Greater stearoyl-CoA desaturase (SCD) enzyme activity in Wagyu adipose tissue may be the biochemical basis for this phenomenon. Eight Angus and eight Wagyu steers were fed a corn-based diet for 244 d or a hay-based diet for 368 d. Subcutaneous adipose tissue, plasma, and digesta samples were collected immediately postmortem. Slip points (a measure of melting point) and SCD enzyme activity were measured in s.c. adipose tissue samples, and fatty acid composition was measured in plasma, digesta, and s.c. adipose tissue. Although no difference was observed between breed types for s.c. adipose tissue fatty acid composition ($P > 0.05$), there was a significant difference between corn- and hay-fed steers. Stearic acid (18:0), and oleic acid (18:1n-9) were higher in s.c. adipose tissue and digesta of corn-fed Angus and Wagyu steers than in hay-fed steers. Palmitic acid (16:0) and palmitoleic acid (16:1n-7) were less in plasma and digesta, and greater in s.c. adipose tissue, of hay-fed Angus and Wagyu steers than in corn-fed steers. Subcutaneous adipose tissue from corn-fed Wagyu and Angus steers

contained more MUFA than hay-fed Wagyu and Angus steers ($P < 0.05$). There was high, negative correlation between the percentage 16:1n-7 and 18:0 ($r = 0.91$; $P < 0.05$) and between the SCD index and slip point ($r = 0.97$; $P < 0.01$). Wagyu steers exhibited both the highest and lowest 16:1n-7/18:0 ratios. Similarly, there was a positive correlation ($r = 0.91$; $P < 0.05$) between percentage 18:0 and slip points. Percentage 18:0 in adipose tissues appeared to have the greatest effect on slip point. Slip points and SCD activities in s.c. adipose tissue were not different between breed types ($P > 0.05$). Wagyu lipids were widely distributed containing the highest (44°C) and lowest (26°C) slip points. Angus slip points were between 37 and 44°C. These data indicated large variation in the Wagyu samples, and suggest that Wagyu adipose tissue of more mature cattle may exhibit a greater degree of unsaturation and SCD enzyme activity. Additionally, corn diets may cause an increase in SCD enzyme activity and a decrease in slip points.

Introduction

Meat from Japanese Black (Wagyu) cattle is characterized by a higher amount of monounsaturated fatty acid (MUFA) in adipose tissues than is observed in adipose tissue of breed types typically produced in the U.S. (Sturdivant et al.,1992; May et al., 1993). Therefore, Wagyu cattle may produce more palatable beef, because saturated fatty acid (SFA), especially myristic (14:0), palmitic (16:0), and margaric (17:0) acids are negatively correlated with juiceness and with "cowy" and "painty" taste characteristics (Camfield et al., 1997).

Stearoyl-CoA desaturase is an endoplasmic reticulum-anchored enzyme that catalyzes the Δ^9-*cis* desaturation of saturated fatty acyl-CoAs, the preferred substrates

being palmitoyl-CoA and stearoyl-CoA (Miyazaki and Ntambi, 2003). An earlier investigation (Cameron et al., 1994) indicated no difference in s.c. adipose tissue SCD enzyme activity or gene expression between Angus and American Wagyu cattle fed to a typical Japanese endpoint, in spite of significantly greater MUFA in Wagyu adipose tissue. We hypothesized that differences in fatty acid composition between Wagyu and Angus cattle may be been due to greater SCD activity earlier in production, which would result in increased MUFA deposition at some point before slaughter. Therefore, we compared Angus and Wagyu cattle fed a corn-based finishing diet and a hay-based finishing diet to a typical U. S. market weight. The goal was to achieve U. S. market weight (approximately 500 kg) at a sufficiently early age to observe differences in SCD enzyme activity.

Materials and Methods

Animals and Experimental Procedures

Eight Angus and eight American Wagyu steers were purchased as weanling calves and assigned to one of two dietary treatments: a high-energy, corn based finishing diet designed to provide maximal rates of marbling adipose tissue accretion; and a medium-energy, hay-based diet that provided submaximal rates of marbling adipose tissue accretion. The high-energy diet was suitable for the commercial production of Angus steers, which typically are slaughtered at 16 to 18 mo of age. The medium-energy diet was designed for the production of the Wagyu cattle, which do not express their ability to accumulate i.m. adipose tissue until after 20 mo of age (Table 3.1).

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 composition[de]				
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] Percentage of dry matter.
[e] Calculated values based on NRC (1996).

Cattle were slaughtered after 8 mo on the high-energy diet or 12 mo on the medium-energy diet in order to attain similar final weights for cattle in both treatment groups. One Angus steer escaped prior to slaughter and was excluded from the study.

Sample Collection

Plasma, digesta, s.c., and i.m. adipose tissues were collected from finished Angus and Wagyu steers. Steers were slaughtered at the Rosenthal Meat Science and Technology Center using standard industry techniques, and tissues were obtained immediately after removal of the hide (approximately 20 min post-exsanguination). The 5th and 8th thoracis rib section of the longissimus muscle was transported to the laboratory in oxygenated 1X Krebs-Henseleit bicarbonate buffer (KHB) (pH = 7.4) with 5 mM glucose at 37^{o}C. The muscle was transferred to the laboratory as soon as possible, and s.c. and i.m. adipose tissues were immediately dissected for analysis or storage. Adipose samples used for lipogenesis from acetate were used immediately. All other samples were processed or stored at –80^{o}C after quick-freezing in liquid nitrogen.

Fatty Acid Composition

Fatty acids were extracted by the methods of Folch et al. (1957) and methylated as described by Morrison and Smith (1964). Fatty acid metyl esters (FAME) were separated with a silica capillary column in Varian gas chromatography (model CP-3800 fixed with a CP-8200 autosampler, Varian Inc., Walnut Creek, CA). Helium was used as the carrier gas (1.2 mL/min). After 32 min at 180°C, oven temperature increased at 20°C per min to 225°C and held for 13.75 min. Total run time was 48 min. Injector and detector temperatures were at 270°C and 300°C, respectively. Standards from Nu-Chek Prep, Inc. (Elysian, MN) were used for identification of individual FAME. Individual FAME were identified as a percentage of total FAME analyzed.

Total Lipid Extraction

Total lipid was extracted by a modification of the methods of Folch et al. (1957). Approximately 1 g of s.c. adipose tissue, 1 mL of plasma, and 5 g of digesta were homogenized with 5 mL of chloroform:methanol (2:1, vol/vol) and held with shaking at room temperature (approximately 20^{o}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 had 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^{o}C under nitrogen.

Slip Point Determination

The melting point of the s.c. adipose tissue lipids was approximated by determining slip points (Smith et al., 1998). After heating to approximately 45^{o}C, the lipids were drawn 1 cm into glass capillary tubes. Duplicate tubes were collected for each sample and frozen at 4 ^{o}C. 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 ^{o}C/min. Stirring of the water bath was utilized to maintain uniform temperatures. Temperature of the water was monitored with a Type K thermocouple (model KTSS-HH, Omega Engineering, Inc., Stamford, CT) and attached to a digital thermometer (model 91100-50,Cole-Parmer Instrument Co., Vernon Hills, IL). Slip point was defined as the temperature at which the lipid moved up the capillary tube and reported as the mean between the duplicate tubes for each sample.

Microsome Extraction and Stearoyl-CoA Desaturase Activity

Subcutaneous and i.m. adipose tissues were 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 mM EDTA, and 1mM 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 mM Tris-HCL buffer (pH = 7.4), fast frozen with liquid nitrogen, and stored at – 80^{o}C until further analysis.

The activity of SCD enzyme activity was determined as described by St. John et al. (1991). The assay system was composed of 100 mM Tris-HCl (pH7.4), 2 mM NADPH 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 MeOH. 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 pmol palmitate converted to palmitoleate per 7 min per mg microsomal protein.

Statistical Analysis

All statistical analysis were performed by the GLM procedure of SAS version 8.1 (SAS Inst. Inc., Cary, NC). Means of SCD activities, cellularity, lipogenesis, and carcass characteristics from Wagyu (n = 8) and Angus (n = 7) steers were compared by two-factor ANOVA. Factor one was diet, and factor two was breed type. The diet x breed type interaction was evaluated. Interaction means were separated using the probability statement of GLM in the significant difference ($P < 0.05$). However, a tendency was indicated by $P < 0.10$.

Results

These data show that average daily gain was different between breed types within a dietary treatment group. Corn-fed Angus steers had greater growth rates than the other three groups (Figure 3.1). 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 and hay groups were predicted to produce similar end-points. However, Wagyu steers had relatively lower growth rates than Angus steers in both diet groups.

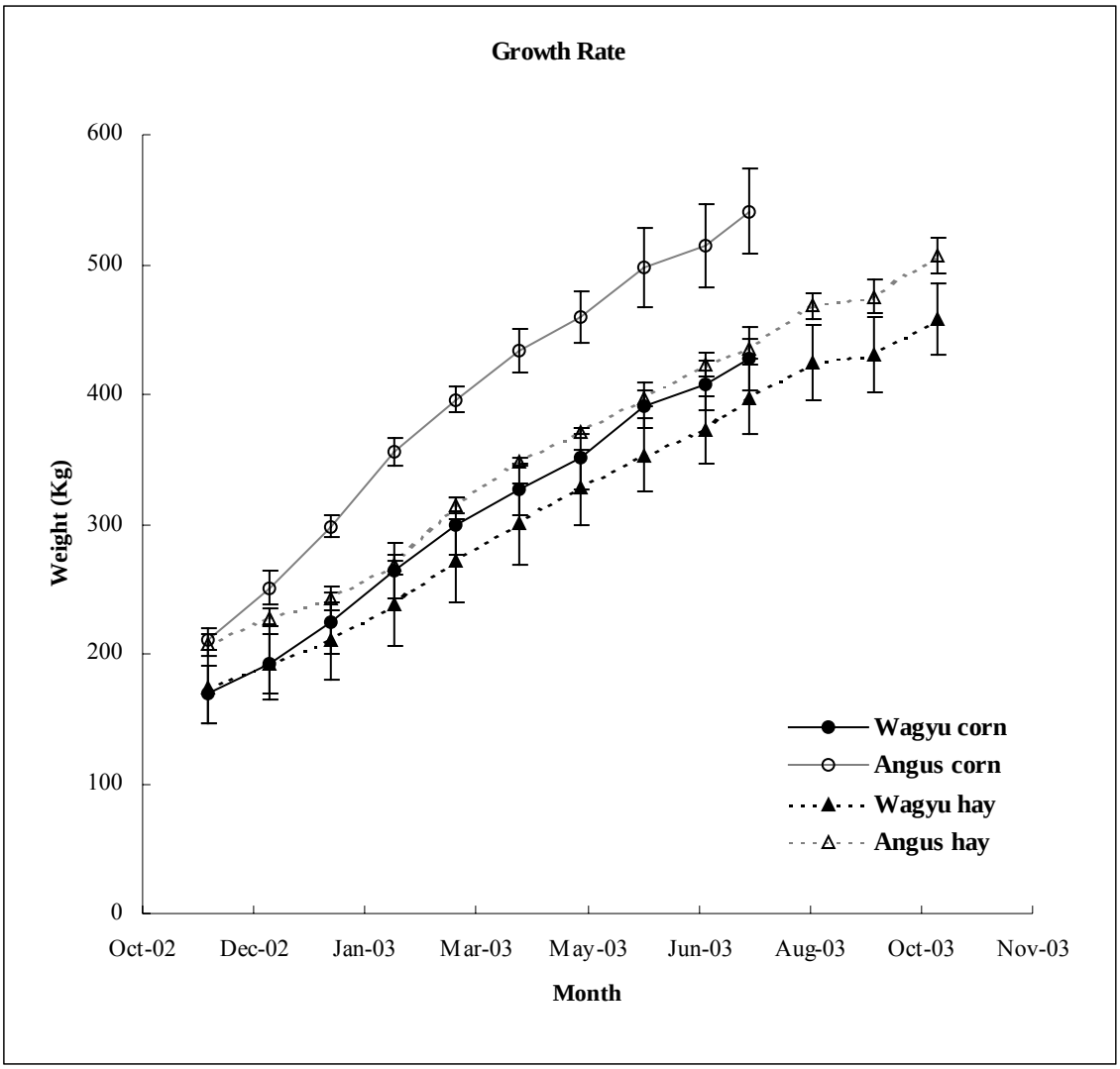

Figure 3.1 Growth rates of Wagyu and Angus steers fed high-corn or high-hay diets.

Slip points were numerically greater in Angus lipids than in Wagyu lipids, although there was no significant difference between breed types ($P = 0.30$). (Table 3.2). However, slip point vs 18:0 or slip point vs SCD index showed that Wagyu steers had considerably more variation than Angus steers (Figure 3.2, Figure 3.3).

Stearoyl-CoA desaturase activity (Table 3.2) was not affected by breed type. The corn-fed Angus (11.3 nmol/(7 min per mg of protein)) and Wagyu (14.5 nmol(7 min per mg of protein)) had higher SCD activities than hay-fed Angus (5.0 nmol/(7 min per mg of protein)) or Wagyu (3.1 nmol(7 min per mg of protein)) ($P < 0.01$, $P = 0.11$). Similar results were observed when rates were expressed per gram adipose tissue.

Table 3.2. Slip points and stearoyl-CoA desaturase activities in subcutaneous adipose tissues of Wagyu and Angus steers fed high-energy (corn) or medium-energy (hay) diets

	Diet/breed types							
	Corn		Hay			*P*-values		
Item	Angus	Wagyu	Angus	Wagyu	SE	Breed	Diet	BxD[x]
Slip point	37.9	35.3	42.8	38.9	5.7	0.3046	0.1791	0.8321
Stearoyl-CoA desaturase activity[a]								
nmol/(7 min per mg of protein)	11.3	14.5	5.0	3.1	4.9	0.6723	0.0044	0.3407
nmol/(7 min per g of adipose tissue)	21.7	28.1	18.6	10.8	9.4	0.9281	0.0500	0.1745

[a] nmol palmitoyl-CoA converted to palmitoleoyl-CoA per 7 min of incubation.
[x] Breed x diet interaction.

The fatty acid composition of s.c. adipose tissue (Table 3.3) showed that hay-fed Angus (52%) and Wagyu (48%) had a higher SFA composition than corn-fed Angus (45%) or Wagyu (42%) steers ($P < 0.06$). Corn-fed Angus (45%) and Wagyu (49%) had more MUFA than hay-fed Angus (35%) or Wagyu (38%) steers ($P < 0.01$). Total PUFA were higher in corn-fed (3.24%) and hay-fed (3.0%) Wagyu than in corn-fed (2.91%) or

hay-fed (2.29%) Angus steers ($P < 0.05$). The desaturase index, the ratio of MUFA:SFA, was not significantly different between diet ($P = 0.36$) or breed groups ($P = 0.18$). Among the SCD indices, only the 16:1n-7:16:0 index was different between diet groups ($P < 0.05$). The amount of vaccenic acid (18:1*trans*-11), the precursor of the CLA, 18:2*cis*-9, *trans*-11, was higher in the hay-fed steers than in the corn-fed steers ($P < 0.05$). However, there were no significant differences in the CLA between diet groups. There was a greater percentage SFA in plasma of steers fed hay than in those fed corn, but no difference in MUFA (Table 3.4). PUFA were greater in plasma of hay-fed steers. The fatty acids 16:0, 16:1n-7, 18:0, and 18:2n-6 were greater in plasma of hay-fed steers than in corn-fed steers. In digesta samples, the area percentage of fatty acids from the highest to the lowest was 18:0 (49.3% and 35.2%), 16:0 (17.2% and 24.8%), and 18:1n-9 (19.1% and 16.5%) in Wagyu and Angus steers, respectively (Table 3.5). There was a statistical significance in digesta for 16:0, 16:1n-7, 18:0, 18:3n-3, and total PUFA ($P < 0.05$). All of these fatty acids except 18:0 were higher in digesta of hay-fed steers than for corn-fed steers. Digesta of hay-fed steers contained less 18:0 than that of corn-fed steers.

There was a high correlation between percentages of 16:1n-7 and 18:0 in s.c. adipose tissue (Figure 3.2A). Wagyu s.c. adipose tissue contained both the highest and lowest of both fatty acids. Slip points were dictated primarily by the percentage of 18:0, so that, the greater the percentage of 18:0 in s.c. adipose tissue, the higher the slip point of lipids from that tissue (Figure 3.2B).

Table 3.3 Percentage of total fatty acids in subcutaneous adipose tissue of Wagyu and Angus steers fed high-energy (corn) or medium-energy (hay) diets

	Diet/breed types							
	Corn		Hay			*P*-values		
Item	Angus	Wagyu	Angus	Wagyu	SE	Breed	Diet	BxD[x]
14:0	3.63	2.83	3.45	2.88	0.68	0.0797	0.8729	0.7475
14:1n-5	1.10	1.35	0.80	0.98	0.65	0.4990	0.3320	0.9133
16:0	26.67	25.40	28.50	26.25	1.75	0.0610	0.1787	0.5999
16:1n-7	3.47	4.73	2.33	2.43	1.60	0.3779	0.0569	0.5011
17:0	0.47	0.56	0.42	0.47	0.12	0.3158	0.2756	0.7613
18:0	15.57	13.98	20.43	19.25	6.01	0.5890	0.1313	0.9481
18:1*trans*-11	2.00	1.55	2.44	2.43	0.42	0.2605	0.0107	0.3368
18:1n-9	41.13	43.05	32.10	34.45	4.18	0.2256	0.0019	0.9225
18:2n-6	3.13	2.30	1.58	2.05	0.70	0.8542	0.0378	0.0988
18:3n-3	0	0.09	0.23	0.30	0.05	0.0553	0.0001	0.6702
18:2*cis*-9,*trans*-11	0.29	0.42	0.38	0.39	0.16	0.4649	0.7675	0.4730
18:2*trans*-10,*cis*-12	0.04	0.24	0.12	0.25	0.15	0.0648	0.6064	0.6489
SFA	45.87	42.20	52.38	48.38	5.79	0.1791	0.0588	0.9569
MUFA	45.70	49.13	35.23	37.85	5.41	0.2049	0.0025	0.8896
PUFA	2.91	3.24	2.29	3.00	0.47	0.0414	0.1159	0.4597
MUFA:SFA	0.91	1.10	0.72	0.87	0.25	0.1861	0.1259	0.9087
Index[y]	0.84	1.04	0.65	0.80	0.25	0.1811	0.1105	0.8510
14:1n-5/14:0	0.29	0.45	0.23	0.35	0.18	0.1461	0.3753	0.8020
16:1n-7/16:0	0.13	0.18	0.08	0.09	0.06	0.2810	0.0436	0.4979
18:1n-9/18:0	2.66	3.87	1.59	2.32	1.39	0.1735	0.0925	0.7508
CLA index[z]	0.15	0.31	0.16	0.18	0.13	0.1881	0.3630	0.3276

[x] Breed x diet interaction

[y] Desaturation index = (14:1 + 16:1 + 18:1*cis*-9 +18:2*cis*-9,*trans*-11)/(14:0 + 16:0 + 17:0 + 18:0 + 18:1*trans*-11).

[z] CLA desaturation index = (18:2*cis*-9,*trans*-11)/(18:1*trans*-11).

Table 3.4 Percentage of total fatty acids in plasma of Wagyu and Angus steers fed high-energy (corn) or medium-energy (hay) diets

	Diet/breed types							
	Corn		Hay			*P*-values		
Item	Angus	Wagyu	Angus	Wagyu	SE	Breed	Diet	BxD[x]
14:0	0.10	0.20	0.96	0.50	0.31	0.1577	0.0050	0.1082
14:1n-5	0.20	0.30	0.50	0.28	0.48	0.7415	0.6252	0.5282
16:0	12.63	10.73	15.78	14.58	1.74	0.0740	0.0025	0.7033
16:1n-7	1.13	1.20	1.18	0.25	0.40	0.0637	0.0390	0.0383
18:0	17.27	18.45	22.18	21.90	1.32	0.8825	0.0001	0.3122
18:1n-9	12.43	12.33	14.15	12.43	2.69	0.4708	0.5565	0.5751
18:2n-6	34.73	42.33	44.63	48.88	6.58	0.1540	0.0372	0.6352
18:3n-3	0.37	0.35	0.48	0.88	0.64	0.5876	0.3352	0.5420
SFA	30.00	29.38	38.91	36.98	2.52	0.1703	0.0001	0.6278
MUFA	13.77	13.83	15.83	12.95	2.84	0.3126	0.7513	0.3423
PUFA	35.10	42.68	45.10	49.75	6.63	0.1438	0.0322	0.6797
MUFA:SFA	0.45	0.47	0.41	0.35	0.06	0.5638	0.0258	0.3192

[x] Breed x diet interaction.

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Table 3.5 Percentage of total fatty acids in digesta of Wagyu and Angus steers fed high-energy (corn) or low-energy (hay) diets

	Diet/breed types							
	Corn		Hay			*P*-values		
Item	Angus	Wagyu	Angus	Wagyu	SE	Breed	Diet	BxD[x]
14:0	0.63	0.63	0.95	0.95	0.43	0.9068	0.1799	0.9855
14:1n-5	0.33	0.95	0.88	0.73	0.36	0.3228	0.5084	0.0675
16:0	19.30	17.18	23.53	24.78	1.97	0.4836	0.0001	0.1281
16:1n-7	0.70	0.43	1.10	1.20	0.41	0.5951	0.0164	0.3981
18:0	57.33	49.28	42.83	35.15	11.48	0.2748	0.0352	0.9750
18:1n-9	15.00	19.05	11.83	16.50	5.61	0.1424	0.3507	0.9167
18:2n-6	4.80	6.28	9.63	13.28	5.62	0.4619	0.0644	0.7172
18:3n-3	0.00	0.25	0.78	1.00	0.25	0.1839	0.0001	0.9246
SFA	77.27	67.08	67.88	60.88	10.09	0.1736	0.1576	0.7266
MUFA	16.03	20.43	13.80	18.43	5.72	0.1431	0.4924	0.9694
PUFA	4.80	6.53	10.40	14.28	5.75	0.4368	0.0446	0.7261
MUFA:SFA	0.21	0.31	0.23	0.32	0.12	0.1586	0.8361	0.9753

[x] Breed x diet interaction.

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Hay-fed cattle had a greater percentage 18:0 in their adipose tissues and plasma, but a lower percentage 18:0 in their digesta (Tables 3.3 to 3.5). However, there was only a weak correlation between s.c. 18:0 and digesta 18:0 (Figure 3.2C), suggesting that digesta 18:0 was not the primary determinant of 18:0 (and therefore, slip points) in s.c. adipose tissue lipids.

The large variation in 18:0 in s.c. adipose tissue was consistent with the large variation in SCD activity (Figure 3.2D). Wagyu had the highest SCD activity and the least 18:0, but they also had the lowest SCD values and highest 18:0. Angus samples were intermediate in SCD activity.

The SCD index and SCD activity were negatively associated with slip points (Figures 3.2E and 3.2F). The weak association of SCD activity with slip points was due primarily to the variation in the SCD assay

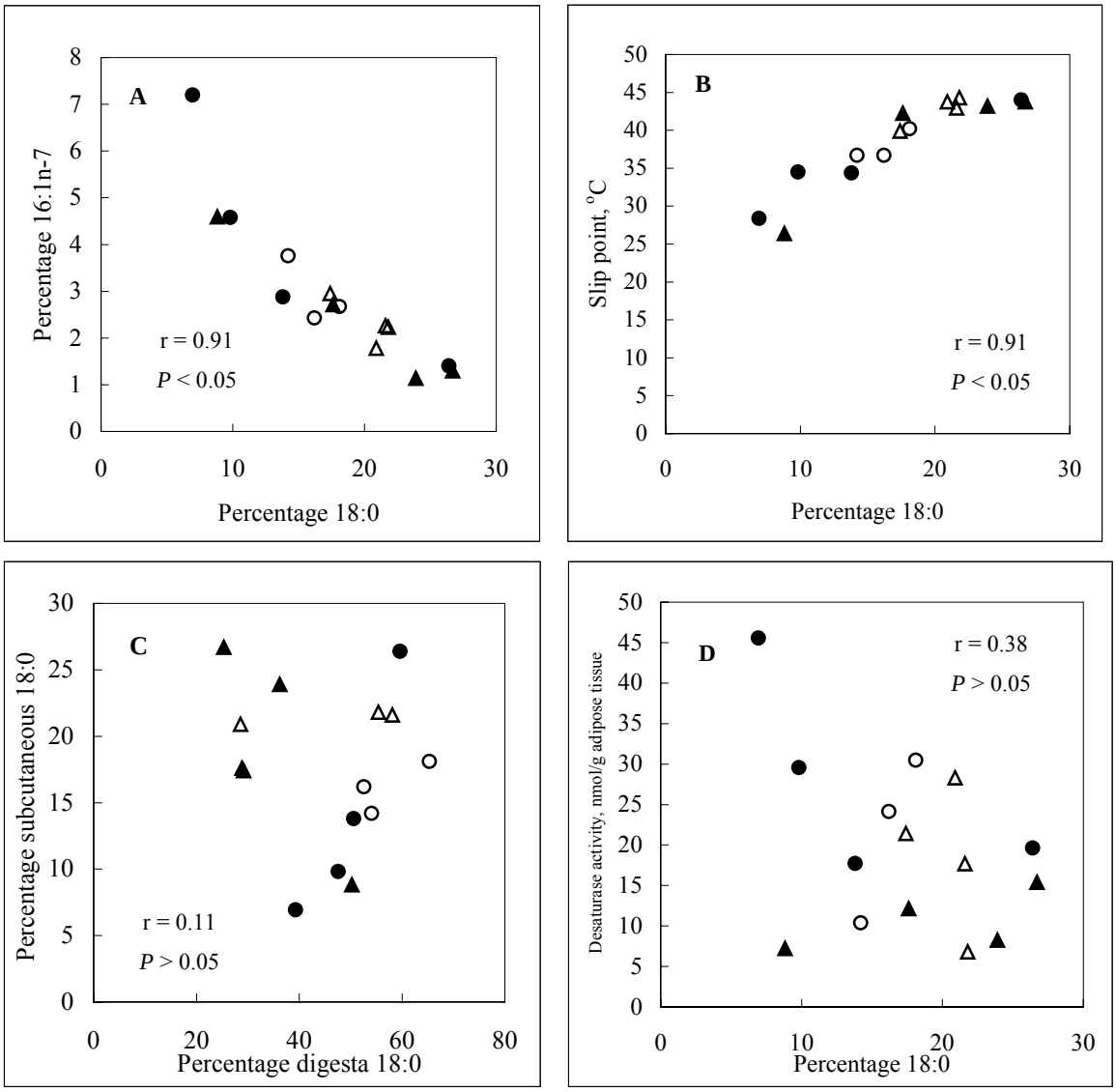

Figure 3.2 Relationships between fatty acid composition and indices of fatty acid desaturation. A: Stearic acid (18:0) versus palmitoleic acid (16:1n-7) in subcutaneous adipose tissue of Wagyu and Angus cattle fed corn or hay. B: Stearic acid (18:0) versus slip point (^{o}C) in subcutaneous adipose tissue of Wagyu and Angus cattle fed corn or hay. C: Stearic acid (18:0) in digesta versus stearic acid (18:0) in subcutaneous adipose tissue of Wagyu and Angus cattle fed corn or hay. D: Stearic acid (18:0) versus stearoyl-CoA desaturase activity (nmol/(g adipose tissue per 7 min)) in subcutaneous adipose tissue of Wagyu and Angus cattle fed corn or hay (●:Wagyu corn, ○:Angus corn, ▲:Wagyu hay, △:Angus hay).

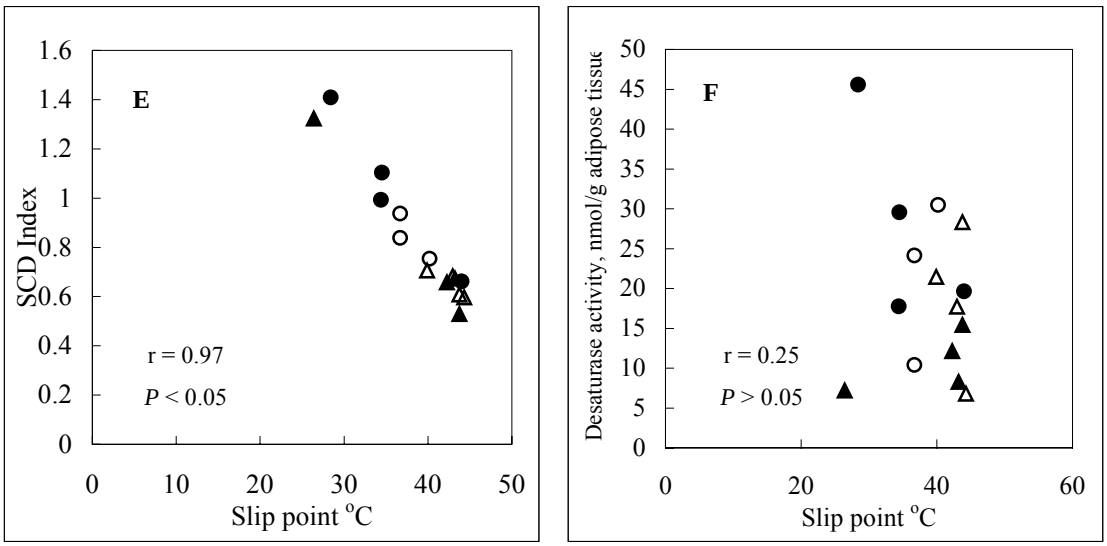

Figure 3.2, continued. E: Slip point (°C) versus SCD index in subcutaneous adipose tissue of Wagyu and Angus cattle fed corn or hay. F: Slip point (°C) versus stearoyl-CoA desaturase activity (nmol/(g adipose tissue per 7 min)) in subcutaneous adipose tissue of Wagyu and Angus cattle fed corn or hay (●:Wagyu corn, ○:Angus corn, ▲:Wagyu hay, △:Angus hay).

Discussion

Japanese Black Wagyu steers were reported by Sturdivant et al. (1992) and May et al. (1993) to inherit a higher percentage of MUFA than Angus steers. Oka et al. (2002) showed that Wagyu steers fed a concentrated diet (45% rolled barley, 30% flaked corn, 20% wheat bran, 5% soybean meal, and 1% mineral mixture) had 51.7% 18:1n-9, 26.1% 16:0, and 8.76% 18:0 in their s.c. adipose tissue. This study had higher percentage of 18:0 (13.9% and 19.2%) and a lower percentage of 18:1n-9 (43.0% and 34.4%) in s.c adipose tissue of corn- and hay-fed steers, respectively. Engle and Spears (2001) indicated that Angus steers fed a corn silage-based diet had a similar s.c. adipose tissue fatty acid composition as reported in this study. It is apparent that fatty acid composition of s.c. adipose tissue is affected not only by breed type but also by diet. This is supported by several studies (Mandell et al., 1998; Engle and Spears. 2001; Oka et al., 2002).

Slip Points and SCD Activity

Cameron et al. (1994) suggested that the fatty acid composition of adipose tissue is determined early in the development of adipose tissue. In this study, animals were slaughtered at 8 mo and 12 mo of age, and significant differences were found between corn-and hay-fed steers in carcass weight, overall maturity, adjusted fat thickness, and yield grade (see chapter 4). The Angus and Wagyu steers may have been at different stages of development, even when slaughtered at the same age. May et al. (1994) reported that i.m. and s.c. adipocytes of Wagyu steers mature at slower rate and have smaller diameters than those of Angus steers.

Slip points of lipids from adipose tissue are related to hardness or softness of fat. This is important because the Japanese market prefers soft fat to hard fat. Pitchford et al. (2002) stated that fat slip point was determined by its fatty acid composition. Higher percentages of MUFA caused lower melting points. We observed significant differences in s.c. fatty acid composition between corn-and hay-fed steers, consistent with the higher slip points of hay-fed steers. SCD activity in s.c. tissue was presented in two ways: 1) it was based on the microsomal protein content; and 2) it was calculated on a per gram adipose tissue basis. Although no differences were found in breed types, s.c. adipose tissue of corn-fed cattle had higher SCD activity than hay-fed cattle ($P < 0.05$). Subcutaneous SCD activity may not fully explain the conversion from 18:0 to 18:1n-9. The total amounts of 18:0 and 18:1n-9 was similar to the results of Oka et al. (2002) (58.5% and 60.3%, respectively). However, this study demonstrated a higher 18:0 and a lower 18:1n-9 in Wagyu s.c. adipose tissue than Oka et al. (2002). This implies that other factors, in addition to SCD, might influence the concentration of MUFA in adipose tissue.

Slip point was highly and positively correlated with percentage of 18:0. Therefore, high percentages of SFA will increase the melting point of adipose tissue lipids. Saturated fatty acids have a highly ordered structure that provides maximum Van der Waals force, which will increase the melting point of fat. Figure 3.3.E and F indicate a negative correlation between slip point and the SCD index. However, SCD enzyme activity and slip points were not well correlated. We recently observed that there was no correlation between SCD enzyme activity and mathematical indices of SCD activity in

Angus steers fed corn, flaxseed, or milo diets (unpublished observations). Thus SCD enzyme activity can explain only a small portion of the difference in fatty acid composition between breed types or across diets.

Smith et al. (1998) reported that cattle finishing with a corn diet produced fat with melting points around 32^{o}C, whereas fat from the highest quality Japanese was liquid at room temperature, with melting points less than 25^{o}C. In this study, the melting point of Wagyu was 35^{o}C. Four of the lowest slip point values were observed in Wagyu steers, as were four of the highest. Three of the cattle with the lowest slip points had the highest SCD activity, and all of the Angus steers had lower SCD activities and higher slip points. These data generally were consistent with the plot of the SCD index vs slip points. The large variability in Wagyu SCD activity and SCD index suggests that the SCD activity is likely to reach its maximum in later development of adipose tissue in Wagyu steers. The large within breed type variation observed for Wagyu steers may be attributed to the heterogeneous population of Wagyu sampled. Wagyu steers in this study were high percentage crossbreds (7/8 or higher) and were therefore expected to be more variable in phenotype than the Angus steers in this study.

Implications

These data indicate that difference in fatty acid composition and slip point in subcutaneous adipose tissue between Wagyu and Angus cattle fed a corn-based finishing diet is due to greater SCD activity than in Wagyu and Angus cattle fed a hay-based finishing diet. No significant difference was found in fatty acid composition and SCD activity between Wagyu and Angus steers. Diet, in addition to the genetic effect, may

influence SCD activity-induced fatty acid composition. Slip point is highly positively related with stearic acid content and highly negatively related with SCD index in s.c. adipose tissue. However, SCD enzyme activity did not correlate with actual SCD activity, which implies that these indices are not valid measured of SCD activity in bovine s.c. or i.m. adipocytes. These data imply that carefully designed animal model studies are required when basing estimates of SCD activity on SCD enzyme activity, because genetic variation causes different enzyme activity between breeds.

CHAPTER IV

CARCASS TRAITS, CELLULARITY AND LIPOGENIC ACTIVITIES IN SUBCUTANEOUS AND INTRAMUSCULAR ADIPOSE TISSUE DIFFER BETWEEN WAGYU AND ANGUS STEERS FED A CORN-BASED FINISHING DIET OR HAY-BASED FINISHING DIET

Overview

Eight Angus and eight Wagyu steers were fed a corn-based diet for 244 d and hay-based diet for 368 d. Subcutaneous and i.m. adipose tissue samples were collected immediately postmortem. ^{14}C-Acetate incorporation into lipids and cellularity were measured in s.c. and i.m. adipose tissue samples from Wagyu and Angus steers. Breed and diet did not affect overall marbling score, USDA quality grade, ribeye area, lean maturity, or kidney pelvic and heart fat ($P > 0.05$), but Wagyu steers had lower USDA yield grade, carcass weight, overall maturity, and adjusted fat thickness than Angus steers for both diets ($P < 0.05$). Lipogenesis from acetate in i.m. adipose tissue was not different between breed types ($P = 0.85$), but tended to be greater ($P = 0.07$) in s.c. adipose tissue of Wagyu steers than in s.c. adipose tissue of Angus steers. Subcutaneous and i.m. adipose tissues had substantially greater rates of lipogenesis in corn-fed steers than in hay-fed steers ($P < 0.06$). Mean s.c. adipocyte diameter and volume were greater in Angus steers than in Wagyu steers ($P < 0.04$), and mean i.m. adipocyte diameter tended to be greater in Angus steers ($P = 0.09$). Cells per gram s.c. adipose tissue tended ($P = 0.08$) to be greater in Wagyu than in Angus steers. Subcutaneous and i.m. adipocyte volumes were

greater ($P < 0.09$) in hay-fed steers than in corn-fed steers, and diameter were also greater ($P < 0.06$) in hay-fed steers than in corn-fed steers. There were no significant breed x diet interactions for lipogenesis or cellularity. The data indicate that the smaller adipocytes of the younger, corn-fed steers had greater rates of lipogenesis than those of the older, hay-fed steers, which would have influenced carcass adiposity. The lower marbling scores and fat thickness of the Wagyu steers was consistent with their smaller adipocytes in both depots.

Introduction

There have been limited studies to document the lipogenic activity in adipose tissues of Wagyu steers. Diet, age, and lipid metabolism are major sources of variation in adiposity of growing steers. Concentrate diets cause faster rates of gain and also cause higher actives of fatty acid synthesis and lipogenic enzymes than roughage diets (Smith et al., 1984). However, age and diet have very limited effects on enzyme activities in i.m. adipose tissue from Angus steers (Smith and Crouse, 1984). Also, 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. Glucose provided 1-10% of the acetyl units in s.c. adipose tissue, but 50-75% in the i.m. depot (Smith and Crouse, 1984). Lactate has not been shown to exhibit significant differences between i.m. and s.c. adipose tissues. Thus, there are many metabolic differences between i.m. and s.c. adipose tissues. Eighteen-mo-old Angus steers gained more weight per day (0.9 kg) than American Wagyu steers (0.7 kg) (Lunt et al., 1993). Angus steers also had greater feed efficiency, carcass weights, and yield grades than American Wagyu steers. However, American Wagyu steers had higher

average USDA marbling scores than Angus (Lunt et al., 1993). Although trained sensory panel analysis showed no differences between steaks from Wagyu and Angus steers, a consumer triangle test indicated that consumers detected a difference between breeds (May et al., 1993). We hypothesized that difference in fatty acid composition and adiposity between Wagyu and Angus cattle may be due to greater lipogenic activity earlier in production, which would have resulted in an increase in fatty acids of endogenous origin at some point before slaughter. Therefore, we compared Angus and Wagyu cattle fed a corn-based finishing diet and a hay-based finishing diet at a typical U. S. market weight. The goal was to achieve U. S. market weight (approximately 500 kg) at a sufficiently early age to observe difference in lipogenesis, cellularity, and carcass characteristics.

Materials and Methods

Animals and Experimental Procedures

Eight Angus and eight American Wagyu steers were purchased as weanling calves and assigned to one of two dietary treatments: a high-energy, corn based finishing diet designed to provide maximal rates of i.m. adipose tissue accretion; and a medium-energy, hay-based diet that provided submaximal rates of i.m. adipose tissue accretion. The high-energy diet was suitable for the commercial production of Angus steers, which typically are slaughtered at 16 to 18 mo of age. The medium-energy diet was designed for the production of the Wagyu cattle, which do not express their ability to accumulate interfacicular adipose tissue until after 20 mo of age. Cattle were slaughtered after 8 mo

on the high-energy diet or 12 mo on the medium-energy diet. One Angus steer escaped prior to slaughter and had to be removed from the study.

Sample Collection

Subcutaneous and i.m. adipose tissues were collected from finished Angus and Wagyu steers. Steers were slaughtered at the Rosenthal Meat Science and Technology Center using standard industry techniques, and tissues were obtained immediately after removal of the hide (approximately 20 min post-exsanguination). The 5^{th}-8^{th} thoracic section of the longissimus muscle was transported to the laboratory in 1X Krebs-Henseleit bicarbonate buffer (KHB) (pH = 7.4) with 5 mM glucose at 37^{o}C. Tissues were transferred to the laboratory as soon as possible, and s.c and i.m. adipose tissues were dissected immediately for analysis or storage. Adipose samples used for lipogenesis from acetate were used immediately. All other samples were processed or stored at –80^{o}C after quick-freezing in liquid nitrogen.

Cellularity

Adipocyte volume and density (cells per gram) were determined by the method of Etherton et al. (1977) with the modifications of May et al. (1994). After tempering the adipose tissue at –20^{o}C, it was sliced into 1-mm thick sections on a chilled glass plate and placed in scintillation vials coated with 3% Sigmacoat (Sigma Chemical Co., St. Louis, MO) in collidine buffer. The samples were then incubated for 72 to 96 h in a 37^{o}C water bath. The osmium solution was removed with a Pasteur pipette and rinsed with 0.154 M NaCl until clear. The NaCl solution was removed, and replaced with 10 ml of 8 M urea in 0.154 M NaCl. The samples were then incubated for 72-96 h at 25^{o}C. Fixed

cells were filtered through 250-, 60-, and 10-μm mesh screens using 0.01% Triton in 0.154 M NaCl. Cell fractions collected from the 60- and 10-μm mesh screens for cell size and number count using a Coulter Counter, Model ZM equipped with a channelizer, Model 256 (Hialeah, FL).

Lipogenesis

All the lipogenesis analytical procedures were based on Page et al. (1997). Fifty to 100 mg of adipose tissue incubated in 3 mL of KHB incubation medium (pH 7.4) containing 5 mM sodium acetate, 5 mM glucose, 10 mM hepes buffer, and 1 μCi [U-^{14}C] acetate for 2 h in a shaking water bath at 37^{o}C. The reactions were stopped by adding 3 mL of 5% tricholoroacetic acid. After rinsing with 0.154 M NaCl and 1X KHB buffer, total lipid was extracted by the methods of Folch et al. (1957). Adipose tissue samples were homogenized in 15 mL of chloroform-methanol (2:1, vol/vol). Remaining unincorporated substrate was separated from the glycerolipids by rinsing with 9 mL of 4% Na_2CO_3. After rinsing three times, the chloroform layers were evaporated completely under N_2 gas at 50^{o}C and resuspended in 10 mL of Econo-Safe scintillation fluid (Research Products International Corp., Mount Prospect, IL). ^{14}C-Labeled lipids were counted on a Beckman liquid scintillation spectrometer (Beckman Instruments, Palo Alto, CA) and calculated as acetate incorporated per 2-h incubation period per 10^5 cells.

Statistical Analysis

All statistical analysis was performed by the GLM procedure of SAS version 8.1 (SAS Inst. Inc., Cary, NC). Means of cellularity, lipogenesis, and carcass characteristics

from Wagyu (n = 8) and Angus (n = 7) steers were compared as a two-factor analysis of variance. Factor one was diet (hay vs corn), and factor two was breed type (Angus vs Wagyu). The diet x breed interaction was included in the model. Means were separated using the probability statement of GLM for significant differences at $P < 0.05$. However, tendencies ($P < 0.10$) are discussed.

Results

Initial weights of the Angus and Wagyu steers were 210 and 170 kg, respectively (see Figure 2.1). Diets were formulated to provide an average daily gain of 1.36 kg/d (approximately 325 kg over the 8-mo period after weaning) for the corn-based diet and 0.9 kg/d (approximately 325 kg over the 12-mo period after weaning) for the hay-based diet for both breed types. These targeted gains were achieved by the Angus steers, which gained an average of 331 kg over the duration of the feeding trial. The Wagyu steers had lower rates of gain, accumulating only 258 kg over the same period. There were no differences in carcass weights between corn- and hay- fed steers (Table 4.1).

The goal was to slaughter the cattle at the same physiological maturity, but overall maturity scores were greater for Angus steers than for Wagyu steers fed either diet ($P < 0.05$). Maturity scores of 143 to 147 are in the midrange of A maturity, the most youthful classification in the U.S. grading system. As expected, overall maturity scores for hay-fed steers were higher than for corn-fed steers ($P < 0.06$). Carcass weight, fat thickness, and USDA yield grade were greater for Angus steers than for Wagyu steers fed corn or hay diets ($P < 0.05$). There were no significant differences in marbling scores, lean maturity, skeletal maturity, quality grade, kidney pelvic and heart fat, or

ribeye area between breeds or diets ($P > 0.1$) (Table 4.1). There was a breed x diet interaction for skeletal maturity ($P = 0.01$). Skeletal maturity was the same between breeds on the corn diet, but greater in Angus steers fed the hay diet.

There were several significant differences in lipogenesis and cellularity of s.c. and i.m. adipose tissues of Wagyu and Angus steers fed corn or hay (Table 4.3). Wagyu steers tended to have more lipogenic activity than Angus steers in s.c. adipose tissue ($P < 0.08$). However, there were no statistical differences between breeds in i.m. adipose tissue ($P < 0.86$). There were diet differences in lipogenesis for both i.m. ($P < 0.06$) and s.c. adipose tissue ($P < 0.01$) (Table 4.3). Lipogenesis from acetate in s.c. adipose tissue was approximately sixfold greater ($P < 0.01$) in steers fed the corn-based diet than in steers fed the hay diet. Lipogenesis in i.m. adipose tissue was approximately fivefold greater in corn-fed steers than in hay-fed steers ($P < 0.06$).

The s.c. adipose tissue of steers fed corn had more ($P < 0.05$) cells per gram of tissue than s.c. adipose tissue of hay-fed steers and there tended ($P < 0.08$) to be a greater number of cells in Wagyu s.c. adipose tissue than in Angus s.c. adipose tissue. The s.c. adipocyte from hay-fed steers tended to have a greater mean volume ($P < 0.09$) and mean diameter ($P < 0.06$) than s.c. adipose tissue of corn-fed steers. Angus steers had greater adipocyte mean volumes ($P < 0.05$) and mean diameters ($P < 0.05$) than Wagyu steers. Hay-fed steers had fewer i.m. adipocytes with volumes greater than 2,000 pL than corn-fed steers (Figure 4.1). Subcutaneous adipose tissue from corn-fed Angus steers had an especially large proportion of adipocytes with volumes greater than 2,000 pL (Figure 4.2).

Total cholesterol, fat, and moisture content of longgissimus were not affected by diet or breed type (Table 4.2). Although there are no differences between treatments, percentage of fat tended to higher in Angus muscle than in Wagyu muscle. There were no significant breeds x diet interactions.

Table 4.1 Carcass characteristics from Wagyu and Angus steers fed high-energy (corn) or medium-energy (hay) diets

	Diet/breed types							
	Corn		Hay			*P*-values		
Item	Angus	Wagyu	Angus	Wagyu	SE	Breed	Diet	BxD[x]
Carcass wt., kg	323.42	252.32	307.77	283.05	32.97	0.0192	0.5966	0.2036
Skeletal maturity[a]	133.33	140.00	165.00	140.00	10.30	0.0552	0.0194	0.0131
Lean maturity[a]	160.00	147.50	160.00	150.00	12.34	0.1059	0.8373	0.8492
Overall maturity[a]	146.67	142.50	162.50	146.25	8.45	0.0250	0.0568	0.1968
Marbling[b]	673.33	612.50	580.00	572.50	142.0	0.7154	0.3993	0.7251
Quality grade[c]	483.33	462.50	443.75	468.75	48.36	0.8480	0.5646	0.3821
Fat thickness, cm	1.44	0.95	1.30	1.05	0.19	0.0040	0.9047	0.2689
Ribeye area, cm^2	78.28	68.39	71.78	68.87	7.56	0.1578	0.4994	0.3936
KPH, %	3.00	2.88	2.63	3.13	0.57	0.4821	0.8987	0.3140
Yield grade	3.33	2.75	3.33	3.08	0.32	0.0280	0.3217	0.3354

[a]A = 100; B = 200; C = 300; D = 400; E = 500.
[b]Practically Devoid = 100; Traces = 200; Slight = 300; Small = 400; Modest = 500; Moderate = 600; Slightly Abundant = 700; Moderately Abundant = 800; Abundant = 900.
[c]Standard = 200; Select = 300; Choice = 400; Prime = 500.
[x] Breed x diet interaction.

Table 4.2 Total cholesterol and fat contents in longissimus muscle tissues of Wagyu and Angus steers fed high-energy (corn) or medium-energy (hay) diets

	Diet/breed types							
	Corn		Hay			*P*-values		
Item	Angus	Wagyu	Angus	Wagyu	SE	Breed	Diet	BxD[x]
mg Cholesterol/100 g tissue	56.39	60.91	62.55	73.63	15.99	0.3879	0.2733	0.7118
% Fat	9.32	6.13	8.34	7.81	2.87	0.2574	0.7320	0.4116
% Moisture	67.68	70.64	68.72	68.12	2.50	0.4029	0.4719	0.2157

[x] Breed x diet interaction.

Table 4.3 Lipogenesis and cellularity of subcutaneous and intramuscular adipose tissues of Wagyu and Angus steers fed high-energy (corn) or medium-energy (hay) diets

	Diet/breed types							
	Corn		Hay			*P*-values		
Item	Angus	Wagyu	Angus	Wagyu	SE	Breed	Diet	BxD[x]
Intramuscular								
Lipogenesis[a]	29.66	54.80	6.44	6.15	16.9	0.8584	0.0598	0.8236
10^5 cells/g tissue	1.71	1.61	2.10	1.94	2.0	0.7113	0.3011	0.9220
Mean diameter, pL	42.5	41.1	53.0	44.6	5.0	0.0868	0.0217	0.2068
Mean volume, pL	146.6	142.6	244.1	176.5	53	0.2230	0.0367	0.2704
Subcutaneous								
Lipogenesis[a]	86.23	212.82	13.21	31.93	2.33	0.0716	0.0065	0.1970
10^5 cells/g tissue	1.77	2.82	1.08	1.61	2.3	0.0767	0.0383	0.5233
Mean diameter, pL	44.9	26.0	48.8	43.8	9.1	0.0367	0.0535	0.1899
Mean volume, pL	233.7	66.6	266.6	207.6	83.3	0.0307	0.0821	0.2570

[a] Lipogenesis from acetate, nmol/(10^5 cells per 2 h).
[x] Breed x diet interaction.

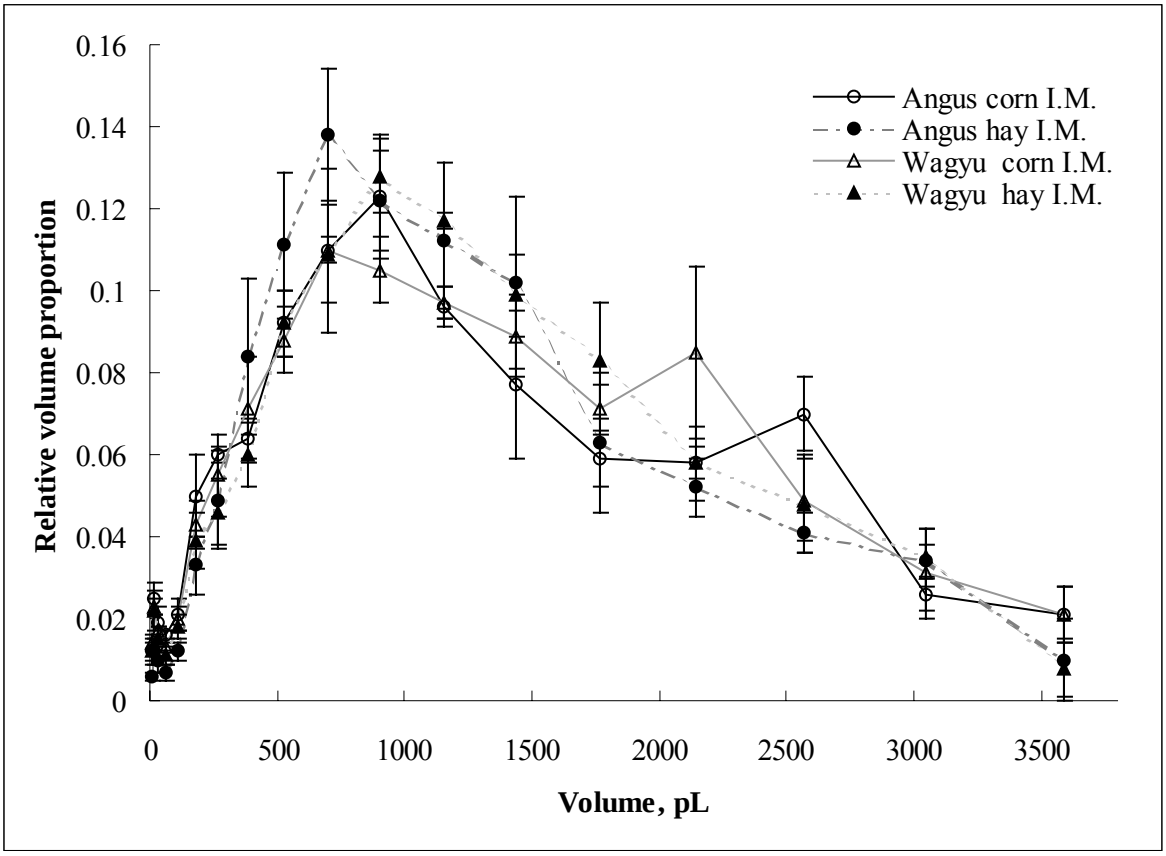

Figure 4.1 Proportional distributions of intramuscular adipocytes from Wagyu and Angus steers fed corn or hay-based diets.

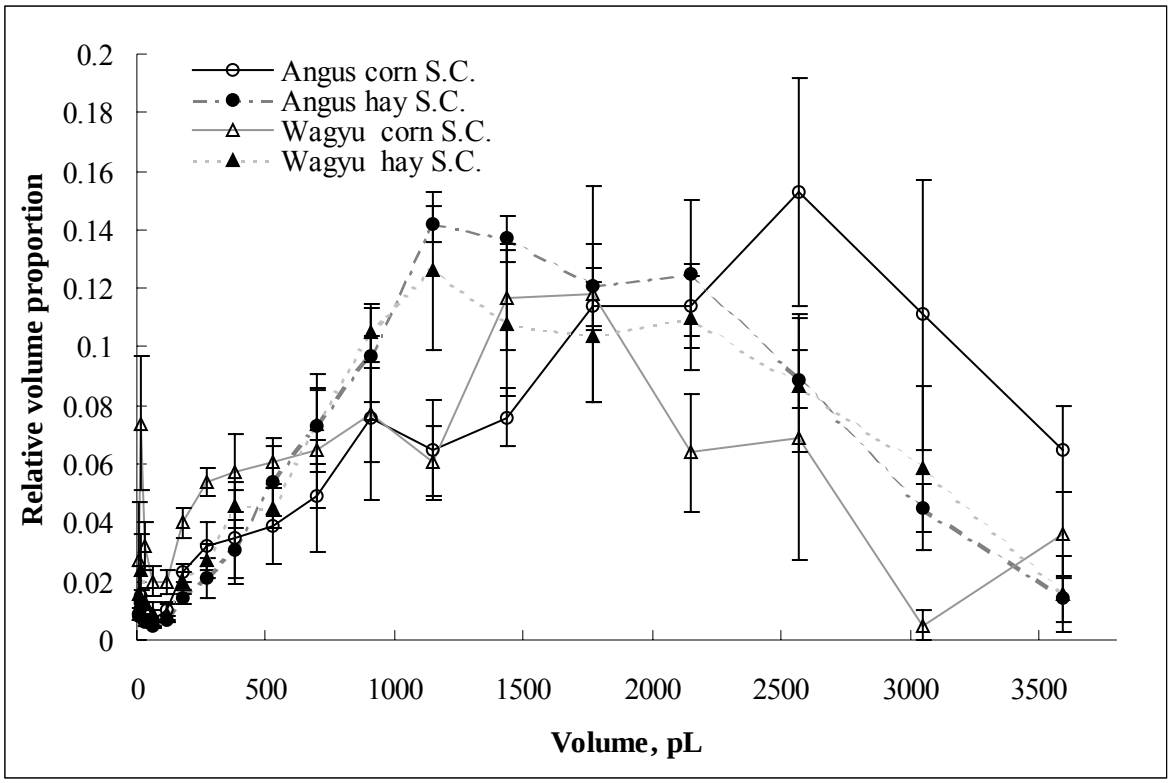

Figure 4.2 Proportional distributions of subcutaneous adipocytes from Wagyu and Angus steers fed corn or hay-based diets.

Discussion

The USDA yield grade is calculated based on carcass weight, ribeye area, adjusted s.c. fat thickness at the 12th thoracic rib, and percentage kidney, pelvic, and heart fat (USDA, 1997). Angus steers had greater carcass weight and fat thickness than Wagyu steers ($P < 0.05$), which caused a significant difference in yield grade. The higher yield grade of the Angus steers indicates the greater carcass adiposity of this breed type, compared to Wagyu steers (Mir et al., 2002). It was previously documented that Wagyu calves have lesser weaning weights and lower rates of weight gain than Angus calves (Lunt et al., 1993). Wagyu cattle are characterized by a greater ability to accumulate i.m. (marbling) adipocytes within the longissimus thoracis and 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 a previous investigation (Zembayashi et al., 1995), it was demonstrated that intramuscular lipid (i.e., marbling) in the thoracis longissimus muscle of Japanese Black cattle increased indefinitely with age (up to 900 d of age), whereas in Charolais x Japanese Black/Horstein crossbred cattle, intramuscular lipid stopped increasing after approximately 500 d of age. The previous results, combined with the data of the present study, indicate that differences in marbling between Wagyu cattle and British or Continental breed types are not evident until the cattle are fed to greater physiological maturity.

In cattle fed the high-corn diet, carcass weight and s.c fat thickness were significantly greater than in cattle fed the hay diet. Plasma glucose tended to be greater

($P < 0.11$) in corn-fed steers than in hay-fed steers, and tended to be greater ($P < 0.16$) in Angus steers than in Wagyu steers of this study (unpublished observations). Smith and Crouse (1984) reported that acetate was the major source of acetyl units for in vitro lipogenesis in s.c. adipose tissue, but only provided 10-25% of the acetyl units in i.m. adipose tissue. On the other hand, glucose was used as the major source of acetyl units for lipogenesis in i.m. adipose tissue, but provided only 1-10% in s.c. adipose tissue. In vitro, glucose can stimulate lipogenesis from acetate over threefold (Smith and Prior, 1981). Glucose is used as a major source of carbon for the pentose cycle, which can contribute most of the NADPH required for lipogenesis in bovine adipose tissues (Smith and Crouse, 1984). However, glucose utilization for fatty acid synthesis is limited by the low activity of lipogenic enzyme in bovine s.c. adipose tissues (Smith and Prior, 1981). The ATP-citrate lyase, NADP-malate dehydrogenase pathway also provides a proportion of NADPH required for lipogenesis, but their activities are lower in i.m. adipose tissue than in s.c. adipose tissue (Smith and Crouse, 1984). The current data suggest that different regulatory actions control fatty acid synthesis in i.m. and s.c. adipose tissues.

The corn diet can activate lipogenic enzymes such as acetyl-CoA carboxylase, fatty acid synthease, ATP-citrate lyase, $NADP^+$ malate dehydrogenase, and hexokinase (Smith et al., 1984). Fatty acid synthesis and lipogenic enzyme activities increase during the feedlot phase, but can decrease at larger body weights (Smith et al., 1984). Therefore, lower rates of lipogenesis in hay-fed steers could have been caused by greater adipose tissue maturity of the hay-fed steers. This is supported by the large volumes of adipocytes in hay-fed steers, relative to corn-fed steers.

Several lipogenic enzymes are regulated by glucose-induced metabolic pathways. Mourrieras et al., (1997) demonstrated that the expression of the fatty acid synthase (FAS) gene and the concentration of glucose metabolites showed positively increase. Among the measured glucose metabolites, glucose 6-phosphate was the most highly correlated metabolite for the glucose-regulated transcription of the FAS gene.

There are earlier studies that investigated adipose tissue cellular differences between bovine breed types. The current study showed patterns similar to past studies. Intramuscular adipose tissue contained more adipocyte number per gram of adipose tissue than s.c. adipose tissue, which means smaller mean cell diameter and smaller mean cell volume than s.c. adipose tissue (Hood and Allen, 1975; Smith and Crouse, 1984; Miller et al., 1991; May et al., 1994). The cell diameters and volumes in Table 4.3 were significantly different between s.c. adipose tissue from Angus and Wagyu steers. Although i.m. adipose tissue mean diameter and volume were not statistically different, these results were similar to an earlier study from this laboratory (May et al., 1994). However, there were more adipocytes number per gram of adipose tissue (thus, smaller adipocytes) in s.c and i.m. than reported by May et al. (1994). The cattle in this study were younger than those of May et al. (1994; see also Lunt et al., 1993), and diets also were different. The differences between this study and that of May et al. (1994) may explain differences in adiposity.

There were no significant differences in cholesterol, fat, or moisture percentages in longissimus muscle of the Wagyu and Angus steers. Our next objective is to

document whether variations in cholesterol ester content of adipose tissue is affected by SCD enzyme activity.

Implications

These data demonstrated that increasing glucose absorption in cattle by feeding a corn-based diet increased carcass weight, fat thickness, USDA yield grade, lipogenesis, and adipocyte volumes, relative to a hay-based diet. Overall maturity, fat thickness, and marbling scores were associated with lipogenesis and adipocyte volume, which differed with age, diet, and breed type. These data imply that diet differences control beef carcass characteristics and have a large impact on lipid quality. Wagyu steers have more potential ability for lipid accumulation as marbling and back fat thickness than Angus steers. However, this was not expressed in the current study. We conclude that Angus steers will have greater adiposity than Wagyu steers regardless of diet, when slaughtered at this endpoint. Furthermore, Angus steers may more consistently produce USDA choice carcass than Wagyu steers when fed to a typical U.S. live weight.

CHAPTER V

CONCLUSIONS

This investigation demonstrated that increasing glucose uptake by feeding a corn-based diet would increase carcass weight, fat thickness, USDA yield grade, lipogenesis, and adipocyte volume, relative to a hay-based diet. Overall maturity, fat thickness, and marbling score were associated with greater rates of lipogenesis and cell volume. These data indicate that diet differences control beef carcass characteristics, especially for aspects of fat quality and quantity. Wagyu steers had more potential ability for lipid accumulation in s.c. adipose tissue, based on lipogenesis, and adipocyte hypertrophy, than Angus steers. However, Angus steers had higher potential ability for yield grade, carcass weight, and overall maturity than Wagyu steers. These data also indicate that difference in fatty acid composition and slip point in s.c. adipose tissue between diets were due to greater SCD activity in Wagyu and Angus cattle fed a corn-based finishing diet. Diet, in addition to genetics, may influence SCD activity-induced fatty acid composition. Slip point was highly positive related with stearic acid content and highly negatively related with SCD index in s.c. adipose tissue. These data imply that carefully designed animal model need to be utilized when basing estimates of SCD activity on differences in SCD enzyme activity, because genetic variations in different breed types cause different enzyme activity between breeds.

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