# Adipocyte fatty acid-binding protein and mitochondrial enzyme activities in muscles as relevant indicators of marbling in cattle[1]

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**ABSTRACT:** Marbling is an important criterion for beef quality grading in many countries. The purpose of the current study was to utilize the natural genetic variation to identify major metabolic indicators of marbling in cattle differing in genotypes. Rectus abdominis (RA, oxidative), semitendinosus (glycolytic), and longissimus thoracis (LT, oxido-glycolytic) muscles were taken from steers of different genotypes that expressed high [Angus, n = 16; and crossbred (Angus × Japanese Black), n = 10] or low (Limousin, n = 12) levels of marbling in their meat. Muscles from Angus and crossbred steers were characterized, as expected, by a greater triacylglycerol (TAG) content ($P < 0.001$) and also by greater protein contents of fatty acid-binding protein specific for heart and muscles (H-FABP; $P < 0.001$ for RA and $P < 0.05$ for LT muscle) or for adipocytes (A-FABP; $P < 0.001$ for RA and LT muscles). Moreover, oxidative enzyme activities ($\beta$-hydroxyacyl-CoA dehydrogenase, citrate synthase, isocitrate dehydrogenase, cytochrome-*c* oxidase) were greater ($P < 0.01$ to 0.001) in the 3 muscles studied, whereas glycolytic enzyme activities (phosphofructokinase and lactate dehydrogenase) were lower ($P < 0.001$) in RA muscle in Angus and crossbred steers compared with Limousin steers. Significant correlations were observed between TAG content and H- and A-FABP protein contents, and oxidative ($r \geq +0.55$, $P < 0.001$) or glycolytic enzyme activities ($r \geq -0.47$, $P < 0.001$), when the 3 genotypes and muscles studied were considered as a whole. In addition, A-FABP protein content and some oxidative enzyme activities were significantly correlated with TAG content independently of the genotype and muscle effects. In conclusion, A-FABP protein content, as well as oxidative enzyme activities, may be used as indicators of the ability of steers from extreme genotypes to deposit intramuscular fat.

**Key words:** triacylglycerol, marbling, fatty acid-binding protein, muscle metabolism, genotype, steer



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## INTRODUCTION

Intramuscular fat (**IMF**) content, commonly called marbling, refers to the visually discernible deposits of fat within muscles. The IMF content affects sensory properties and nutritional value of meats (Geay et al., 2001). The IMF content can reach up to 20 to 30% in superior quality Japanese beef. Because Australia exports a high proportion of its beef production to Japan, Australian researchers wish to understand which biological mechanisms may increase IMF but at the same time limit total carcass adiposity. In contrast, in France, the IMF content is very low, at 5% on average (Bas and Sauvant, 2001). However, with young European bulls, the IMF content can be too low (less than 2.5%) to ensure a sufficiently enjoyable taste for beef consumers. Whatever the country, it is thus important to understand the genetic and biological mechanisms that could lead to new rearing strategies favorable to IMF deposition.

Triacylglycerol (**TAG**), the major component of IMF in muscles, is stored to a minor extent within the myofibers and mostly within the intramuscular adipocytes

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(Pethick et al., 2004). The IMF content results from the balance between synthesis and degradation of TAG. Consequently, many metabolic pathways in adipocytes and myofibers could contribute to the variation of IMF content, including the ability of muscle to utilize circulating lipoprotein-associated TAG via the lipoprotein lipase activity, the intracellular trafficking of fatty acids by the fatty acid-binding proteins (**FABP**), the muscle capacity for de novo fatty acid synthesis from carbon precursors, and the activities of key enzymes that direct fatty acids toward oxidation within mitochondria or storage within intramuscular adipocytes (Hocquette et al., 1998).

The aim of this work was to take advantage of the natural genetic variation among genotypes to develop marbling to identify precisely the major muscular metabolic differences. The contribution of some of them to the variability of fat deposition within muscle was also studied and considered as major metabolic indicators of marbling.

## MATERIALS AND METHODS

### *Animals and Samples*

This study was carried out in compliance with the French recommendations and those of the Animal Care and Use Committee of INRA for the use of experimental animals including animal welfare and appropriate conditions (guidelines April 18, 1988).

Four groups of steers from 3 different genotypes were used: 2 groups of Angus and 1 group of crossbred, Angus × Japanese Black with a marbled meat, and 1 group of Limousin producing less marbled meat. All animals were subjected to a long finishing period (6 or 10 mo) with a cereal-rich diet, which allowed them to express their genetic potential for intramuscular fat deposition.

In Exp. 1, 1 group of 12 Limousin steers and 2 groups of 10 Angus and 10 crossbred, Angus × Japanese Black steers were finished for 6 mo with a similar diet on a DM basis [flattened wheat (47 to 50%); triticale (17 to 18%); hay of natural prairie (14 to 18%); lupins (9%); ME, 12 KJ/kg of DM; CP, 15% of DM;]. Limousin and Angus steers were slaughtered at 23 mo of age, and crossbred steers were slaughtered at 28 mo of age. In Exp. 2, 1 group of 8 Angus steers was finished for 10 mo and slaughtered at 28 mo of age. These extremely fat animals were compared with the extremely lean Limousin of Exp. 1. Angus steers from Exp. 1 and 2 were termed Angus-1 and Angus-2, respectively.

Limousin steers were finished in the experimental animal facilities of the Herbivore Research Unit of the National Institute for Agricultural Research (INRA, Institut National de la Recherche Agronomique), Clermont-Ferrand/Theix, France. Angus-1 and crossbred Angus × Japanese Black were reared at the Department of Primary Industries, Victoria research station at Rutherglen, Victoria, whereas Angus-2 steers were reared on the Western Australia Department of Agriculture's experimental research farm at Vasse, Western Australia. The French and Australian cattle were slaughtered at the research center's experimental slaughterhouse and a commercial slaughterhouse, respectively, without the use of electrical immobilization or stimulation.

At the time of slaughter, 3 muscle types were sampled from Limousin steers: rectus abdominis (**RA**, oxidative), longissimus thoracis (**LT**, oxido-glycolytic), and semitendinosus (**ST**, glycolytic). The RA and ST samples were taken from Angus-1 and crossbred, Angus × Japanese Black steers (Exp. 1), and LT samples were taken from Angus-2 steers (Exp. 2). Samples were removed from the center of each muscle; LT samples at the 6th rib and ST samples in the superficial portion, taken within 30 min of stunning, immediately frozen in liquid nitrogen, and kept at –80°C awaiting analyses. The Australian samples were maintained in dry ice during the transfer from Australia to France.

### *TAG and Total Protein Contents*

Total lipids were extracted from muscle samples using chloroform-methanol (2:1, vol/vol) according to the method of Folch et al. (1957), and TAG were determined from total lipid extracts, as described by Leplaix-Charlat et al. (1996). Total protein contents of muscles were determined by the method of Bradford (1976).

### *FABP Protein Contents*

The FABP protein contents were determined by ELISA, as described by Piot et al. (2000), on cytosolic protein preparations from muscles, using a polyclonal antibody raised against rat heart and skeletal muscle (**H-FABP**) or rat adipocyte (**A-FABP**) isoforms, respectively. The results were expressed as arbitrary densitometric units because pure bovine FABP were not available to convert the data to micrograms per gram of wet tissue weight.

### *A-FABP and H-FABP mRNA Contents*

Quantifications of A-FABP and H-FABP mRNA were performed for LT muscle only, at least in quadruplicate, with real-time reverse transcription (**RT**)-PCR using the Light Cycler System (Roche Diagnostics, Meylan, France) technology relative to a standard curve. Total RNA was extracted using TRIzol Reagent (Invitrogen SARL, Cergy-Pontoise, France) and purified using the Nucleospin RNA II kit (Macherey Nagel, Hoerdt, France). Purity and concentration of RNA was checked with the RNA 600 Nano Assay kit using the Bioanalyzer (Agilent Technologies, Waldbronn, Germany). For the assay, cDNA was synthesized by RT from 2.5 μg of total RNA in a 20-μL final volume using 100 U of Superscript II reverse transcription (Rnase H-; Invitrogen Life Technologies, Cergy-Pontoise, France) and 10 pmol of oligo(dT), as described in the manufacturer's protocol. Amplification was performed in a total volume of 20 μL from 2 μL of cDNA (diluted 1:4, vol/vol sterile water) with the LightCycler-FastStart DNA Master Hybrid-

ization Probes reaction mix according to the manufacturer's protocol.

For the PCR, the following primers were used to amplify a fragment of the *A-FABP* gene: forward: 5′-GGT ACCTGGAAACTTGTCTCC-3′; reverse: 5′-CTGATTT AATGGTGACCACAC-3′ (0.5 μ*M* final concentration each, MWG-Biotech, Courtabeuf, France). The sequence of the fluorescent probe was 6FAM-ACATGAA AGAAGTGGGCGTGGGCT XT–PH (0.2 μ*M* final concentration; TIB MOLBIOL, Berlin, Germany). These sequences were based on the available A-FABP sequences in GenBank database (human, BC003672 and NM_001442; pig, Y16039; and rat, U75581). For the PCR, the following primers were used to amplify a fragment of the *H-FABP* gene: forward: 5′-CCTCTCCTTC CACTGACTGC-3′; reverse: 5′-TTGACCTCAGAGCAC CCTTT-3′ (0.2 μ*M* final concentration each, MWG-Biotech). The sequence of the fluorescent probe was 6FAM-CACCAGATTGCCTCATTTTTCTCC XT C'PH (0.2 μ*M* final concentration; TIB MOLBIOL). These sequences were based on the available H-FABP sequences in GenBank database (bovine, NM_174313; Billich et al., 1988).

The amplified fragments were sequenced to check their specificity. The cycling conditions of PCR included a first denaturation at 95°C for 8 min and 40 cycles of denaturation at 95°C for 5 s followed by a hybridation/elongation phase of 60 s at 60°C. The standard was a pool of cDNA obtained from the 2 breeds (Limousin and Angus-2). All the samples (diluted 1:4, vol/vol sterile water) and the standard of the PCR (diluted 1, 1:2, 1:4, 1:16, 1:32, vol/vol sterile water) were analyzed within the same run. Different PCR runs were performed so that, for each sample, at least 2 RT and 2 PCR per RT were performed and analyzed relative to the standard curve for 3 amounts of total RNA (10, 50, and 100 ng). Efficiency of the PCR ranged from 95 to 100%. The results were expressed in arbitrary units per milligram of total RNA. The technical variability between assays was equal to 30 to 40% on average. Variability between individuals ranged from 64 to 82% depending on the breed. For each mRNA, the levels were expressed by comparison with the level of cyclophilin mRNA, a housekeeping gene, measured by real-time RT-PCR, as described previously (Bonnet et al., 2000).

### *Glycolytic and Oxidative Enzyme Activities*

Maximal activity levels of the enzymes reflecting the glycolytic metabolic pathway (phosphofructokinase [**PFK**], lactate dehydrogenase [**LDH**]), or the potential for fatty acid β-oxidation (hydroxyacyl-CoA dehydrogenase [**HAD**]), mitochondrial density (isocitrate dehydrogenase [**ICDH**], citrate synthase [**CS**]), and oxidative phosphorylation (cytochrome-*c* oxidase [**COX**]; for review see Hocquette et al., 1998) were measured spectrophotometrically according to the methods of Beutler (1971), Ansay (1974), Bass et al. (1969), Briand et al. (1981), Shepherd and Garland (1969), and Smith and Conrad (1956), respectively. The detailed protocols were reported by Jurie et al. (2006). One unit of the enzyme was defined as the amount that catalyzes per minute the disappearance of 1 μmol of NADH for PFK, LDH, and HAD; the reduction of 1 μmol of NADP for ICDH; the liberation of 1 μmol of coenzyme A for CS; and the oxidation of 1 μmol of cytochrome-*c* for COX. The results were expressed in micromoles or nanomoles·minute$^{-1}$·milligram of protein$^{-1}$.

### *Statistical Analysis*

The SAS software (SAS Inst. Inc., Cary, NC) was used for all statistical analyses. Data from animal characteristics were analyzed, for Exp. 1 and 2 separately, using the GLM procedure in a model that contained the genotype fixed effect. Prior to statistical analysis, data from muscle characteristics were submitted to log transformation and were analyzed using the MIXED procedure. Data from genotypes (Limousin, Angus-1, and Angus × Japanese Black steers) and RA and ST muscles (Exp. 1) were analyzed in a model that contained the genotype, muscle, and genotype × muscle interaction as fixed effects, with animal nested within genotype and being treated as a random effect. Data from genotypes (Limousin and Angus-2 steers) and LT muscle (Exp. 2) were analyzed in a model that contained the genotype as the fixed effect. Cyclophilin mRNA levels were introduced as a covariate in the statistical model for the analysis of FABP mRNA levels, as recommended by Hocquette and Brandstetter (2002). When significant effects were detected, differences in means were further separated by the PDIFF option of SAS. All results were presented as means ± SEM. Because the main objective of this study was not to compare the genotypes but to use the natural genetic variability to describe the variability in muscle characteristics, simple correlation coefficients were calculated using the CORR procedure of SAS.

## RESULTS

### *Animal Characteristics*

At the beginning of the finishing period, Limousin steers from Exp. 1 presented an initial live weight greater ($P < 0.001$) than Angus-1 and crossbred steers (+34 and +19%, respectively). At slaughter, Limousin steers always presented a live weight and a carcass weight greater ($P < 0.001$) than Angus-1 (+19 and +32%, respectively) and crossbred steers (+16 and +30%, respectively), although the average daily gain of the Angus-1 steers was significantly greater (1,165 g/d, $P < 0.05$) than the Limousin (1,022 g/d) and the crossbred (984 g/d) steers. Fat thickness at the 11th rib cut was greater ($P < 0.05$) for Angus-1 and crossbred than Limousin steers (+29 and +45%, respectively; Table 1).

The Angus steers of Exp. 2 (Angus-2) were characterized by a longer finishing period than the steers of the

**Table 1.** Means ± SEM for finishing period and slaughter characteristics from steers of different genotypes

| Item | Genotype | | | |
|---|---|---|---|---|
| | Limousin | Angus-1 | Crossbred[1] | Angus-2 |
| Experiment | 1 and 2 | 1 | 1 | 2 |
| No. of animals | 12 | 10 | 10 | 8 |
| Finishing period | | | | |
| Initial live weight, kg | 553 ± 6$^{gG}$ | 414 ± 6$^{h}$ | 463 ± 9$^{i}$ | 418 ± 10$^{iH}$ |
| Duration of finishing, mo | 6 | 6 | 6 | 10 |
| Average daily gain, g/d | 1,022 ± 38$^{b}$ | 1,165 ± 64$^{a}$ | 984 ± 39$^{b}$ | 1,150 ± 70 |
| Slaughter | | | | |
| Final age, mo | 23 | 23 | 28 | 28 |
| Final live weight, kg | 738 ± 10$^{g}$ | 622 ± 13$^{h}$ | 639 ± 10$^{h}$ | 755 ± 17 |
| Carcass weight, kg | 464 ± 7$^{gD}$ | 351 ± 8$^{h}$ | 356 ± 7$^{h}$ | 420 ± 11$^{E}$ |
| Fat thickness at the 11th rib cut, mm | 9.6 ± 1.0$^{bH}$ | 12.4 ± 0.8$^{a}$ | 13.9 ± 0.6$^{a}$ | 19.0 ± 2.2$^{G}$ |

$^{a,b}$Within a row, means without a common superscript letter differ ($P < 0.05$; Exp. 1).
$^{D,E}$Within a row, means without a common superscript letter differ ($P < 0.01$; Exp. 2).
$^{g,h,i,G,H}$Within a row, means without a common superscript letter differ ($P < 0.001$; lower-case letter, Exp. 1 and upper-case letter, Exp. 2).
[1]Angus × Japanese Black.

first experiment (10 vs. 6 mo). At slaughter, they were as heavy as Limousin steers but with a lower carcass weight (–10%, $P < 0.01$). Compared with crossbred steers slaughtered at the same age (28 mo), Angus-2 steers were heavier (Table 1).

### *Difference in TAG and Total Protein Contents Between Muscles and Genotypes*

In the first 3 groups of animals (Exp. 1), the TAG contents were greater ($P < 0.001$) in RA than in ST muscle: on average across genotypes (+245%, 5.17 vs. 1.50 g/100 g of fresh muscle, respectively). The TAG contents were also greater ($P < 0.001$) in Angus-1 and crossbred than in Limousin steers: on average across muscles (+349 and +332%, 4.58 and 4.41 vs. 1.02 g/100 g of fresh muscle, respectively), but the difference was slightly greater in RA than in ST muscle, the muscle × genotype interaction tending to be significant ($P = 0.07$). The TAG contents in LT muscle were intermediate between those of RA and ST muscles for Limousin steers and were 10-fold greater ($P < 0.001$) in Angus-2 than in Limousin steers (Exp. 2; Table 2).

Protein content was significantly greater in Limousin than in Angus-2 and crossbred steers: 177.1 vs. 174.4 and 166.8 mg/g ($P < 0.05$) for RA muscle and 189.3 vs. 174.2 and 175.4 mg/g ($P < 0.001$) for ST muscle. Similarly, the protein content was greater ($P < 0.01$) in Limousin than in Angus-2 steers for LT muscle, 194.5 and 173.0 mg/g, respectively (data not shown in table).

### *Differences in H-FABP and A-FABP Protein and mRNA Contents Among Muscles and Genotypes*

On average for the 3 genotypes studied (Exp. 1), H-FABP and A-FABP protein contents were greater ($P < 0.001$) in RA than in ST muscle (H-FABP: +171%, 4.04 vs. 1.49 arbitrary units, and A-FABP: +264%, 48.4 vs. 13.3 arbitrary units). However, significant muscle × genotype interactions ($P < 0.001$) were observed in H-FABP and A-FABP protein contents (Table 3). In RA muscle, H-FABP and A-FABP protein contents were greater ($P < 0.001$) from Angus-1 and crossbred compared with Limousin steers (H-FABP: +76 and +121%, respectively, and A-FABP: +159 and +335%, respectively), whereas no significant differences between genotypes were observed in ST muscle.

The data from LT muscle (Exp. 2) were similar to those obtained from RA muscle: H-FABP was greater (+64%, $P < 0.02$) and A-FABP protein content was greater (+261%, $P < 0.001$) from Angus-2 compared with Limousin steers (Table 3). Similarly, H-FABP and A-FABP mRNA levels (Exp. 2) were greater ($P < 0.001$) in LT muscle from Angus-2 compared with Limousin steers (+348 and +300%, respectively; Figure 1).

### *Differences in Metabolic Enzyme Activity Among Muscles and Genotypes*

***Glycolytic Muscle Catabolism.*** For animals of Exp. 1, glycolytic enzyme activities (PFK, LDH) were on average greater ($P < 0.001$) in ST than in RA muscle: PFK (+55%, 0.17 vs. 0.11 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ of protein) and LDH (+18%, 5.2 vs. 4.4 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ of protein). But significant muscle × genotype interactions ($P < 0.01$) were observed for PFK and LDH activities (Table 4). In RA muscle, PFK activity was greater ($P < 0.001$) in Limousin than in Angus-1 and crossbred steers (+67 and +50%, respectively), whereas in ST muscle the differences among genotypes were not significant. The LDH activity was greater in Limousin than Angus-1 and crossbred steers in RA muscle (+36 and +38%, respectively; $P < 0.001$) and in ST muscle (+19 and +12%, respectively; $P < 0.01$), thus the differences among genotypes were lower in ST than in RA.

**Table 2.** Means ± SEM for TAG content (g/100 g of fresh muscle) in rectus abdominis (RA) and semitendinosus (ST) muscles (Exp. 1) and in longissimus thoracis (LT) muscle (Exp. 2) from steers of different genotypes

| | Genotype | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|
| Muscle | Limousin | Angus-1 | Crossbred[1] | Angus-2 | Muscle | Genotype | Muscle × genotype |
| RA | 1.50 ± 0.22[b] | 6.95 ± 0.93[a] | 7.07 ± 0.71[a] | | 0.001 | 0.001 | 0.07 |
| ST | 0.54 ± 0.05[b] | 2.20 ± 0.12[a] | 1.75 ± 0.20[a] | | | | |
| LT | 1.07 ± 0.12[b] | | | 10.03 ± 0.53[a] | | 0.001 | |

[a,b]Within a row, means without a common superscript letter differ ($P < 0.001$).
[1]Angus × Japanese Black.

Moreover, PFK and LDH enzyme activities in LT muscle were generally similar to those in RA muscle, with no significant difference between Limousin and Angus-2 steers (Exp. 2; Table 4).

***Oxidative Muscle Catabolism.*** For animals of Exp. 1, no significant muscle × genotype interactions were observed for the 4 oxidative enzyme activities (HAD, CS, ICDH, COX) studied. The HAD, ICDH, and COX activities were greater ($P < 0.001$) in RA than in ST muscle: on average across genotypes HAD was +50% higher (11.9 vs. 7.9 μmol·min$^{-1}$·mg$^{-1}$ of protein), ICDH was + 88% higher (8.0 vs. 4.3 μmol·min$^{-1}$·mg$^{-1}$ of protein), and COX was +130% higher (63.8 vs. 27.7 μmol·min$^{-1}$·mg$^{-1}$ of protein, respectively), whereas CS activity did not significantly differ between RA and ST muscles (19.2 vs. 19.4 μmol·min$^{-1}$·mg$^{-1}$ of protein, respectively; Table 5). In addition average CS activity across RA and ST muscles was +31 and +29% greater ($P < 0.001$) in Angus-1 and crossbred compared with Limousin steers (21.1 and 20.7 vs. 16.1 μmol·min$^{-1}$·mg$^{-1}$ of protein, respectively). Similarly, average ICDH activity across muscles was +59 and +67% greater ($P < 0.001$, 6.9 and 7.3 vs. 4.4 μmol·min$^{-1}$·mg$^{-1}$ of protein); and average COX activity across muscles +64 and +116% higher (47.0 and 61.9 vs. 28.6 μmol·min$^{-1}$·mg$^{-1}$ of protein), in Angus-1 and crossbred compared with Limousin steers, respectively. The HAD activity was significantly greater ($P < 0.004$) in crossbred only than in compared with Limousin steers: +24% (11.3 vs. 9.1 μmol·min$^{-1}$·mg$^{-1}$ of protein; Table 5).

Oxidative enzyme activities in LT muscle were generally similar to those of RA muscle, and the above differences among genotypes were confirmed in LT muscle in Exp. 2: HAD, CS, ICDH, and COX activities were greater (+68, +67, +80, and +134%, respectively, $P < 0.001$) in Angus-2 compared with Limousin steers (Table 5).

### *Relationships Between Intramuscular TAG and Muscle Metabolic Characteristics*

All the studied parameters in the 2 experiments were characterized by significant correlation coefficients ($P < 0.001$) with intramuscular TAG content when data of the 3 muscles (RA, ST and LT) from the 4 groups of steers were taken into account (n = 84 observations, Table 6). Whereas glycolytic enzyme activities (PFK, LDH) were correlated negatively with intramuscular TAG content (r = –0.47 and –0.55, respectively), H-FABP (r = +0.56) and A-FABP (r = +0.59) protein contents and oxidative enzyme activities, HAD (r = +0.64), CS (r = +0.55), ICDH (r = +0.78), and COX (r = +0.74) were correlated positively with TAG content. These correlations were generally also observed for each muscle taken alone, except for the relationships between TAG content and H-FABP and A-FABP protein contents in

**Table 3.** Means ± SEM for contents of fatty acid-binding proteins specific to muscle fibers (H-FABP) or intramuscular adipocytes (A-FABP; arbitrary units) in rectus abdominis (RA) and semitendinosus (ST) muscles (Exp. 1) and in longissimus thoracis (LT) muscle (Exp. 2) from steers of different genotypes

| | | Genotype | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | Muscle | Limousin | Angus-1 | Crossbred[1] | Angus-2 | Muscle | Genotype | Muscle × genotype |
| H-FABP | RA | 2.44 ± 0.18[d] | 4.29 ± 0.29[c] | 5.40 ± 0.19[c] | | 0.001 | 0.001 | 0.001 |
| | ST | 1.44 ± 0.10 | 1.54 ± 0.14 | 1.50 ± 0.07 | | | | |
| | LT | 1.34 ± 0.20[b] | | | 2.20 ± 0.31[a] | | 0.04 | |
| A-FABP | RA | 18.3 ± 3.9[d] | 47.4 ± 7.6[c] | 79.6 ± 9.5[c] | | 0.001 | 0.04 | 0.001 |
| | ST | 15.5 ± 1.0 | 14.7 ± 3.2 | 9.6 ± 1.6 | | | | |
| | LT | 11.3 ± 2.2[d] | | | 40.8 ± 4.6[c] | | 0.001 | |

[a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).
[c,d]Within a row, means without a common superscript letter differ ($P < 0.001$).
[1]Angus × Japanese Black.

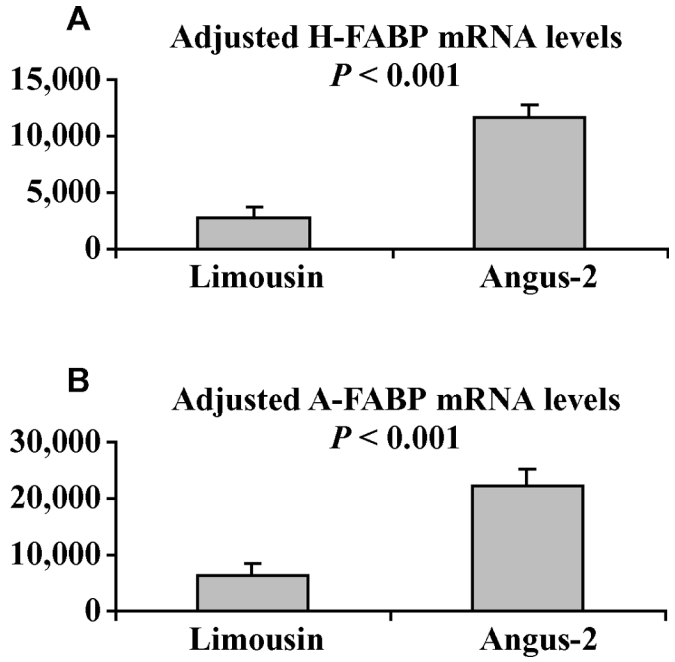


**Figure 1.** The least squares means ± SEM for (A) heart fatty acid-binding protein (H-FABP) and (B) adipocyte fatty acid-binding protein (A-FABP) mRNA levels adjusted for cyclophilin levels (the statistical model contained cyclophilin levels as a covariate) in longissimus thoracis muscle from Limousins and Angus-2 steers (Exp. 2). Results are expressed in arbitrary units per milligram of total RNA.

ST muscle and with HAD activity in RA muscle, which were not significant. Furthermore, the relationships between TAG content and H-FABP protein content in LT muscle were not significant (r = +0.40, $P$ = 0.08). In addition, for LT muscle data (n = 20 observations), the correlation coefficients were r = +0.82 ($P$ < 0.001) and r = +0.78 ($P$ < 0.001) between TAG content and H-FABP or A-FABP mRNA levels, respectively (data not shown). Moreover, residual correlation coefficients were computed after the genotype and muscle effects had been removed from RA and ST pooled data (n = 64 observations). Following this analysis, 4 of the various parameters (A-FABP protein content, HAD, CS, and ICDH activities) were still characterized by significant correlation coefficients (r ≥ +0.31) with intramuscular TAG content, which are therefore independent of genotype and muscle effects.

## DISCUSSION

Our study was conducted with steers of extreme genotypes and with different muscle types in order to display a wide range of IMF content. It is well known that animals from early- and late-maturing breeds, such as Angus and Limousin breeds, respectively, differ in growth, carcass composition, and marbling, as confirmed in recent papers (Chambaz et al., 2003; Cuvelier et al., 2006a,b). Similarly, Albrecht et al. (2006) described large differences among German Angus, Galloway, Holstein-Friesian, and double-muscled Belgian Blue bulls in IMF contents and marbling scores. These were the reasons why steers from 3 beef genotypes (Limousin, Angus, Angus × Japanese Black) greatly different in muscle development, maturity, and marbling were used in the current study. Furthermore, 3 muscles with divergent metabolic potentials, as recently confirmed for RA and ST muscles (Jurie et al., 2006), were chosen for this study. This allowed the identification of some metabolic indicators linked to fatty acid intracellular trafficking and catabolism, which may reveal major differences and hence explain part of the variability of fat deposition within muscles.

### *Differences in H- FABP and A-FABP Expression Among Muscles and Genotypes*

Both A-FABP and H-FABP are members of the FABP family that is composed of a group of small cytosolic proteins that specifically bind and transport fatty acids intracellularly. The H-FABP is expressed in various

**Table 4.** Means ± SEM for glycolytic enzyme activities[1] in rectus abdominis (RA) and semitendinosus (ST) muscles (Exp. 1) and in longissimus thoracis (LT) muscle (Exp. 2) from steers of different genotypes

| | | Genotype | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | Muscle | Limousin | Angus-1 | Crossbred[2] | Angus-2 | Muscle | Genotype | Muscle × genotype |
| PFK | RA | 0.15 ± 0.01[c] | 0.09 ± 0.01[d] | 0.10 ± 0.01[d] | | 0.001 | 0.001 | 0.01 |
| | ST | 0.18 ± 0.01 | 0.16 ± 0.01 | 0.16 ± 0.01 | | | | |
| | LT | 0.12 ± 0.01 | | | 0.10 ± 0.01 | | 0.41 | |
| LDH | RA | 5.39 ± 0.20[c] | 3.95 ± 0.12[d] | 3.90 ± 0.17[d] | | 0.001 | 0.001 | 0.01 |
| | ST | 5.74 ± 0.24[a] | 4.84 ± 0.20[b] | 5.15 ± 0.14[b] | | | | |
| | LT | 5.30 ± 0.16 | | | 4.94 ± 0.22 | | 0.18 | |

[a,b]Within a row, means without a common superscript letter differ ($P$ < 0.01).
[c,d]Within a row, means without a common superscript letter differ ($P$ < 0.001).
[1]Phosphofructokinase (PFK) and lactate dehydrogenase (LDH; μmol·min$^{-1}$·mg$^{-1}$ of protein).
[2]Angus × Japanese Black.

**Table 5.** Means ± SEM for oxidative enzyme activities[1] in rectus abdominis (RA) and semitendinosus (ST) muscles (Exp. 1) and in longissimus thoracis (LT) muscle (Exp. 2) from steers of different genotypes

| | | Genotype | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | Muscle | Limousin | Angus-1 | Crossbred[2] | Angus-2 | Muscle | Genotype | Muscle × genotype |
| HAD | RA | 11.2 ± 0.5$^{b}$ | 10.5 ± 1.0$^{b}$ | 13.9 ± 0.5$^{a}$ | | 0.001 | 0.004 | 0.20 |
| | ST | 7.0 ± 0.5$^{b}$ | 8.2 ± 0.7$^{ab}$ | 8.6 ± 0.3$^{a}$ | | | | |
| | LT | 7.6 ± 0.3$^{h}$ | | | 12.8 ± 0.8$^{g}$ | | 0.001 | |
| CS | RA | 16.7 ± 0.9$^{b}$ | 20.1 ± 1.6$^{a}$ | 20.8 ± 4.1$^{a}$ | | 0.96 | 0.001 | 0.32 |
| | ST | 15.5 ± 1.3$^{e}$ | 22.0 ± 1.6$^{d}$ | 20.6 ± 1.2$^{d}$ | | | | |
| | LT | 16.2 ± 1.0$^{h}$ | | | 27.0 ± 1.4$^{g}$ | | 0.001 | |
| ICDH | RA | 5.6 ± 0.5$^{h}$ | 8.9 ± 0.7$^{g}$ | 9.5 ± 0.3$^{g}$ | | 0.001 | 0.001 | 0.86 |
| | ST | 3.1 ± 0.3$^{h}$ | 4.9 ± 0.4$^{g}$ | 5.0 ± 0.3$^{g}$ | | | | |
| | LT | 6.6 ± 0.4$^{h}$ | | | 11.9 ± 0.6$^{g}$ | | 0.001 | |
| COX | RA | 41.0 ± 2.4$^{ch}$ | 62.3 ± 3.8$^{b}$ | 88.2 ± 9.9$^{ag}$ | | 0.001 | 0.001 | 0.62 |
| | ST | 16.1 ± 1.4$^{h}$ | 31.6 ± 4.2$^{g}$ | 3.5.5 ± 2.8$^{g}$ | | | | |
| | LT | 39.2 ± 2.4$^{h}$ | | | 91.8 ± 9.1$^{g}$ | | 0.001 | |

$^{a\text{-}c}$Within a row, means without a common superscript letter differ ($P < 0.05$).
$^{d,e}$Within a row, means without a common superscript letter differ ($P < 0.01$).
$^{g,h}$Within a row, means without a common superscript letter differ ($P < 0.001$).
[1]$\beta$-hydroxyacyl-CoA dehydrogenase (HAD), citrate synthase (CS), isocitrate dehydrogenase (ICDH), and cytochrome-*c* oxidase (COX; nmol·min$^{-1}$·mg$^{-1}$ of protein).
[2]Angus × Japanese Black.

tissues but predominantly in cardiac and oxidative skeletal muscles, whereas A-FABP is exclusively expressed within adipocytes (for a review, see Zimmerman and Veerkamp, 2002). Therefore, the relevance of A-FABP in the muscle tissue is a relative measurement of the amount of intramuscular adipose tissue or of the number of intramuscular adipocytes, which represents a small amount of the total muscle volume. To our knowledge, there are only a limited number of data available on the H-FABP expression in bovine muscles apart from the study of Moore et al. (1991) and 2 recent papers from our group (Piot et al., 2000; Brandstetter et al., 2002). By contrast, no data have been reported on the A-FABP expression in bovine muscles. So, an important contribution of this work was to describe the expression of H- and A-FABP in bovine muscles. The H- and A-FABP protein contents were greater in RA, an oxidative muscle, than in ST, a glycolytic muscle. Our results confirm thus that H-FABP protein expression levels differ between muscles with different metabolic traits, as previously observed in rats (Veerkamp and Van Moerkerk, 1993) and in cattle such as preruminant calves (Piot et al., 2000) and bulls and steers (Brandstetter et al., 2002). Similarly, A-FABP protein expression level was greater also in oxidative muscles, which suggests the same positive association between A-FABP expression and muscle oxidative metabolism.

Moreover A- and H-FABP protein contents were greater in RA and LT muscles for Angus and crossbred genotypes, which deposit much more intramuscular fat, than for Limousin breed, which produces a leaner meat. However, no significant difference in A- and H-FABP protein contents was observed between genotypes for ST muscle, although significant differences in TAG contents were observed between genotypes for this muscle. In addition, the differences in FABP protein contents

**Table 6.** Correlation coefficients (r) between triacylglycerol (TAG) muscle content and the different parameters studied

| | Fatty acid-binding protein contents[1] | | Glycolytic enzyme activities[2] | | Oxidative enzyme activities[3] | | | |
|---|---|---|---|---|---|---|---|---|
| Item | H-FABP | A-FABP | PFK | LDH | HAD | CS | ICDH | COX |
| All muscles[4] (n = 84) | +0.56*** | +0.59*** | –0.47*** | –0.55*** | +0.64*** | +0.55*** | +0.78*** | +0.74*** |
| RA muscle (n = 32) | +0.73*** | +0.70*** | –0.57*** | –0.68*** | +0.29 | +0.44* | +0.71*** | +0.64*** |
| ST muscle (n = 32) | +0.05 | –0.33 | –0.21 | –0.42* | +0.36* | +0.62*** | +0.75*** | +0.54** |
| LT muscle (n = 20) | +0.40 | +0.71*** | –0.12 | –0.17 | +0.87*** | +0.78*** | +0.82*** | +0.85*** |
| Residual[5] (n = 64) | 0.00 | +0.43*** | –0.05 | +0.01 | +0.54*** | +0.36*** | +0.31* | +0.01 |

[1]H-FABP = heart FABP; and A-FABP = adipocyte FABP.
[2]PFK = phosphofructokinase; and LDH = lactate dehydrogenase.
[3]HAD = $\beta$-hydroxyacyl-CoA dehydrogenase; CS = citrate synthase; ICDH = isocitrate dehydrogenase; and COX = cytochrome-*c* oxydase.
[4]RA = rectus abdominis; ST = semitendinosus; LT = longissimus thoracis; and n = number of observations.
[5]Residual r were computed after the genotype and muscle effects have been removed from RA and ST pooled data.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

between genotypes were confirmed by the results in H- and A-FABP mRNA levels in LT muscle from Angus and Limousin steers in which a high intramuscular fat content such as in the Angus steers was related to high expression of both FABP. Moreover, independently of genotype and muscle effects, the A-FABP protein content was significantly correlated with TAG content, so this present finding strengthened our initial hypothesis in which the A-FABP expression would be associated with the intramuscular fat content.

### *Differences in Metabolic Enzyme Activities Among Muscles and Genotypes*

A greater intramuscular TAG content was associated with greater HAD, ICDH, and COX enzyme activities, associated with oxidative nutrient catabolism and lower PFK and LDH enzyme activities, associated with glycolytic nutrient catabolism. However, the activity of CS, an enzyme of the Krebs cycle, did not follow the same pattern as the other 3 oxidative enzyme activities. This enzyme is in fact involved in the catabolism of glucose-derived pyruvate, unlike HAD and COX. The enzyme activities were in accordance with the fiber type frequencies in the muscles of steers. In Charolais steers, the RA muscle was composed of 34% oxidative slow-twitch (**SO**) fibers, 16% oxido-glycolytic fast-twitch (**FOG**) fibers and 50% glycolytic fast-twitch (FG) fibers, whereas the ST muscle was made of 13% SO fibers, 26% FOG fibers, and 62% FG fibers (our unpublished data). Moreover, according to Gotoh (2003), LT muscle from Japanese Black steers contained 31% SO fibers, 19% FOG fibers, and 50% FG fibers. Thus, in RA and LT muscles, half of the myofibers were oxidative. The differences reported here in oxidative enzyme activities depending on TAG content did not agree with recently published data in pigs, except for CS activity (Damon et al., 2006). In fact, these authors failed to find any differences in HAD and CS activities in longissimus muscle from crossbred Large White × Duroc pigs exhibiting a high or a low lipid content. But unlike our different bovine genotypes, the 2 studied pig populations did not differ in muscle fiber types. The differences between our genotypes were therefore greater than the differences between the 2 groups of pigs studied by Damon et al. (2006). In addition, the differences between Limousin and Angus-2 steers for PFK and COX activities in LT muscle were also described by Cuvelier et al. (2006b) for bulls from Belgian Blue, Limousin, and Aberdeen Angus breeds raised in the same experimental farm. But these authors described a significant difference in LDH activity and a not significant difference in CS activity between breeds as opposed to this current study. This discrepancy could be due to the type of animals (bulls vs. steers) or to the expression of enzyme activities: per gram of fresh muscle (Cuvelier et al., 2006b) and per milligram of protein (our study), the protein content being significantly different among genotypes.

As expected, the intramuscular TAG contents were indeed much greater for Angus and crossbred genotypes than for Limousin genotype independently of the muscle. In addition, Angus and crossbred steers were characterized by a high oxidative muscle metabolism and Limousin steers by a high glycolytic muscle metabolism. It was hypothesized that when TAG were catabolized to a large extend their deposition was reduced, and so more aerobic muscles would accumulate less TAG. Actually, the opposite was observed because, in our study, greater intramuscular TAG were clearly associated with high oxidative enzyme activities. So, a lower capacity for nutrient oxidation cannot explain the variability in TAG content in our experimental design.

### *Major Metabolic Indicators of Intramuscular TAG*

Based on correlation coefficients, H-FABP and A-FABP expressions at protein and mRNA levels were among the best indicators of intramuscular TAG deposition in our experiment. Earlier study failed to demonstrate any relationship between FABP activity of muscle and marbling in cattle when interfascicular adipose tissue was exhaustively removed (Moore et al., 1991); however, the techniques available at that time did not allow to distinguish between A- and H-FABP. But these authors demonstrated substantial differences in FABP activity among Charolais × Hereford, young Angus, and older Angus cattle in longissimus muscle that likely contained some marbling. More recently, polymorphisms in *A-FABP* and *H-FABP* genes have been shown to be significantly associated with genetic variation in intramuscular fat content in a Duroc pig population (Gerbens et al., 1998, 1999). Furthermore, A- and H-FABP mRNA levels, but not protein expression, were significantly related to intramuscular fat content (Gerbens et al., 2001). In contrast, Damon et al. (2006) reported an association between intramuscular fat and A-FABP protein content, but not at mRNA level in pigs. These inconsistent results could be due to the pig populations studied, which exhibited a low variability in intramuscular fat content, to the sensitivity of the techniques used (RT-PCR-ELISA and protein ELISA), or to different mechanisms of A-FABP regulation (i.e., transcriptional between breeds in our study vs. posttranscriptional within breed, Damon et al., 2006). Nevertheless, expression of the *A-FABP* gene is clearly more elevated in fat Japanese Black steers than in leaner Holstein steers in the longissimus dorsi muscle (Wang et al., 2005). Furthermore, because A-FABP was expressed within adipocytes (and not within muscle fibers), it was likely that the accumulation of A-FABP was an indicator of adipocyte numbers within the muscle tissue. This is in agreement with Cianzio et al. (1985), who demonstrated that intramuscular adipocyte number was a good predictor of marbling score, and with Damon et al. (2006), who showed in pigs positive correlations between A-FABP protein content and both

adipocyte number and intramuscular fat. Moreover, the expression level of *BTG2* gene, which was involved in preadipocyte antiproliferation, was found to be greater in low-marbled steers than in high-marbled steers (Sasaki et al., 2005). The relevance of A- and H-FABP protein contents for the prediction of marbling in our experiment was likely to be associated with the great variability among muscles and genotypes in this study.

In addition, other important indicators of TAG deposition were evidenced in the 3 studied muscles among the oxidative metabolic markers, namely HAD, CS, ICDH, and COX activities. Unlike A-FABP and H-FABP protein contents, these oxidative activities were also significantly correlated with TAG content in ST muscle, which was the leanest muscle studied. The COX activity was also shown to explain 47% of variability in intramuscular fat content between Belgian Blue, Limousin, and Aberdeen Angus young bulls (Barnola et al., 2005). In addition, cytosolic $NADP^+$-ICDH was identified as more expressed in Japanese Black than in Holstein steers in the study of Wang et al. (2005). These results were in line with those of Gondret et al. (2004) who demonstrated that intramuscular fat content variability in rabbits could not be assigned only to 1 specific energy metabolic pathway, but rather resulted from a balance between fatty acid oxidation and synthesis. Moreover, HAD, CS, and ICDH activities were significantly correlated with TAG content independently of genotype and muscle effects. So, these results suggest that fatty acid turnover, known to be greater in oxidative than in glycolytic muscles, may favor TAG deposition.

In conclusion, evidence was provided that A-FABP expression as mRNA level and protein content, indicators of intramuscular adipocyte number, and oxidative enzyme activities and associated with fatty acid catabolism and hence fat turnover, may be major metabolic indicators of the ability of animals to deposit intramuscular fat according to muscle types and breeds. Moreover, a part of the variability in intramuscular fat content may be linked to other metabolic pathways such as de novo lipogenesis, which indeed differed between different bovine genotypes (M. Bonnet, personal communication) and between groups of rabbits at different ages (Gondret et al., 2004). It was likely that the contribution or the relevance of these other indicators were different between muscle types or breeds because A-FABP expression did not differ in ST despite variability in TAG content.

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