# Relationship of Fatty Acid Composition to Intramuscular Fat Content in Beef from Crossbred Wagyu Cattle[1]

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**ABSTRACT:** The deposition of i.m. fat, or marbling, in cattle is recognized as a desirable carcass trait in North American beef grading schemes. In order to investigate the relationship between degree of marbling and fatty acid composition of whole bovine muscle, we extracted the total lipid from pars costalis diaphragmatis (PCD) (n = 23) and longissimus (n = 36) muscles from Wagyu crossbred cattle that were assigned Canadian Grading Agency marbling scores ranging from 1 to 8 on an inverse 10-point scale (i.e., a score of 1 indicated "very abundant" marbling and a score of 10 would be assigned to a carcass "devoid" of marbling). Fatty acid methyl esters (FAME) of the total lipid and triacylglycerol fractions were resolved and quantified through GLC. Marbling scores were negatively associated with total lipid from both PCD ($r = -.57$, $P < .01$) and longissimus ($r = -.80$, $P < .001$). Differences between PCD and longissimus were found for almost all FAME studied from both lipid fractions, but no differences ($P > .05$) were seen when the monounsaturated:saturated fatty acid (MUFA/SFA) ratios were compared. Heifers had higher ($P < .05$) oleic acid content and lower ($P < .05$) palmitic acid content in lipid extracted from both muscles, resulting in higher ($P < .05$) MUFA/SFA ratios than those for steers. The relative amount of myristic acid increased as the lipid content (total lipid and triacylglycerol) increased in either longissimus (r values from .48 to .55; n = 36; $P < .01$) or PCD muscles (r from .67 to .76; n = 23; $P < .001$). The relative amount of linoleic acid (*cis*-9, *cis*-12 isomer) from total lipid was negatively associated with all chemical measurements of lipid from the longissimus (r from –.52 to –.64; n = 36; $P < .001$) and PCD muscles (r from –.75 to –.85; n = 23; $P < .001$). This association was not significant ($P > .1$) for either muscle when linoleic acid from the triacylglycerol fraction was examined, suggesting the negative association between this fatty acid and lipid content was due to a dilution of membrane phospholipids with increasing triacylglycerol. Indices of fatty acid elongase activity, calculated from FAME data, implicated the balance between this enzyme activity and fatty acid synthase as a source of variation between animals displaying various degrees of marbling and worthy of further investigation to better understand the process of marbling fat deposition in beef cattle.

Key Words: Beef Cattle, Carcass Quality, Fatty Acids, Lipids, Meat Composition, Skeletal Muscle

 J. Anim. Sci. 1999. 77:1717–1725

## Introduction

Lipid fatty acid composition in any feedstuff is a major determinant of product quality. In beef, fatty acid composition affects its shelf-life, palatability, and nutritive value. For example, oleic acid seems to be beneficial for reducing plasma total cholesterol and total low-density lipoprotein cholesterol in humans (Grundy, 1986, 1989), and it contributes to better taste panel evaluations for portions of cooked beef (Dryden and Marchello, 1970). The anticarcinogenic properties of conjugated linoleic acid have been

[1]The authors are thankful for the support of the Beef Industry Development Fund (BIDF), Agriculture and Agri-Food Canada's Matching Investments Initiative, and the Agritech International Corp. The authors would like to thank the reviewers of this manuscript for many useful comments and criticisms.

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Received June 16, 1998.
Accepted February 12, 1999.

documented (Ha et al., 1987; Ip et al., 1991), and beef has been shown to be relatively rich in this fatty acid (Chin et al., 1992).

Well-marbled beef is rewarded with better scores in North American beef grading schemes, and much of the lipid present in closely trimmed cuts of beef is contributed by the i.m. or marbling fat (i.e., i.m. or interfascicular adipose tissue). There have been some inconsistent reports of changes in i.m. fatty acid composition as this depot gets larger; depot size is often based on the visually assessed marbling score. Waldman et al. (1968) found no significant relationships between fatty acid composition and marbling score. Skelley et al. (1973) reported weak negative relationships between marbling score and myristic (C14:0) and stearic (C18:0) acids from longissimus and a positive relationship between marbling score and oleic acid (C18:1). In contrast, Dryden and Marchello (1970) found a positive correlation between myristic acid and marbling score and negative correlations between marbling score and two fatty acids, pentadecanoic (C15:0) and linoleic (C18:2) acid.

The objective of this study was to determine possible associations between relative amounts of fatty acids and the quantity of lipid present in bovine muscles. This was accomplished using two different muscles from crossbred Wagyu cattle that exhibited a wide variation in marbling.

## Materials and Methods

### *Animals and Diet*

Crossbred steers (n = 15) and heifers (n = 21) were used in this study and were either 50 or 75% Wagyu in genetic makeup. The 50% Wagyu cattle were progeny of Angus dams bred with Wagyu semen, and 75% Wagyu animals were progeny of crossbred Wagyu dams (50% Angus, 50% Holstein, or 50% Simmental) bred with Wagyu semen. All animals were cared for according to guidelines set by the Canadian Council on Animal Care (CCAC, 1984), were fed the same diet, and were reared in individual pens. The finishing diet consisted of 79% rolled barley, 20% barley silage, and 1% mineral mix on a dry matter basis. The composition of the mineral mix (per 100 kg) was as follows: 47.7 kg NaCl; 15.0 kg $Ca_2PO_4$; 15.0 kg $CaCO_3$; 10.0 kg Dynamate (Premiere Agri-Feeds, Lethbridge, AB, Canada); 1.3 kg $ZnSO_4$; 1.5 kg $MnSO_4$; 492 g $CuSO_4$; 7.8 g ethylene-diamine-dihydraiodide (80%); 5.5 g $Na_2SeO_3$; 6.0 g $CoSO_4$; and 9.0 kg vitamins A, D, and E. The vitamin concentrations (per kilogram of vitamin mix) were as follows: $10 \times 10^6$ IU vitamin A, $1 \times 10^6$ IU vitamin D, and 100,000 IU vitamin E.

Time of slaughter was determined by both live weight (75% Wagyu > 450 kg; 50% Wagyu > 490 kg) and backfat thickness (> 10 mm) as determined by ultrasonography (Park et al., 1994). Animals were slaughtered between the ages of 509 and 560 d.

### *Tissue Lipid Extractions*

Samples of the pars costalis diaphragmatis (PCD) muscle from 23 of the 36 carcasses were obtained within 30 min after exsanguination, frozen in liquid $N_2$, and stored at −80°C until required. Immediately prior to lipid extraction, each PCD sample was thawed, any visible external fat and connective tissue were removed, and approximately 10 g of the remaining tissue was minced with a scalpel to ensure homogeneity.

Total lipids were extracted using a hexane-isopropanol solvent system (Hara and Radin, 1978). After gravimetric determination of total lipid, extracted from approximately 1 g of minced PCD, the lipid extract was dissolved in 3 mL of hexane and stored under $N_2$ gas at −20°C until subsequent methylation.

Carcasses were assessed by a federal grader 24 h postmortem. After grading, samples of longissimus muscle (seventh to ninth rib) were obtained from all 36 animals, ground three times through a 3.2-mm plate, and held frozen (−20°C) prior to measurement of solvent-extractable fat. Lipids from the longissimus muscle were extracted using the procedure described above.

### *Hydrolysis, Esterification, and Separation of Lipid Fractions*

In order to separate the triacylglycerol fraction from total lipid, 50 μL of each total lipid mixture (containing between .1 and .5 mg lipid) was applied to a prerun HPTLC-Fertigplatten Kieselgel 60 plate (VWR Canlab, Mississauga, ON, Canada), with a triolein standard applied in a separate lane. The TLC plate was developed in hexane:diethyl ether:acetic acid (80:20:1), fatty acids were visualized with $I_2$ vapor, and the corresponding sections in lanes adjacent to the standard (triacylglycerol) were scraped into test tubes. To determine the total acyl lipid in the total lipid samples, 50 μL of the original extract in hexane was added directly to a tube. Heneicosanoic acid (C21:0) in hexane (12.5 μg; Sigma-Aldrich, Oakville, ON, Canada) was added to all tubes (triacylglycerol and total acyl lipid) as an internal standard to determine the proportions of triacylglycerol and total acyl lipid as a percentage of total lipid. Methylation was carried out using methanolic HCl in sealed tubes at 50°C for 24 h as described by Christie (1992). Upon cooling, .25 mL of water was added to each tube, and fatty acid methyl esters were extracted three times with 3 mL of hexane. The combined organic fraction was dried at 40°C under a stream of $N_2$, resuspended in .5 mL of hexane, transferred to a GLC vial, flushed briefly with $N_2$, and sealed.

Separation of fatty acid methyl esters (**FAME**) was performed on a flame ionization gas chromatograph (model 5890, Hewlett Packard, Mississauga, ON,

Canada) equipped with a J&W Scientific 30-m DB-23 Megabore column (Chromatographic Specialties, Brockville, ON, Canada) with helium as the carrier gas at a flow rate of 12 mL/min. Initial temperature was 180°C for 5 min, increased to 230°C by 2°C/min. Peaks were assigned by comparing retention times to those of FAME standards from two suppliers (Nu-Chek-Prep, Elysian, MN; Sigma-Aldrich), and relative proportions were determined as percentages of summed peak areas.

### *Statistical Analyses*

Data were analyzed using JMP IN statistical software (version 3.2.1, Duxbury Press, Toronto, ON, Canada) according to procedures outlined by Sall and Lehman (1996) and least squares analysis of variance using the GLM procedure (SAS, 1990). The model for the latter included breed and sex as main effects. Age at slaughter was used initially as a covariate but was excluded from the model after it was determined not to be a significant source of variation. Paired *t*-tests were performed between muscles for individual FAME concentrations from both lipid fractions (total lipid and triacylglycerol). Pearson correlation coefficients were generated between individual FAME concentrations and other carcass traits (e.g., marbling score, gravimetric total lipid, and age). For comparative analyses between the two muscles, data were limited to the 23 animals for which samples of both longissimus and PCD were obtained.

## Results and Discussion

Beef carcass marbling is measured on a 10-point inverse scale in Canada, with a score of 1 indicating "very abundant" marbling and a score of 10 representing a carcass "devoid" of marbling. The carcasses in this study displayed a large variation in marbling, scoring between 1 and 8 with a mean (5.0; SEM = .30) that translated to a "moderate" degree of marbling. When compared to a 1991 survey of over 20,000 Canadian beef carcasses, for which the average score was just over 8 ("slight" marbling; Jones et al., 1991), the crossbred Wagyu animals in the present study support the reputation of this breed to deposit greater amounts of i.m. fat compared with other breeds of beef cattle (May et al., 1993).

Average total lipid extracted from the longissimus muscle (8.7% wet weight; SEM = .74) was found to be two to three percentage points higher than typical values from previous studies of various types of beef cattle (Marchello et al., 1968; Skelley et al., 1973; Miller et al., 1981). Total lipid from the PCD muscle (mean of 18.0% wet weight; SEM = 1.22) was approximately twice that from longissimus and supported visual observations that the PCD contained considerably more i.m. fat than longissimus muscle in any given animal. Surveys of total lipid extracted from various bovine muscles have been reported (Hornstein et al., 1967; O'Keefe et al., 1968; Terrell et al., 1969b), and of these only the psoas major (Terrell et al., 1969b) and PCD (Hornstein et al., 1967) had more extractable lipid than longissimus. Hornstein et al. (1967) also found that the PCD from four Angus steers had the highest lipid content and the highest phospholipid content in five muscles examined. In agreement with our results, the average total lipid extracted from the PCD in their study was slightly more than twice that extracted from the longissimus. The high lipid content of PCD is indicative of the marbling found in this muscle. Intramuscular adipose tissue can be separated from the muscle and connective tissues through dissection (Middleton et al., 1998). The relative abundance of i.m. adipose tissue and ease of access at most commercial abattoirs make this muscle useful for studying bovine i.m. fat depots.

Fatty acid profiles from total acyl and triacylglycerol fractions of both muscles are shown in Table 1. Total acyl lipid represented the fraction of total extracted lipid from which methyl esters were formed (i.e., including phospholipids and triacylglycerol). Trace species, defined as representing less than .5% of total fatty acids, are not shown (i.e., C14:1, C15:0, C18:2 t9t12, C18:2 c9t11, C18:3, and C20:4) and together accounted for 1 to 2% of the total FAME from each lipid fraction. Trace data, however, were included in the calculation of total FAME, as well as monounsaturated fatty acid (**MUFA**) to saturated fatty acid (**SFA**) ratios. Indices of $\Delta^9$-desaturase and elongase enzymes (Malau-Aduli et al., 1997, 1998) were calculated and are shown in Tables 1 and 2. In general, the FAME profiles presented herein agree with previous reports of fatty acid composition of whole bovine muscle (e.g., Marchello et al., 1968; Hecker et al., 1975; Eichhorn et al., 1986), as well as a study involving FAME from dissected i.m. fat and longissimus from crossbred Wagyu steers (Sturdivant et al., 1992).

Differences between the two sample muscles were found for all the fatty acids studied (Table 1), except for the predominant form of linoleic acid (C18:2 c9c12) from total acyl lipid and C17:0 from the triacylglycerol fraction. The MUFA/SFA ratios derived from the data did not differ between longissimus and PCD and were comparable to ratios calculated from data presented in the literature, which ranged from 1.02 to 1.12 for neutral and total extracted lipid from various muscles (O'Keefe et al., 1968; Marmer et al., 1984; Siebert et al., 1996). Sturdivant et al. (1992) showed the MUFA/SFA ratio to be closer to 2 in three tissues (s.c. adipose, i.m. adipose, and longissimus muscle) from steaks obtained from purebred Japanese Wagyu cattle, a result of increased amounts of palmitoleic (C16:1) and oleic (C18:1) acids, as well as a decrease in stearic acid (C18:0) content. In a

Table 1. Fatty acid composition of total acyl lipid and triacylglycerol fractions in longissimus and pars costalis diaphragmatis (PCD) muscles from Wagyu crossbred cattle[a]

| | Total acyl lipid | | Triacylglycerol | |
|---|---|---|---|---|
| FAME[b] | Longissimus | PCD | Longissimus | PCD |
| Total, % of wet wt | 8.07$^{w}$ ± .72 | 16.68$^{x}$ ± 1.31 | 7.73$^{y}$ ± .71 | 15.53$^{z}$ ± 1.22 |
| C14:0 | 3.02$^{w}$ ± .13 | 1.72$^{x}$ ± .13 | 3.32$^{y}$ ± .10 | 1.65$^{z}$ ± .15 |
| C16:0 | 31.04$^{w}$ ± .38 | 26.03$^{x}$ ± .39 | 31.69$^{y}$ ± .34 | 26.32$^{z}$ ± .44 |
| C16:1 c9[c] | 4.02$^{w}$ ± .12 | 2.18$^{x}$ ± .06 | 4.10$^{y}$ ± .12 | 2.13$^{z}$ ± .07 |
| C17:0 | .76$^{s}$ ± .04 | .90$^{t}$ ± .07 | .86 ± .04 | .93 ± .07 |
| C18:0 | 12.60$^{w}$ ± .31 | 18.79$^{x}$ ± .49 | 12.55$^{y}$ ± .32 | 19.08$^{z}$ ± .52 |
| C18:1 t11[d] | .39$^{s}$ ± .09 | .79$^{t}$ ± .12 | .33$^{u}$ ± .08 | .71$^{v}$ ± .13 |
| C18:1 c9[e] | 42.58$^{w}$ ± .53 | 44.92$^{x}$ ± .73 | 42.55$^{y}$ ± .52 | 45.33$^{z}$ ± .78 |
| C18:1 c11[f] | 1.54$^{w}$ ± .04 | 1.44$^{x}$ ± .04 | 1.46$^{q}$ ± .03 | 1.41$^{r}$ ± .04 |
| C18:2 c9c12[g] | 1.88 ± .09 | 2.00 ± .09 | .98$^{q}$ ± .06 | 1.21$^{r}$ ± .09 |
| MUFA/SFA[h] | 1.05 ± .02 | 1.05 ± .03 | 1.02 ± .02 | 1.05 ± .03 |
| $\Delta^9$-Desaturase (C16)[i] | 11.43$^{w}$ ± .26 | 7.73$^{x}$ ± .20 | 11.43$^{y}$ ± .27 | 7.49$^{z}$ ± .21 |
| $\Delta^9$-Desaturase (C18)[j] | 77.18$^{w}$ ± .47 | 70.48$^{x}$ ± .78 | 77.24$^{y}$ ± .49 | 70.34$^{z}$ ± .81 |
| Elongase (C16–C18)[k] | 61.13$^{w}$ ± .55 | 69.28$^{x}$ ± .50 | 60.60$^{y}$ ± .52 | 69.34$^{z}$ ± .55 |

[a]Data for individual FAME presented as mean percentage of total ± SE.
[b]FAME = fatty acid methyl esters.
[c]*Cis*-9-hexadecenoic (palmitoleic) acid.
[d]*Trans*-11-octadecenoic acid.
[e]*Cis*-9-octadecenoic (oleic) acid.
[f]*Cis*-11-octadecenoic acid.
[g]*Cis*-9-*cis*-12-octadecadienoic (linoleic) acid.
[h]MUFA = monounsaturated fatty acids (sum of C14:1, C16:1, and C18:1); SFA = saturated fatty acids (sum of C14:0, C15:0, C16:0, C17:0, and C18:0).
[i]$\Delta^9$-Desaturase (C16): index of C16 desaturase activity = [C16:1/(C16:0 + C16:1)]100.
[j]$\Delta^9$-Desaturase (C18): index of C18 desaturase activity = [C18:1c9/(C18:0 + C18:1c9)]100.
[k]Elongase (C16–C18): index of C16 to C18 elongase activity = [(C18:0 + C18:1c9)/(C16:0 + C16:1 + C18:0 + C18:1c9)]100.
[q,r,s,t,u,v,w,x,y,z]Means with these pairs of superscripts within the same row differ significantly (paired *t*-test): [qr]($P < .05$), [st]($P < .01$), [uv]($P < .01$), [wx]($P < .001$), [yz]($P < .001$).

different experiment within the same report, dissected i.m. fat and longissimus muscle from crossbred Wagyu steers revealed MUFA/SFA ratios of 1.22 and 1.19, respectively.

Zembayashi et al. (1995) reported significantly higher MUFA/SFA ratios in intramuscular neutral lipid from purebred and 3/4 Japanese Black (Wagyu) steers compared with Holstein and 1/4 Japanese Black crossbred steers, suggesting a genetic predisposition for increased synthesis and(or) deposition of MUFA in the Wagyu breed. Supporting this conclusion, May et al. (1993) showed that 3/4 and 7/8 Wagyu steers had significantly higher MUFA/SFA ratios than their Angus counterparts in longissimus muscle lipid extracts, as well as s.c. and i.m. adipose tissues. Our results (Table 1) were considerably lower and were closer to values obtained from Angus (May et al., 1993), Holstein (Zembayashi et al., 1995), and Hereford (Skelley et al., 1973) breeds than to values from Wagyu. We also found no difference ($P > .1$) in MUFA/SFA ratios between the breed types for either muscle (data not shown).

The explanation for the relatively low MUFA/SFA ratios we report here likely has more to do with age at slaughter than with Wagyu genetic influence. The animals in the present study were slaughtered between 17 and 19 mo of age, compared with approximately 27 mo for the crossbred Wagyu steers studied by Sturdivant et al. (1992), which was closer to the typical age at slaughter for Wagyu cattle in Japan (30 to 34 months; Sturdivant et al., 1992). Fatty acids from bovine muscle and s.c. fat have been shown to increase in the degree of unsaturation with age (Waldman et al., 1968; Hecker et al., 1975). Therefore, animals closer to maturity would exhibit higher MUFA/SFA ratios than younger animals. We also found no relationships between age at slaughter and MUFA, SFA, or the MUFA/SFA ratio ($P > .1$), supporting the claim that the animals in the present study were slaughtered before changes in degree of unsaturation would have become apparent.

The 75% Wagyu animals were approximately 25 d older ($P < .001$) than 50% Wagyu cattle at slaughter, a direct result of the maturity requirements described in Materials and Methods, but there were no breed effects ($P > .1$) with respect to marbling scores or measures of lipid content in either muscle. Similarly, breed did not influence the content of individual FAME ($P > .05$, data not shown). Stearic acid (C18:0) in both the total acyl and triacylglycerol fractions from longissimus was the only fatty acid to exhibit a trend with age (data not shown). The relationship was

negative and not significant ($P = .07$) but suggested that the activity of $\Delta^9$-stearoyl-CoA desaturase, the enzyme responsible for converting stearic to oleic acid, may have increased in level of expression or activity during the period over which the animals were slaughtered. This hypothesis, however, was not supported by any trends between indices of $\Delta^9$-desaturase and age for either muscle ($P > .1$). There were no significant age × breed or age × muscle interactions (data not shown).

Differences were found between muscles ($P < .001$) in the calculated desaturase indices (Table 1). The relative proportions of conversion of palmitic to palmitoleic acid, and stearic to oleic acid, were both substantially higher in longissimus than in PCD, suggesting that $\Delta^9$-desaturase activity was higher in longissimus. Stearoyl-CoA desaturase, the enzyme responsible for the conversion of C14:0, C16:0, and C18:0 to their (*n*-9) monounsaturated counterparts, is quantitatively more important in adipose than in muscle tissue (Sturdivant et al., 1992). When the increased adiposity of the PCD compared to longissimus is considered, one would expect to see higher desaturase indices from the PCD data, but the opposite trend was observed ($P < .001$). Although the proportion of oleic acid was indeed higher in the PCD than in longissimus, the amount of saturated precursor (i.e., C18:0) was disproportionately higher in the PCD, leading to the lower index.

The elongase index, which provided an estimate of C16 to C18 fatty acid elongase activity, was higher ($P < .001$) with PCD than with longissimus data, suggesting that this enzyme was more active in PCD than in longissimus (Table 1). Supporting this, both stearic and oleic acid were more prevalent ($P < .001$) in the PCD, and myristic, palmitic, and palmitoleic acids were more abundant ($P < .001$) in longissimus lipids (Table 1). The relatively high phospholipid content of the PCD (Hornstein et al., 1967) could not be the reason for this observation, because the trend was found in the total acyl and triacylglycerol lipid fractions.

The influence of gender was apparent in several of the measurements taken. There were no differences ($P > .1$) between sexes in the amount of solvent-extracted fat (i.e., total lipid and triacylglycerol), but heifers received better marbling scores than steers ($P < .05$). Heifers also had higher MUFA/SFA ratios than steers in both total lipid and triacylglycerol extracted from PCD ($P < .05$) and longissimus ($P < .01$), a

Table 2. Fatty acid composition of the total acyl lipid fraction of longissimus and pars costalis diaphragmatis (PCD) muscles from Wagyu crossbred heifers and steers[a]

| | Longissimus | | PCD | |
|---|---|---|---|---|
| FAME[b] | Heifers | Steers | Heifers | Steers |
| Total, % of wet wt | 8.44 ± .66 | 7.45 ± .78 | 15.73 ± 1.83 | 17.71 ± 1.91 |
| C14:0 | 2.85 ± .14 | 3.13 ± .16 | 1.54 ± .18 | 1.92 ± .19 |
| C16:0 | 30.34$^{u}$ ± .36 | 31.48$^{v}$ ± .43 | 25.31$^{w}$ ± .50 | 26.82$^{x}$ ± .52 |
| C16:1 c9[c] | 4.03 ± .11 | 3.98 ± .14 | 2.19 ± .09 | 2.17 ± .09 |
| C17:0 | .74 ± .04 | .83 ± .04 | .77$^{w}$ ± .08 | 1.05$^{x}$ ± .09 |
| C18:0 | 12.33 ± .31 | 12.96 ± .36 | 18.45 ± .69 | 19.16 ± .72 |
| C18:1 t11[d] | .45 ± .09 | .28 ± .11 | .61 ± .15 | .99 ± .16 |
| C18:1 c9[e] | 43.65$^{u}$ ± .52 | 41.83$^{v}$ ± .62 | 46.55$^{w}$ ± .89 | 43.14$^{x}$ ± .93 |
| C18:1 c11[f] | 1.60 ± .04 | 1.49 ± .04 | 1.49 ± .05 | 1.38 ± .05 |
| C18:2 c9c12[g] | 1.71 ± .08 | 1.93 ± .10 | 1.99 ± .13 | 2.01 ± .14 |
| MUFA/SFA[h] | 1.10$^{y}$ ± .02 | 1.00$^{z}$ ± .03 | 1.12$^{w}$ ± .04 | .98$^{x}$ ± .04 |
| $\Delta^9$-Desaturase (C16)[i] | 11.70 ± .26 | 11.20 ± .31 | 7.96 ± .28 | 7.48 ± .29 |
| $\Delta^9$-Desaturase (C18)[j] | 77.97$^{u}$ ± .47 | 76.38$^{v}$ ± .55 | 71.65 ± 1.04 | 69.20 ± 1.09 |
| Elongase (C16–C18)[k] | 61.94 ± .53 | 60.69 ± .63 | 70.24$^{w}$ ± .64 | 68.24$^{x}$ ± .67 |

[a]Data for individual FAME presented as mean percentage of total ± SE.
[b]FAME = fatty acid methyl ester.
[c]*Cis*-9-hexadecenoic (palmitoleic) acid.
[d]*Trans*-11-octadecenoic acid.
[e]*Cis*-9-octadecenoic (oleic) acid.
[f]*Cis*-11-octadecenoic acid.
[g]*Cis*-9-*cis*-12-octadecadienoic (linoleic) acid.
[h]MUFA = monounsaturated fatty acids (sum of C14:1, C16:1, and C18:1); SFA = saturated fatty acids (sum of C14:0, C15:0, C16:0, C17:0, and C18:0).
[i]$\Delta^9$-Desaturase (C16): index of C16 desaturase activity = [C16:1/(C16:0 + C16:1)]100.
[j]$\Delta^9$-Desaturase (C18): index of C18 desaturase activity = [C18:1c9/(C18:0 + C18:1c9)]100.
[k]Elongase (C16–C18): index of C16 to C18 elongase activity = [(C18:0 + C18:1c9)/(C16:0 + C16:1 + C18:0 + C18:1c9)]100.
[u,v,w,x,y,z]Means with these pairs of superscripts within the same row differ significantly (ANOVA/*t*-test): [uv]($P < .05$), [wx]($P < .05$), [yz]($P < .01$).

Table 3. Simple correlation coefficients between selected fatty acid methyl ester(s) (FAME) and marbling score and properties of the lipid from crossbred Wagyu longissimus muscle[a]

| FAME (TAL)[b] | TL[c] | TAG[d] | Marbling score |
|---|---|---|---|
| C14:0 | .48** | .55** | –.28† |
| C16:0 | .10 | .09 | –.07 |
| C16:1 c9[e] | .32† | .27 | –.20 |
| C18:0 | –.34* | –.31† | .34* |
| C18:1 c9[f] | –.23 | –.22 | .12 |
| C18:2 c9c12[g] | –.64*** | –.52*** | .73*** |
| Elongase[h] | –.31† | –.29† | .20 |
| Marbling score | –.80*** | –.60*** | — |

[a]n = 36.
[b]FAME from the total acyl lipid fraction.
[c]Total lipid (percentage of wet weight).
[d]Triacylglycerol (percentage of wet weight).
[e]*Cis*-9-hexadecenoic (palmitoleic) acid.
[f]*Cis*-9-octadecenoic (oleic) acid.
[g]*Cis*-9,*cis*-12-octadecadienoic (linoleic) acid.
[h]Elongase: index of C16 to C18 elongase activity = [(C18:0 + C18:1c9)/(C16:0 + C16:1 + C18:0 + C18:1c9)]100.
†$P < .10$.
*$P < .05$.
**$P < .01$.
***$P < .001$.

result of both higher ($P < .05$) amounts of oleic acid and lower ($P < .05$) amounts of palmitic acid (Table 2). These results are supported by reports of the effects of gender on C16:0 and C18:1 content in bovine adipose (Waldman et al., 1968; Terrell et al., 1969a; Hecker et al., 1975) and muscle (Waldman et al., 1968; Hecker et al., 1975) tissues. Heifers also had a higher ($P < .05$) desaturase index in longissimus and a higher ($P < .05$) elongase index in PCD than did steers (Table 2). No sex differences were found in the relative concentrations of myristic and linoleic acids, which are discussed in more detail below.

Marbling score was negatively associated with total lipid and triacylglycerol content of both muscles (Tables 3 and 4). The correlations were negative because the Canadian grading system uses a scale from 10 ("devoid" of marbling) to 1 ("very abundant" marbling) and were stronger with longissimus (Table 3) than with PCD data (Table 4). The latter observation reflected the anatomical distance of the sampling sites from the grading site. There were also significant correlations between the two muscles in the amount of extracted lipid (Table 5), indicating that i.m. fat deposition in cattle is coordinated among muscles that display marbling. This is supported by a report of total lipid extracted from three muscles of Hereford steers (Marchello et al., 1968). Using two different extraction procedures, they found that marbling score was positively correlated with the amount of lipid retrieved from all muscles, with r values ranging from .56 (semimembranosus) to .94 (triceps brachii and longissimus). These observations support the general premise that, while assessed at a specific point on the beef carcass (i.e., longissimus muscle), marbling score reflects the relative amount of i.m. fat deposition in the entire carcass.

The relative amount of myristic acid (C14:0) in total acyl lipid was positively associated with all chemical lipid measurements of the longissimus (Table 3, Figure 1b) and the PCD muscles (Table 4, Figure 1a). The results of previous studies comparing this fatty acid to carcass fat measurements have been mixed. In a study of Angus and Polled Hereford steers, Skelley et al. (1973) found a weak but significant correlation between myristic acid and lipid content of longissimus. Several other reports have indicated that lipid content is unrelated to this fatty acid (Waldman et al., 1965; Terrell et al., 1968; Waldman et al., 1968). In agreement with the present study, Dryden and Marchello (1970) found positive relationships between myristic acid and marbling score and between myristic acid and total i.m. lipid from three muscles from a group of Hereford steers. The correlation was essentially unaffected when data from the longissimus, triceps brachii, and semimembranosus muscles were combined ($r = .57$, $P < .01$).

The myristic acid found in the PCD and longissimus reported here was likely generated through de novo synthesis. With respect to dietary origin, myristic acid has not been found in barley (Hilditch and Williams, 1964), but it has been detected in small amounts in the barley silage used in this study (1.5% of total FAME). The silage contained 1.4% total lipid, and the diet contained 20% barley silage, resulting in a diet

Table 4. Simple correlation coefficients between selected fatty acid methyl ester(s) (FAME) and marbling score and properties of the lipid from crossbred Wagyu pars costalis diaphragmatis (PCD) muscle[a]

| FAME (TAL)[b] | TL[c] | TAG[d] | Marbling score |
|---|---|---|---|
| C14:0 | .76*** | .67*** | –.55** |
| C16:0 | .44* | .39† | –.40† |
| C16:1 c9[e] | .29 | .45* | –.49* |
| C18:0 | –.35 | –.44* | .51* |
| C18:1 c9[f] | –.38† | –.32 | .15 |
| C18:2 c9c12[g] | –.75*** | –.85*** | .52* |
| Elongase[h] | –.59** | –.59** | .52* |
| Marbling score | –.57** | –.62** | — |

[a]n = 23.
[b]FAME from the total acyl lipid fraction.
[c]Total lipid (percentage of wet weight).
[d]Triacylglycerol (percentage of wet weight).
[e]*Cis*-9-hexadecenoic (palmitoleic) acid.
[f]*Cis*-9-octadecenoic (oleic) acid.
[g]*Cis*-9,*cis*-12-octadecadienoic (linoleic) acid.
[h]Elongase: index of C16 to C18 elongase activity = [(C18:0 + C18:1c9)/(C16:0 + C16:1 + C18:0 + C18:1c9)]100.
†$P < .10$.
*$P < .05$.
**$P < .01$.
***$P < .001$.

Table 5. Simple correlation coefficients between selected fatty acid methyl ester(s) (FAME) and lipid quantities of two muscles from crossbred Wagyu cattle[a]

| | Longissimus | | | | | |
|---|---|---|---|---|---|---|
| | C14:0 | | C18:2[b] | | | |
| Pars costalis diaphragmatis | TAL[c] | TAG[d] | TAL[c] | TAG[d] | % TL[e] | % TAG[f] |
| C14:0 (TAL)[c] | .36[†] | .29 | –.45* | –.26 | .28 | .35[†] |
| C14:0 (TAG)[d] | .12 | .14 | –.43* | –.21 | .35[†] | .24 |
| C18:2[b] (TAL)[c] | –.11 | –.18 | .64*** | .47* | –.38[†] | –.34 |
| C18:2[b] (TAG)[d] | .36[†] | .15 | .43* | .72*** | .08 | .01 |
| % TL[e] | .27 | .33 | –.44* | –.08 | .48* | .43* |
| % TAG[f] | .31 | .33 | –.55** | –.17 | .49* | .46* |

[a]n = 23.
[b]*Cis*-9,*cis*-12-octadecadienoic (linoleic) acid.
[c]Fatty acid from the total acyl lipid fraction.
[d]Fatty acid from the triacylglycerol fraction.
[e]Total lipid content (percentage of wet weight).
[f]Triacylglycerol content (percentage of wet weight).
[†]$P < .10$.
*$P < .05$.
**$P < .01$.
***$P < .001$.

that contained less than .01% C14:0 on a fresh weight basis (and at most .3% C14:0 in the total dietary fat). This finding, combined with the fact that the feed was identical for all animals in the study, makes it unlikely that differences we observed in myristic acid content were dietary or ruminal. Regarding de novo synthesis, myristic acid can result from premature release from the fatty acid synthase complex (Gurr and Harwood, 1991), the typical end product being palmitic acid. Little is known about myristic acid with respect to differences in rates of de novo synthesis or of its incorporation into muscle or adipose lipids in cattle.

The proportion of stearic acid in the lipid extracted from both muscles was positively associated with marbling score (Tables 3 and 4). Although this relationship was stronger than that between myristic acid and marbling score in longissimus (Table 3), the associations between myristic acid and the objective measurements of fat content (i.e., total lipid and triacylglycerol) were stronger than those seen with stearic acid (Tables 3 and 4).

The relative content of linoleic acid (C18:2 c9c12) from the total acyl fractions of whole muscle tissue exhibited a negative correlation ($P < .001$) with all objective measures of lipid taken from both muscles and a positive association with Canadian marbling score (Tables 3 and 4). These findings are supported by those reported by Dryden and Marchello (1970); linoleic acid content from three muscles was shown to be negatively correlated with U.S. marbling score ($r = –.47$, $P < .05$). Similarly, statistically nonsignificant correlations of –.20 and –.16 were described by Waldman et al. (1968) and Skelley et al. (1973), respectively, between linoleic acid from longissimus and marbling score.

Linoleic acid is an essential fatty acid, and the ingested form is largely hydrogenated (70 to 90%) by ruminal microflora (Doreau and Ferlay, 1994). It is the predominant polyunsaturated fatty acid in bovine tissues (Eichhorn et al., 1986; St. John et al., 1987; Kinney Sweeten et al., 1990), and it is most prevalent in the phospholipid fraction (Eichhorn et al., 1986; Kinney Sweeten et al., 1990). The increased amount of linoleic acid in phospholipids leads to the hypothesis that the negative association observed between linoleic acid and extracted lipid may have been due to a dilution of total membrane lipids with increasing amounts of triacylglycerol. This, in fact, turned out to be the case in the present study. When linoleic acid from the triacylglycerol fraction was examined with respect to muscle lipid content, the relationship was absent ($P > .1$, data not shown), as suggested by the correlation coefficients between muscles (Table 5). The negative association between this fatty acid and measures of muscle lipid content reported by others (Waldman et al., 1968; Dryden and Marchello, 1970; Skelley et al., 1973) was also likely due to such a dilution effect, because none of these groups separated the triacylglycerol fraction from their lipid extracts.

Compared with previous studies, the negative correlations of linoleic acid with lipid content of both longissimus and PCD in this study were stronger. This might be explained through our choice of solvents for lipid extraction (i.e., hexane-isopropanol). In a study comparing phospholipid recovery using several lipid extraction procedures, Kolarovic and Fournier (1986) reported that hexane-isopropanol yielded more phospholipid for 9 of the 10 phospholipid classes they studied than three separate methods based on the chloroform-methanol solvent system. Thus, one would expect the negative relationship between linoleic acid

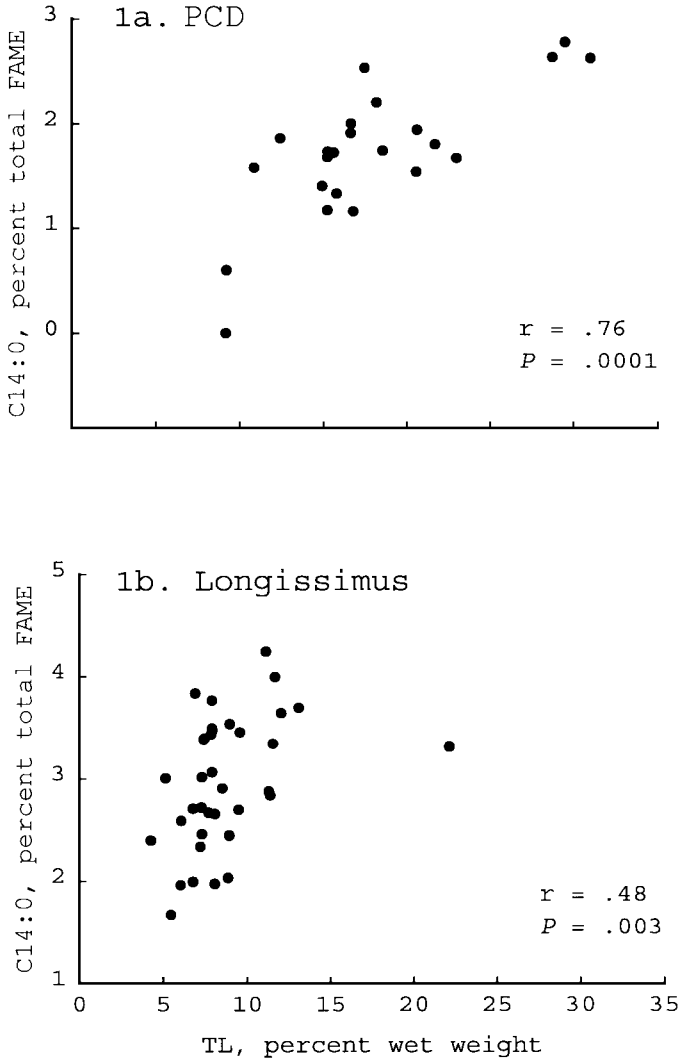


Figure 1. Relationship between myristic acid (C14:0) content and total lipid (TL) extracted from (a) pars costalis diaphragmatis (PCD, n = 23) and (b) longissimus (n = 36) muscles from crossbred Wagyu cattle. Simple correlation coefficients and probability levels are shown.

and lipid content to be stronger, and more significant, with a solvent system exhibiting greater phospholipid and comparable neutral lipid recovery (Kolarovic and Fournier, 1986).

There seemed to be two general and opposite trends between C18 and C14/C16 fatty acids with respect to lipid content in both muscles. The proportions of myristic, palmitic, and palmitoleic acids showed a general increase with increasing lipid content, and the proportions of stearic and oleic acids tended to decrease (Tables 3 and 4). These data suggest that the enzyme responsible for the conversion of C16 to C18 fatty acids, fatty acid elongase, may have been unable to keep pace with de novo production of palmitic acid in animals that deposited greater amounts of marbling fat. Such an imbalance between the activities of fatty acid synthase and elongase enzymes could lead to the trends presented here. The correlation coefficients between elongase indices and measures of fat content from both muscles also support this idea (Tables 3 and 4), although not significantly for longissimus data.

The trends between fatty acid composition and lipid content of bovine muscles presented here hint at possible physiological mechanisms responsible for differential i.m. fat deposition. A study involving younger animals that controls for diet, breed, age, and gender effects might reveal similar trends and(or) when the described trends become apparent in cattle. If these (or similar) associations are shown to exist in younger animals, the results may prove useful in bull and(or) cow selection in breeding programs aiming to improve the general population for the marbling trait.

## Implications

The relative proportions of fatty acids in lipid extracted from two muscles obtained from Wagyu-cross beef cattle at slaughter and the lipid content of the muscles are related. The relative proportion of myristic acid in the lipid increased and the relative proportion of linoleic acid in the lipid decreased as marbling increased. The greater proportions of myristic acid in animals with superior marbling was likely the result of de novo synthesis, rather than the influence of diet, and merits further investigation. The negative relationship between linoleic acid and marbling may be due to a simple dilution of this fatty acid, a major component of membrane phospholipids, with increasing amounts of triacylglycerol. Based on an index of fatty acid elongase activity, the balance in activity between this enzyme and fatty acid synthase may be related to differences among animals in their propensity to deposit marbling fat.

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