Marbling: Management of cattle to maximize the deposition of intramuscular adipose tissue

*Stephen B. Smith, Regents Professor, Department of Animal Science, Texas A&M University, College Station, TX 77843*

*Bradley J. Johnson, Gordon W. Davis Regent’s Chair, Department of Animal and Food Science, Texas Tech University, Lubbock, TX 79409*
Introduction
Consumers in the U.S., Japan, and Korea have valued highly marbled beef for nearly a century. Since the development of a feedlot industry in Australia (to include the production of Japanese Black cattle), Australian consumers have had access to more highly marbled beef, and Korea has increased the marbling of their native Hanwoo cattle by more than 30% over the last two decades. Much more recently, China has initiated grain feeding of cattle to satisfy the growing domestic demand for highly marbled “snow” beef. In spite of growing interest in pasture-fed (or grass-fed) beef in the United States, most consumers still prefer beef that is reasonably marbled and juicy. Recently, Hunt et al. (2014) reported that Top Choice longissimus (loin) muscle (6.85% intramuscular lipid) was rated more highly in a consumer panel than Select longissimus muscle (2.95% intramuscular lipid) for tenderness, juiciness, flavor, and overall liking, and similar results were observed for the gluteus medius (sirloin) muscle. Also, juiciness was highly correlated with overall liking (r = 0.73), tenderness (r = 0.64), and flavor (r = 0.63) (Hunt et al., 2014). Similar results were reported previously (Killinger et al., 2004; O’Quinn et al., 2012). Furthermore, monounsaturated fatty acids (MUFA) such as oleic acid were positively correlated with flavor (Garmyn et al., 2011).

Scientists have taken a two-prong approach to understand the biology of marbling development. Biochemists, molecular biologists, and geneticists have worked to gain a better understanding of the intracellular and extracellular factors that regulate the development of marbling adipose tissue, whereas beef cattle nutritionists have worked to optimize diets and time on feed to provide high-quality beef carcasses without exacerbating carcass adiposity. Thus, this review will address 1) the biology and biochemistry of marbling, and 2) the effects of production systems on carcass and fat quality.

Fat content and beef palatability
The contribution of fat content of meat to overall meat palatability has been established for decades (see Smith et al., 2004 for review). Savell and Cross (1988) established a “Window of Acceptability” for beef (Figure 1), indicating that overall palatability of beef is optimal between 3 and 7.5% intramuscular lipid. Muscle devoid of marbling contains approximately 1% intramuscular lipid, so that any increase in intramuscular lipid above 1% is associated with differentiation and lipid filling of marbling adipocytes.

The relationship between percent fat and overall palatability underscores the importance of intramuscular lipid in beef quality. What is not addressed in these data is that, as percent fat increases, there is a dramatic change in the extent of intramuscular preadipocyte differentiation and concomitant changes in fatty acid composition of intracellular lipid. As described below, an increase in fat percentage is invariably accompanied by a decrease in the proportion of saturated fatty acids (SFA) and trans-fatty acids and corresponding increase in oleic acid (18:1n-9) and other MUFA. This has a direct effect on the palatability and healthfulness of beef.

Fatty acids and flavor
Monounsaturated fatty acids in meat have been shown to influence beef palatability (Waldman et al., 1968; Westerling and Hedrick, 1979). These early studies demonstrated that the more oleic acid in beef, the greater the overall palatability of the beef. Some portion of the effect of oleic acid on increasing palatability of beef may be due to the fat softness associated with this fatty acid (Smith et al., 1998; Wood et al., 2004; Chung et al., 2006b). This provides a more fluid mouthfeel, which most perceive as more desirable.
Fatty acid composition of beef and risk factors for cardiovascular disease

In recent years, research has demonstrated that high-oleic acid ground beef may reduce risk factors for cardiovascular disease (Adams et al., 2010; Gilmore et al., 2011, 2013). When participants consumed ground beef formulated from long-fed, grain-fed steers for 5 weeks (5 patties per week), high-density lipoprotein (HDL) cholesterol increased significantly in normocholesterolemic men and postmenopausal women (Figure 2). Furthermore, ground beef from long-fed, grain-fed cattle did not reduce low-density lipoprotein (LDL) particle diameters (an independent risk factor for cardiovascular disease). In none of the studies did the ground beef interventions increase LDL cholesterol or any inflammatory risk factors.

Participants in the Adams et al. (2010) study consumed ground beef containing 34% fat, whereas the participants in the Gilmore et al. (2011) and Gilmore et al. (2013) studies consumed ground beef containing 24 and 20% fat, respectively. Therefore, all of the participants in these studies consumed a large amount of beef fat. Two important conclusions can be made from these studies: Even at this level of fat intake, ground beef had no negative effects on lipoprotein cholesterol metabolism in normocholesterolemic men, and ground beef low in SFA (high-MUFA) had positive health benefits. For this reason, it is important to understand factors that control both the development and the composition of beef adipose tissues, and especially marbling adipose tissue, as it contributes to the fatty acid composition of virtually all beef cuts and products.

Consumer evaluation of high MUFA and lower MUFA beef

A consumer triangle test between highly marbled beef (Wagyu) and lower marbled beef (Angus) demonstrated that many consumers could distinguish between levels of marbling (May et al., 1993). The major qualitative traits used to describe differences in eating quality were tenderness, juiciness, and flavor (Figure 3). When consumers were asked to indicate which traits they used to determine the difference between high marbling and lower marbling beef samples, the majority of the respondents used a combination of tenderness and juiciness, while other consumers indicated the singular traits of flavor and tenderness. The highly marbled Wagyu beef also was higher in MUFA than the Angus beef.

Figure 2. Changes in HDL cholesterol (HDL-C; upper panel) and LDL particle diameter (lower panel) in mildly hypercholesterolemic men (Hyper-C men, n = 10), normocholesterolemic men (Normo-C men, n = 27) and postmenopausal women (PM women, n = 19). In all three studies, participants consumed 5, 4-ounce ground beef patties/week for 5 weeks. Fat content was held constant within a study, and patties were formulated to be low (grass-fed or chub pack) or high in total MUFA (grain-fed for extended periods). Low MUFA ground beef decreased HDL-C and LDL size in hypercholesterolemic men (Adams et al., 2010) and LDL size was reduced in postmenopausal women after consuming chub pack ground beef (Gilmore et al., 2013). High MUFA ground beef increased HDL cholesterol in normocholesterolemic men (Gilmore et al., 2011) and postmenopausal women (Gilmore et al., 2013). There were no effects of the ground beef interventions on LDL cholesterol, plasma triglycerides, or inflammatory risk factors, but ground beef decreased plasma insulin in normocholesterolemic men (Gilmore et al., 2011). *Indicates significant change from baseline (habitual diets).

This study was one of many (e.g., Killinger et al., 2004; O’Quinn et al., 2012; Hunt et al., 2014) that demonstrated that increasing the amount of marbling, and concomitantly increasing oleic acid in beef, contributes substantially to...
the ability of consumers to discriminate between levels of marbling in beef.

The Biology of Marbling

Histology of marbling

Marbling adipose tissue, also known as interfascicular or intramuscular adipose tissue, represents a unique adipose tissue depot. It can be distinguished from other fat depots by its location within perimysial connective tissues alongside muscle fibers (Moody and Cassens, 1968; Brooks et al., 2011b; Figure 4a & 4b). For this reason, marbling adipocytes are thought to arise from the differentiation and lipid filling of fibroblasts within the perimysial connective tissue strands between muscle bundles.

Marbling consists of clusters of individual cells (adipocytes), and intramuscular adipocytes increase both in number and size in cattle as beef progresses from being Practically Devoid to higher marbling scores (Moody and Cassens, 1968; Schiavetta et al., 1990; May et al., 1994; Brooks et al., 2011b).

Marbling or steatosis?

In cattle as well as other livestock species, intramuscular adipocytes are almost invariably located within the perimysial connective tissue, between muscle fiber bundles (fasciculi) (Moody and Cassens, 1968; Brooks et al., 2011b; Figure 4a & 4b). However, in *longissimus* muscle from Japanese Black A5 cattle (the highest quality grade in Japan), marbling adipocytes have been observed within muscle bundles (Smith et al., 2000; Figure 5), indicating that a cell type unique from perimysial connective tissue fibroblasts can be induced to differentiate into marbling adipocytes. The presence of marbling adipocytes in muscle from Japanese Black cattle, together with the markedly reduced number of myofibers per bundle, suggests the conversion (transdifferentiation) of muscle cells (satellite cells) to adipocytes.

This raises the question as to whether marbling adipose tissue should be considered an unusual metabolic condition in some beef cattle breed types. Muscle growth and repair are dependent on the proliferation and fusion of muscle
satellite cells to existing muscle fibers. Under the right conditions, satellite cells can develop into adipogenic (fat-forming) cells. Velleman et al. (2010) demonstrated marbling adipocytes in pectoralis muscle of chicks that were feed-restricted immediately post hatch (Figure 6). Adipocytes formed between muscle bundles (like true marbling) as well as within muscle bundles (as seen in muscle from Japanese Black cattle). The deposition of adipocytes within muscle bundles in chicken pectoralis muscle is consistent with the conversion of muscle satellite cells to adipocytes.

To date, the mechanisms responsible for the trans-differentiation of muscle cells to adipocytes in beef cattle have not been identified, as this process is quite difficult to measure in livestock species. However, cell culture studies suggest that fatty acids derived from marbling adipocytes within muscles actually may promote this process. Subcutaneous and marbling adipose tissue and muscle fibers from Japanese Black cattle are exceptionally high in oleic acid (Sturdivant et al., 1992; May et al., 1993; Chung et al., 2006b). Can endogenously produced oleic acid by adipose tissues and/or muscle fibers promote trans-differentiation of muscle cells to marbling adipocytes?

Chung and Johnson (2009) demonstrated that treating muscle cells in culture with 100 µM oleic acid and other growth factors up-regulated CCAAT enhancer building protein β (C/EBPβ) and peroxisome proliferator-activated receptor gamma (PPARγ) gene expression and down-regulated myogenin gene expression (Figure 7). Increased expression of C/EBPβ and PPARγ is associated with differentiation and lipid filling of adipocytes, whereas reduced myogenin gene expression indicates a depression in muscle fiber development. Thus, the combination of oleic acid and growth factors caused bovine muscle cells to change into lipid-filling adipocytes, which may explain the extreme marbling in beef from Japanese A5 cattle. However, to date research has not demonstrated infiltration of muscle bundles with marbling adipocytes in U.S. domestic cattle, so the contribution of muscle myogenic cells to normal marbling development is questionable.

**Glucose and acetate metabolism in marbling fat**

Early research from the author’s laboratory demonstrated that marbling adipose tissue preferentially uses glucose as the carbon source for fat synthesis, whereas subcutaneous adipose tissue preferentially uses acetate (Smith and Crouse, 1984; Figure 8a). In marbling adipose tissue, acetate and lactate contributed less than 20% of the carbon for fat synthesis, whereas glucose contributed approximately 70% of the carbon. The reverse was seen for subcutaneous adipose tissue; in fact, glucose contributed less than 5% of the total carbon for fat synthesis in this adipose tissue depot.

As cattle become fatter, the contribution of glucose to fat synthesis decreases while the use of acetate for fat synthesis increases, especially in marbling adipose tissue (Figure 8b). Thus, providing sources of dietary glucose (or precursors for glucose) at early ages may promote marbling development more than if glucose or...
its precursors are fed at later stages of development. Myers et al. (1999) and Meyer et al. (2005) demonstrated that early weaning of beef steers promotes greater marbling development at slaughter than normal weaning of steers, and this may have been caused by increased glucose availability (from the grain-based rations) at the early stages of marbling development.

Because of ruminal metabolism of cellulose and starch, very little free glucose is absorbed from the gastrointestinal tract of cattle, and ruminants must rely on the ruminal production of propionate to satisfy their...
biological requirement for glucose. Propionate is converted to glucose in the liver, a process that requires vitamin B12 (also provided by ruminal bacteria). Glucose synthesis from propionate is energy-consuming and blood glucose levels in cattle are lower than in monogastrics such as pigs and humans. For this reason, bovine subcutaneous adipose tissue uses acetate as the building material for fatty acids and spares glucose for tissues with an absolute requirement for glucose such as red blood cells. Marbling adipose tissue is unique in that it is highly dependent on glucose as a precursor for fatty acid synthesis, particularly in young cattle.

Fatty acid composition of bovine adipose tissue

The most abundant fatty acid in beef is oleic acid (Figure 9a). The SFAs palmitic (16:0) and stearic (18:0) contribute substantially to the overall fatty acid composition of beef and beef fat, whereas there is very little linoleic acid (18:2n-6) in beef fat. There are even lesser amounts of α-linoleic acid (18:3n-3, an omega-3 fatty acid) and conjugated linoleic acid (18:2cis-9, trans-11). Marbling adipose tissue contains more stearic and less oleic acid than subcutaneous adipose tissue (discussed in detail below), which affects both the healthfulness and functionality of marbling adipose tissue in meat products.

The melting point of beef fat is determined by the ratio of MUFA to SFA. Saturated fatty acids have melting points around 70°C, whereas MUFA have melting points below room temperature (around 20°C). Linoleic acid (a polyunsaturated fatty acid that is common in corn oil) has a very low melting point, -20°C, but there is little linoleic acid in beef. Monounsaturated fatty acids have low melting points because of their chemical structure, which contains a single double bond located approximately in the middle of the molecule (Figure 9b). The double bond causes a kink in the molecule, and this hinders the formation of the crystalline structure of solidified fat. Therefore, the more double bonds present in a fatty acid, the lower the melting point.

Marbling development and total MUFA

Across a broad range of production conditions, there is a significant correlation between the concentration of MUFA and amount of intramuscular lipid in longissimus muscle (Chung et al., 2006b; reviewed in Smith et al., 2006). This is especially evident in cattle with the genetic propensity to marble who are fed grain-based diets for extended periods of time (Figure 10). In addition to providing carbon for marbling adipose tissue development, grain-based diets also increase MUFA in marbling by stimulating the expression of stearoyl coenzyme A desaturase (SCD; Δ9-desaturase). Angus steers fed high-starch diets had more elevated longissimus muscle SCD expression, as well as expression
of other genes associated with adipogenesis, than did steers fed a low starch diet (Martin et al., 1999; Chung et al., 2007; Duckett et al., 2009; Graugnard et al., 2010). The promotion of SCD gene expression therefore leads to increased MUFA in muscle concurrent with the differentiation of marbling adipocytes.

The use of bovine preadipocyte cell lines has provided additional insight into the regulation of SCD gene expression and MUFA concentrations in bovine adipose tissue. Preadipocytes are isolated from fat depots, and lipid filling can be stimulated powerfully by the addition of PPARγ agonists (such as pioglitizone), insulin, and dexamethasone (Figure 11a). Early differentiation is characterized by the expression of genes such as those encoding SCD, leading to an increase in MUFA (Figure 11b). The cell culture data, combined with information from growing cattle, indicate that promoting marbling in beef cattle with grain-based diets also will increase total MUFA in beef.

The \textit{trans}-10, \textit{cis}-12 isomer of conjugated linoleic acid (t10,c12 CLA) strongly depresses SCD gene expression and thereby decreases the synthesis of MUFA (Chung et al., 2006a [Figure 11b]). This is unusual in light of the fact that t10,c12 CLA is a product of rumen fermentation, and its accumulation would effectively block the conversion of trans-vaccenic acid (a primary product of ruminal fermentation) to \textit{cis}-9, \textit{trans}-11 CLA (c9,t11 CLA). The strong depression of SCD gene expression by t10,c12 CLA in bovine preadipocyte culture systems suggests that any production strategy to increase t10,c12 CLA in beef ultimately would reduce the endogenous production of the c9,t11 isomer in beef. It also would depress the synthesis of MUFA in general, which would lead to increases in SFA, especially stearic acid.
**Melting points of lipids and fatty acid composition**

Melting points of lipids directly affect the perception of juiciness in beef, and fatty acids directly influence and have a profound effect on lipid melting points. This is best demonstrated in comparisons across species or dietary treatments (Figure 12). The melting point of lipids from pigs fed standard finishing diets is approximately 30°C (86°F), but is less than 25°C (77°F) in pigs fed canola oil (which is rich in MUFA and polyunsaturated fatty acids). Conversely, sheep fat contains over 30% stearic acid and only about 30% oleic acid, and for this reason has a high melting point (approximately 40°C or 104°F).

Cattle fed a standard, corn-based finishing diet in the United States produce backfat and marbling fat that have consistently low melting points (approximately 86°F). The situation is different in Australia, where grains such as barley or wheat are fed in place of corn. When these grains are fed in combination with whole cottonseed or rumen-protected cottonseed oil, the melting point of the fat can exceed 45°C (113°F). This fat is very hard because it is very high in SFA. When Australian cattle are fed a corn-based diet, the melting point of the fat is reasonably low, and resembles the backfat of feedlot cattle produced in the United States. Lipids extracted from the fat of Japanese Black cattle or Murray Grey cattle raised in Japan have melting points as low as 24°C (75°F) (Figure 12).

**Figure 12.** Melting points of lipids extracted from backfat of various species. Fat samples were obtained from pigs or pigs fed canola oil (Can. Pigs); from grass-fed sheep; from grain-fed cattle; from corn-fed cattle; from cattle fed whole cottonseed (CS-fed) or protected CS (Prot. CS-fed); from Japanese Black cattle, or from Murray Grey cattle. The J. Black and M. Grey cattle were raised in Japan by traditional practices, and the other animals were raised in Australia. Adapted from Smith et al. (1998)

**Figure 13a.** Fatty acid composition of intramuscular adipose tissue (marbling) dissected from the longissimus (loin) muscle of U.S. crossbred cattle or Japanese Black cattle raised in Japan. Adapted from Sturdivant et al. (1992)

**Figure 13b.** Slip point as a function of the concentration of stearic acid in lipids extracted from 8 bovine subcutaneous fat depots from 50 carcasses. Turk and Smith (2009)
Subcutaneous fat from Japanese cattle contains much less stearic acid, and much more oleic acid, than carcass fat from typical U.S. domestic cattle (Figure 13a). The same is true for marbling fat from Japanese Black cattle (Sturdivant et al., 1992). The primary determinant of lipid melting points (measured as slip points) is stearic acid (Figure 13b). Brisket fat, like the carcass fat from Japanese cattle, is very high in oleic acid (and proportionately low in stearic acid), so it also has a very low melting point. Conversely, fat from the flank and plate (commonly used in the production of ground beef) is very high in stearic acid and concomitantly has a high melting point. This information indicates that genetics and fat depot both influence the fatty acid composition and fat functionality.

Brisket subcutaneous fat is unique in that oleic acid is elevated even in young cattle (9 months of age) and increases only slightly between 9 and 16 months of age (Smith et al., 2012) (Figure 14a). In contrast, oleic acid is low in rib subcutaneous and marbling fat at 8 months of age, but increases substantially from 8 months of age onward (Figure 14a). Several studies have documented the expression of the genes associated with adipose tissue differentiation and lipid metabolism, including SCD, C/EBPβ, and PPARγ (Cameron et al., 1994; Martin et al., 1999; Chung et al., 2006a; Duckett et al., 2009; Brooks et al., 2011a; Smith et al., 2012; Choi et al., 2013; 2014a,b). As seen for fatty acid composition, there are profound differences in C/EBPβ, PPARγ, and SCD gene expression among fat depots (Figures 14b – 14d). SCD and C/EBPβ gene expression increased between 14 and 16 months of age in marbling fat, whereas PPARγ gene expression increased earlier in growth, between 12 and 14 months of age. These data confirm that, compared to subcutaneous fat, marbling develops later in growth.

Brisket fat warrants additional comment, as its fatty acid composition and pattern of adipogenic gene expression were quite unusual. Although oleic acid was highest in brisket fat at all ages tested (Figure 14a), SCD gene expression was low in brisket fat and did not change with age (Figure 14b). C/EBPβ and PPARγ gene expression declined to nearly undetectable levels in brisket fat, but increased in rib intramuscular and subcutaneous fat between either 12 and 14 or 14 and 16 months of age (Figures 14c & 14d). The low level of SCD gene expression and decline in C/EBPβ and PPARγ gene expression over time suggests that brisket subcutaneous fat is a more mature adipose tissue than rib fat or marbling. From a
practical standpoint, lean and fat trims from the brisket can be used from cattle of any practical age to produce a product that contains elevated levels of oleic acid. Conversely, oleic acid does not increase in marbling until much later in growth, and the concentration of oleic acid in marbling (as well as in muscle) never is as high as in fat trim from the loin and brisket.

Management factors that influence the extent and/or composition of marbling adipose tissue

**Calf-feeding versus yearling-feeding**

Calf-fed steers are fed high-concentrate finishing diets at weaning, whereas yearling-fed steers typically are fed native pasture until approximately 12 months of age. Thus, calf-fed steers are younger at slaughter. In the recent past, when corn was relatively inexpensive and especially during times of drought, calves were adapted to corn soon after weaning. However, the use of grains for the production of ethanol has increased the price of grains such that producers often are feeding more distillers grains or are keeping their calves on pasture for longer periods of time. This is especially attractive to that producer segment who market beef as coming from grass-fed cattle.

Relative to calf feeding, backgrounding calves on pasture until 12 months of age may promote harder, more saturated fat. Angus steers fed hay-based diets have depressed SCD gene expression and SCD enzyme activity compared to steers fed corn-based diets (Chung et al., 2007), and correspondingly lower concentrations of MUFA in subcutaneous fat (Chung et al., 2006b). Therefore, initial backgrounding of calves on pasture could elicit a similar depression in SCD gene expression, reducing the concentration of MUFA in beef.

In one study, steers were adapted at 8 months of age to a corn-based finishing diet (calf-fed), or were allowed to graze native pasture until 12 months of age (yearling-fed) (Brooks et al., 2011a,b). Because of poor pasture conditions, at 10 months of age the yearling-fed steers were supplemented with sufficient concentrate to provide 2 pounds per day average daily gain. Cattle were sampled at 8, 12 and 16 months of age. The yearling-fed steers also were sampled at 17.5 months of age, at which time they had achieved the same body weights as the calf-fed steers (1,200 pounds). As predicted, fat thickness increased at a greater rate in the calf-fed steers, but the yearling-fed steers had the same fat thickness by the final sampling time (Figure 15a). Similarly, the yearling-fed steers had similar marbling scores as the calf-fed steers by the final sampling period (i.e., at the same body weight) (Figure 15b).

The MUFA:SFA ratios in yearling-fed subcutaneous fat never achieved the values observed in subcutaneous fat from calf-fed steers (Figure 15c). This suggests that the accumulation of SFA in subcutaneous fat of the yearling-fed calves prior to weaning and during the time on pasture diluted the MUFA that subsequently were synthesized and deposited in their adipose tissues. Dilution of MUFA by preformed fatty acids was not observed in marbling, probably because so little lipid had accumulated in the marbling of the yearling-fed steers prior to being switched to the high-corn diet. It also is clear from these data and previous reports (Sturdivant et al., 1992; May et al., 1993; Archibeque et al., 2005) that marbling represents a more saturated fat depot than subcutaneous fat.
SCD gene expression (Figure 15d). SCD gene expression was barely detectable in marbling and subcutaneous fat at 12 months of age in the yearling-fed steers, and then increased once the steers were adapted to the corn-based, finishing ration. This supports data from previous studies that have reported that beef from grass-fed cattle is higher in SFA, and lower in MUFA, than beef from grain-fed steers (Chung et al., 2006b; Leheska et al., 2008; Duckett et al., 2009; Adams et al., 2010; Gilmore et al., 2011).

**Conclusions**

For beef cattle, the development of marbling is more complex than the development of subcutaneous fat. The results of these studies indicate that grain-based diets are necessary to promote the development of marbling. Furthermore, grain-based diets increase the healthfulness and juiciness of beef by promoting the production of oleic acid in marbling and other fat depots.

The depression in MUFA caused by backgrounding cattle on pasture apparently was caused by a depression in...


References


