Glucose-6-phosphate dehydrogenase and leptin are related to marbling differences among Limousin and Angus or Japanese Black x Angus steers

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Glucose-6-phosphate dehydrogenase and leptin are related to marbling differences among Limousin and Angus or Japanese Black × Angus steers[1,2]

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ABSTRACT: This work investigated the metabolic basis for the variability of carcass and i.m. adiposity in cattle. Our hypothesis was that the comparison of extreme breeds for adiposity might allow for the identification of some metabolic pathways determinant for carcass and i.m. adiposity. Thus, 23- to 28-mo-old steers of 3 breeds, 2 with high [Angus or Japanese Black × Angus (J. Black cross)] and 1 with low (Limousin) i.m. and carcass adiposity, were used to measure activities or mRNA levels, or both, of enzymes involved in de novo lipogenesis [acetyl-coA carboxylase, fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme], circulating triacylglycerol (TAG) uptake (lipoprotein lipase), and fatty acid esterification (glycerol-3-phosphate dehydrogenase), as well as the mRNA level of leptin, an adiposity-related factor. In a first study, enzyme activities were assayed in the s.c. adipose tissue (AT), the oxidative rectus abdominis, and the glycolytic semitendinosus muscles from steers finished for 6 mo. Compared with Angus or J. Black cross, Limousin steers had a 27% less ($P = 0.003$) rib fat thickness, and 23 and 29% less ($P \leq 0.02$) FAS and G6PDH activities in s.c. AT. In rectus abdominis and semitendinosus, the 75% less ($P < 0.001$) TAG content was concomitant with 50% less ($P < 0.001$) G6PDH activity. In a second study, enzyme activities plus mRNA levels were assayed in an oxido-glycolytic muscle, the longissimus thoracis (LT), in the i.m. AT dissected from LT, and in s.c. AT from the same Limousin steers and from Angus steers finished for 10 mo. Compared with Angus, the 50% less ($P < 0.001$) rib fat thickness in Limousin contrasted with the 1.1- to 5.8-fold greater ($P \leq 0.02$) mRNA levels or activities, or both, of acetyl-coA carboxylase, G6PDH, lipoprotein lipase, and glycerol-3-phosphate dehydrogenase in s.c. AT. Conversely, the 90% less ($P < 0.001$) TAG content in Limousin LT was concomitant to the 79 and 83% less ($P \leq 0.002$) G6PDH activity and leptin mRNA level. Such differences could arise from a greater number of adipocytes in LT from Angus steers because no difference was found between Limousin and Angus for G6PDH activity and leptin mRNA in i.m. AT. We conclude that FAS and G6PDH in s.c. AT could be involved in differences in carcass adiposity, but this relationship disappeared when the fatness increased strongly. Leptin and G6PDH are related to the expression of marbling whatever the body condition and thus could be relevant indicators of marbling in beef cattle.

Key words: adipose tissue, bovine, leptin, lipogenic activity, muscle

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INTRODUCTION

Because of the importance of i.m. adipose tissue (marbling) for meat sensory quality and the economic factors involved in meat animals with adequate i.m. vs. extramuscular adipose tissue repartition, it could be useful to identify metabolic pathways involved in site-specific development of various adipose sites. In cattle, the re-

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partition of adipose sites results from specific capacities for hyperplasia and hypertrophy of adipocytes, which differ depending on age, breed, sex, and plane of nutrition (Vernon, 1980, 1986; Hood, 1982; Chilliard and Robelin, 1985).

Among these factors, breed induces strong variability in the repartition of adipose tissue [e.g., dairy breeds deposit a greater proportion of their total adipose tissue in the abdominal cavity and less subcutaneously than beef breeds (Lister, 1980; Kempster, 1981)]. Likewise, i.m. adipose tissue is more developed in the early-maturing, fat Angus and Hereford than in the late-maturing, lean Charolais (Johnson, 1987). However, the metabolic origins of such breed differences remain poorly understood despite the metabolic peculiarities reported for enzymes involved in lipogenesis (May et al., 1994; Mendizabal et al., 1999; Eguinoa et al., 2003) or glucose oxidation (Miller et al., 1991). Moreover, adipose site-specific development results from nutrient partitioning between adipose and muscle cells as well as from their proportion in the muscle. However, little attention has been paid to the lipid uptake and metabolism in whole muscles (Vernon et al., 1987; Belk et al., 1997; Kazala et al., 2003).

To address the metabolic basis for carcass and muscular adiposity in cattle, we hypothesized that the relevant metabolic pathways could be highlighted by comparing extreme breeds for adiposity. Thus, metabolic pathways of lipid anabolism were compared in a breed and a crossbreed that accumulate adipose tissue readily [Angus and Japanese Black × Angus (J. Black cross)] with another breed that does not (Limousin).

MATERIALS AND METHODS

Animals and Diet

This study was carried out in compliance with the French recommendations and those of Animal Care and Use Committee of INRA for the use of experimental animals including animal welfare and appropriate conditions (guidelines 18 April 1988). The objective of these experiments was not to compare breeds per se, but to induce a high variability in adipose tissue deposition in carcass or in muscles to find related metabolic differences that could be considered as major metabolic indicators. This was achieved by comparing a lean breed reared in France to 2 fat ones reared in Australia, taking care to distribute the same diets in both locations. It is worth noting that because breeds or crossbreeds were reared in 2 countries, we could not exclude an effect of the different environments added to the breed effect, although the effects of either genetic factors or energy level of the diet were shown to be much more pronounced than the effects of photoperiod, temperature, or ingredient composition of the diet on the regulation of lipid metabolism in the bovine (Vernon, 1980; Chilliard et al., 2005). Thus, the high variability in adipose tissue deposition in carcass or muscle has to be considered as the result of a global breed × location interaction.

Table 1. Composition of the finishing diet fed to the Limousin and Angus steers and Japanese Black × Angus crossbred steers

Item	Limousin[1]	Angus or Japanese Black × Angus[2]
Ingredient,[3] % of DM		
Hay	17.6	14.0
Lupin	8.9	9.0
Soya	1.7	—
Triticale	17.5	18.5
Rolled wheat	47.5	49.5
Lime	—	1.0
Molafos	6.9	8.0
Vitamin mineral premix[4]	1.2	—
Chemical composition		
ME, KJ/kg of DM	12.5	11.9
Crude protein, g/kg of DM	155	151

[1]Limousin steers were reared in the experimental unit of INRA Theix Research Center and finished for 6 mo.

[2]Angus and crossbred Japanese Black × Angus steers were reared in the experimental farm of Murdoch University in Australia and were finished for 6 mo for the steers used in the first study or 10 mo for the Angus steers used in the second study.

[3]All steers received a similar finishing diet with a cereal-rich (approximately 75%) diet to allow them to express their genetic potential for the development of adipose tissue.

[4]Minerals, %: Ca, 18; P, 12; Mg, 4; Na, 2; trace elements, mg/Kg: Zn, 11,300; Mn, 8,400; Cu, 2,250; I, 120; Co, 30; and Se, 24; vitamins, UI or mg/kg: vitamin A, 2.7 mg; vitamin D_3, 135,000 UI; and vitamin E, 500 mg.

For our purpose, 2 separate studies were conducted using 4 groups of steers from 3 different breeds or crossbreeds: Angus (2 groups) and J. Black cross (F_1; sire, Japanese Black; dam, Angus), known to produce a marbled meat, were reared in the experimental farm of Murdoch University in Australia, and Limousin steers, which produce a weakly marbled meat, were reared in the experimental unit of INRA Theix Research Center in France. Before the experiments, steers were grown on improved pasture (clover plus rye grass). During the experiments, all animals had a long finishing period with a similar cereal-rich (around 75%) diet (Table 1) to allow them to express their genetic potential for development of adipose tissue. All steers had free access to water.

In study 1, 12 Limousin, 10 Angus, and 10 J. Black cross steers were slaughtered after a 6-mo finishing period with a similar diet (Table 1). At slaughter, samples of s.c. adipose tissue, rectus abdominis (**RA**), and semitendinosus (**STN**) muscles were frozen in liquid nitrogen and stored at –80°C. To maximize the difference for adiposity and to assay other putative metabolic markers, a second study was performed. For study 2, 8 Angus steers were slaughtered after a 10-mo finishing period with the same diet as in the previous group (Table 1). At slaughter, samples of longissimus thoracis (**LT**) muscle, s.c. adipose tissue, and i.m. adipose tissue dissected from the LT were frozen in liquid nitrogen

and stored at –80°C. These extremely fat Angus steers were then compared with the Limousin steers used in study 1, from which i.m. adipose tissue and LT were also sampled.

Adipose Tissue and Muscle Measurements

Total lipid content of muscle tissues was extracted by mixing 4 g of frozen tissue with chloroform:methanol, 2:1 (vol/vol) according to the method of Folch et al. (1957). Triacylglycerols (**TAG**) were determined from total lipid extracts, as described by Leplaix-Charlat et al. (1996). Briefly, after elimination of phospholipids by absorption on silicic acid, TAG were saponified overnight at room temperature with 4 *M* KOH in 100% ethanol, and then neutralized with 4 *M* HCl in water. The resulting free glycerol was determined enzymatically using a TAG test kit (PAP 1000, Biomérieux S. A., Craponne, France).

The following lipogenic enzymes were assayed: fatty acid synthase (**FAS**), which is involved in de novo fatty acid synthesis; malic enzyme, also called nicotinamide adenine dinucleotide phosphate-malate dehydrogenase (**MD**), and glucose-6-phosphate dehydrogenase (**G6PDH**), which are involved in maintaining the reduced nicotinamide adenine dinucleotide phosphate (**NADPH**) supply for de novo fatty acid synthesis; lipoprotein lipase (**LPL**), the rate-limiting enzyme in TAG-rich lipoprotein catabolism, which provides TAG-derived fatty acids to adipose tissue for storage and to muscle for energy production; and glycerol-3-phosphate dehydrogenase (**G3PDH**), which is involved in glycerol 3-phosphate synthesis from glucose and thus in the esterification of the fatty acids. The FAS, G6PDH, MD, and G3PDH activities were assayed spectrophotometrically in s.c. and i.m. adipose tissues as well as in RA, STN, and LT muscles, as described previously (Chilliard et al., 1991). Activity of LPL was measured in s.c. and i.m. adipose tissues as well as in RA, STN, and LT muscles using an artificial emulsion containing ^{3}H-triolein after a detergent (ammonia-HCL, 25 m*M*; EDTA, 5 m*M*; Triton X100, 0.8% wt/vol; and SDS, 0.01% wt/vol) extraction procedure (Hocquette et al., 1998). Enzyme activities were expressed as nanomoles of released fatty acids (LPL) or as nanomoles of reduced (G6PDH, MD) or oxidized (FAS, G3PDH) nucleotides per minute and per milligram of protein, after measurement of soluble protein content in enzyme homogenates (Bradford, 1976) using bovine serum albumin as the standard (BioRad Protein Assay, Marnes La Coquette, France).

The following mRNA levels were assayed: LPL, FAS, acetyl-coA carboxylase (**ACC**, the alpha isoform expressed in adipocytes and involved in de novo fatty acid synthesis), leptin (a hormone mainly synthesized in adipose tissue at a rate strongly related to adiposity in ruminants; Chilliard et al., 2005), and peroxisome proliferator-activated receptor γ2 (**PPARγ2**; which regulates adipogenesis and lipid metabolism-related genes). Total RNA was extracted as described previously (Bonnet et al., 2000). The levels of LPL, ACC (the alpha isoform expressed in adipocytes), and FAS mRNA were quantified by real-time quantitative reverse transcription (**RT**)-PCR in adipose tissues and muscles, using the fluorescent TaqMan methodology and a Light Cycler System (Roche Diagnostics, Meylan, France), as previously described (Bonnet et al., 2000; Bernard et al., 2005). Leptin mRNA was quantified with the same methodology (Bonnet et al., 2002), using specific primers (Genosys Biotechnology, Cambridge, UK): 5′-GTCAGCAATGGGTCAGTTGAG-3′ (forward) and 5′-TCCTCCTTTGTTCTGCTGCAC-3′ (reverse) and a TaqMan probe: 5′-CAGGACCAGCCCCCAGGA GCC-3′. The level of PPARγ2 mRNA was quantified by real time quantitative RT-PCR in adipose tissues and muscles, using the fluorescent SYBR Green I methodology (Roche Diagnostics) and specific primers 5′-GCGT TCCCAAGTTTTACTGC-3′ (forward) and 5′-CACGAC TCCCACCGATATTT-3′ (reverse). For each mRNA, the transcript level was related to the level of cyclophilin mRNA, a housekeeping gene, measured by real time RT-PCR, as described previously (Bonnet et al., 2000).

Statistical Analysis

Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). The model used to compare data in RA and STN among Limousin, Angus, and J. Black cross (study 1) or data in s.c. and i.m. adipose tissues between Limousin and Angus (study 2) accounted for the breed plus location (**BL**), the anatomical site (**S**), and their interaction (**BL** × **S**) as fixed effects, and animal nested within breed plus location was treated as a random effect. The model used to compare data in s.c. adipose tissue among Limousin, Angus, and J. Black cross (study 1) or data in LT between Limousin and Angus (study 2) or data in s.c. adipose tissue between J. Black cross (from study 1) and Angus (from study 2) accounted for BL as the fixed effect. For the different models and when applicable, a multiple comparison of means was performed using the LSMEANS statement. Differences between BL or anatomical sites, or both, were considered to be significant when $P \leq 0.05$.

RESULTS

Animals and Carcass Data, and Protein Contents in Adipose Tissues and Muscles

Compared with J. Black cross or Angus steers in study 1, Limousin were characterized (Table 2) by greater ($P < 0.001$) BW at the beginning of the finishing phase (+19 and +34%), BW at slaughter (+15 and +19%), HCW (+30 and +32%), dressing percent (+13 and +12%), and less ($P = 0.003$) fat thickness at the 11th rib (–30 and –23%). Moreover, ADG was greater ($P = 0.031$) for the Angus steers than for the Limousin (+14%) and J. Black cross (+19%) steers. When the same

Table 2. Growth performances, carcass characteristics, and protein content in tissue homogenates of Limousin, Japanese Black × Angus and Angus steers[1]

Item	Limousin	Japanese Black × Angus	Angus, study 1[2]	Angus, study 2[3]	*P*-value, study 1	*P*-value, study 2
No. of animals	12	10	10	8		
Finishing phase						
Initial age, mo	17	22	17	20		
Initial BW, kg	553 ± 6[a]	463 ± 9[b]	414 ± 6[c]	418 ± 10	<0.001	<0.001
Period, mo	6	6	6	10		
Slaughter						
Age, mo	23	28	23	28		
BW, kg	738 ± 10[a]	639 ± 10[b]	622 ± 13[b]	755 ± 17	<0.001	0.337
ADG, kg/d	1.02 ± 0.04[b]	0.98 ± 0.04[b]	1.17 ± 0.06[a]	1.11 ± 0.10	0.031	0.100
HCW, kg	464 ± 7[a]	356 ± 7[b]	350 ± 8[b]	420 ± 11	<0.001	0.002
Dressing percent, %	62.9 ± 0.2[a]	55.5 ± 0.6[b]	56.4 ± 0.3[b]	55.6 ± 0.6	<0.001	<0.001
Rib (11th) fat thickness, mm	9.6 ± 1.0[b]	13.9 ± 0.6[a]	12.4 ± 0.8[a]	19.0 ± 2.2	0.003	<0.001
Protein content in homogenates, mg/g						
Subcutaneous AT[4]	3.1 ± 0.2[a]	2.0 ± 0.1[b]	1.9 ± 0.3[b]	2.3 ± 0.2	0.001	0.010
Intramuscular AT	13.4 ± 0.6	—	—	10.4 ± 1.3	—	0.036
Rectus abdominis	35.3 ± 3.4[a]	32.2 ± 1.1[b]	29.7 ± 0.9[b]	—	0.002	—
Semitendinosus	34.9 ± 0.8	32.4 ± 1.9	34.0 ± 0.9	—	0.119	—
Longissimus thoracis	40.7 ± 0.9	—	—	36.8 ± 0.7	—	0.006

[a–c]Means within a row with different letters differ between Limousin, Japanese Black × Angus, and Angus in the first study ($P < 0.05$).
[1]Values are means ± SEM.
[2]In study 1, breed plus location differences were studied using 23-mo-old Limousin and Angus and 28-mo-old Japanese Black × Angus steers slaughtered after a 6-mo finishing period with a similar diet.
[3]In study 2, breed plus location differences were studied using 23-mo-old Limousin and 28-mo-old Angus steers after a 6- or 10-mo finishing period, respectively, with a similar diet.
[4]AT = adipose tissue.

Limousin steers were compared with Angus steers finished for 10 mo (study 2, Table 2), they had greater BW at the beginning of the finishing phase (+34%, $P < 0.001$) but similar BW at slaughter and ADG. Limousin steers also had a greater ($P = 0.002$) HCW (+11%) and dressing percent (+13%) as well as much less (–49%, $P < 0.001$) fat thickness at the 11th rib.

Compared with J. Black cross or Angus steers finished for 6 mo (study 1), protein content was greater ($P = 0.002$) in Limousin s.c. adipose tissue (+55 and +63%, respectively) and RA (+9 and +19%, respectively), but similar in STN (Table 2). Moreover, Limousin steers had a greater ($P < 0.04$) protein content in s.c. adipose tissue (+35%), i.m. adipose tissue (+29%), and LT (+11%) than the Angus steers (Table 2) finished for 10 mo (study 2).

Study 1: Lipogenic Activities in s.c. Adipose Tissue, and in RA and STN from Limousin, Angus, and J. Black Cross Steers

In s.c. adipose tissue (Figure 1), FAS ($P = 0.05$) and G6PDH ($P = 0.02$) activities were greater in Angus than Limousin (+39 and +59%, respectively) steers and were intermediate in the J. Black cross, which did not differ significantly from either. The activity of G3PDH was the least ($P = 0.02$) in J. Black cross s.c. adipose tissue (–29 and –31% relative to Limousin and Angus, respectively). Activity of LPL was the greatest ($P < 0.001$) in the Limousin (+276 and +227% relative to Angus and J. Black cross steers, respectively). A trend ($P = 0.15$) for greater MD activity in s.c. adipose tissue from Angus or J. Black cross than Limousin steers was observed.

In the RA and STN muscles, de novo lipogenesis and uptake and esterification of fatty acids differed depending on the breed plus location, the anatomical site or both (Figure 2). The content of TAG and G6PDH activity were greater ($P < 0.001$) in muscles from Angus or J. Black cross than from Limousin steers, and differences were greater ($P < 0.001$) in RA (+362 and +370% for TAG content in Angus and J. Black cross vs. Limousin steers; +124 and +94% for G6PDH activity) than in STN (+307 and +224% for TAG content; +101 and +79% for G6PDH activity) due to an interaction ($P = 0.01$) between the breed plus location and anatomical site. Activity of MD differed between breeds plus location ($P = 0.008$) and was slightly greater in RA from J. Black cross than from Limousin steers (+16%, $P = 0.015$) and was intermediate in Angus but did not differ significantly from either. In STN, MD activity was the greatest in J. Black cross steers (+18%, $P = 0.01$ and +19%, $P = 0.007$ relative to Angus and Limousin, respectively). Activity of LPL differed between breed plus location ($P = 0.02$) and was less in STN (–72%, $P = 0.008$) or tended to be less in RA (–24%, $P = 0.10$) from J. Black cross than from Limousin steers. Additionally, there was an interaction between the effects of breed plus location and muscle type for FAS activity ($P = 0.01$), which was greater (+32%, $P = 0.001$) in Angus than in Limousin steers but only in RA, and for G3PDH activity ($P < 0.001$) giving the following ranking: STN J. Black cross > STN Angus = STN Limousin = RA Limousin >

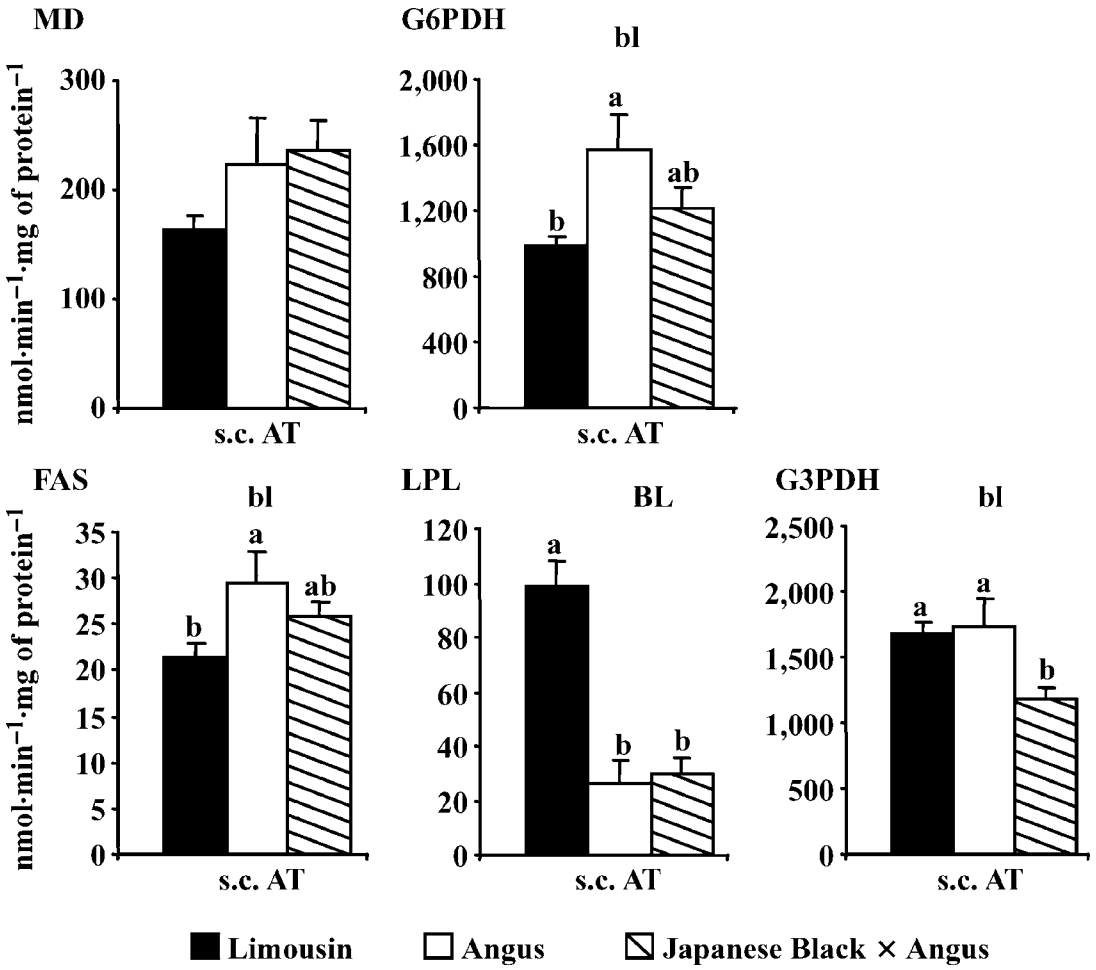

Figure 1. Activities (nmol·min^{-1}·mg of protein^{-1}) of malic enzyme (MD), glucose-6-phosphate dehydrogenase (G6PDH), fatty acid synthase (FAS), lipoprotein lipase (LPL) and glycerol-3-phosphate dehydrogenase (G3PDH) in subcutaneous (s.c.) adipose tissue (AT) from Limousin, Angus, or Japanese Black × Angus steers finished during 6 mo (study 1). Results are means ± SEM. bl or BL = a significant effect of breed plus location ($P < 0.05$ or $P < 0.001$, respectively). [a,b]Means without a common superscript letter differ ($P < 0.05$).

RA J. Black cross = RA Angus. Whatever the breed plus location, differences between anatomical sites were observed for TAG content, MD, G6PDH, FAS, and LPL, which were 4-, 1.1-, 4-, 1.2-, and 2.6-fold greater ($P \leq 0.004$) in oxidative RA than in glycolytic STN muscle, respectively. Conversely, G3PDH was 1.3- greater ($P < 0.001$) in STN than in RA.

Study 2: mRNA Levels or Activities, or Both, of Lipogenic Enzymes, Leptin, and PPARγ2 in s.c. and i.m. Adipose Tissues, and LT from Limousin and Angus Steers

Compared with Angus, Limousin steers were characterized (Figure 3) by greater G6PDH (+21%, $P = 0.01$) and G3PDH activities (+99%, $P < 0.001$) and LPL mRNA level and activity (+39%, $P = 0.02$ and +454%, $P < 0.001$, respectively) only in s.c. adipose tissue due to an interaction between breed plus location and anatomical site ($P \leq 0.05$). Limousin adipose tissues had greater ACC ($P = 0.003$) and tended to have less PPARγ2 ($P = 0.06$) mRNA levels than in the Angus, but the differences between breed plus location reached significance only in s.c. adipose tissue for ACC (+34%, $P = 0.002$) and in i.m. adipose tissue for PPARγ2 (–37%, $P = 0.03$) mRNA levels when data were analyzed by multiple comparison of the means. Whatever the breed plus location, s.c. adipose tissue had several-fold greater ($P < 0.001$) MD (5.5), G6PDH (4.3), FAS (3.9), and LPL (4.5) activities as well as leptin (1.7), FAS (3.0), ACC (3.5), and PPARγ2 (1.7) mRNA levels than i.m. adipose tissue. Differences between adipose tissue sites for G3PDH depended on the breed plus location ($P < 0.001$ for the interaction), given that the activity was either greater in s.c. adipose tissue than i.m. adipose tissue from Limousin (+30%, $P = 0.007$) or greater in i.m. than s.c. adipose tissue from Angus (+55%, $P = 0.008$) steers.

In LT muscle, differences between breed plus location were observed for TAG content, MD, and G6PDH activities as well as leptin mRNA level, which were 9.4-, 1.3-, 4.7-, 5.8-fold greater ($P \leq 0.002$) in Angus than in

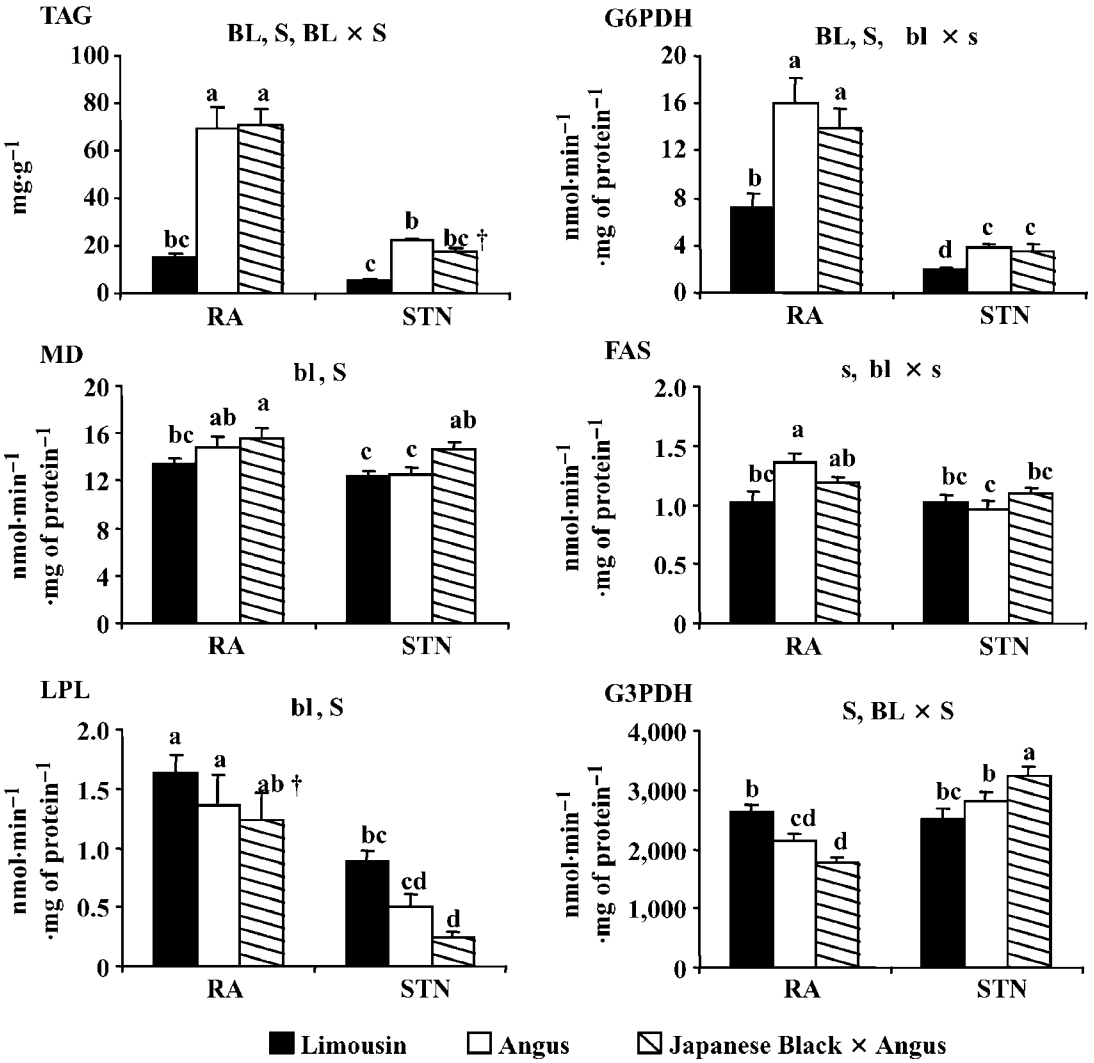

Figure 2. Triacylglycerol content (TAG, mg·g^{-1}) and activities (nmol·min^{-1}·mg of protein^{-1}) of glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (MD), fatty acid synthase (FAS), lipoprotein lipase (LPL), and glycerol-3-phosphate dehydrogenase (G3PDH) in rectus abdominis (RA) and semitendinosus (STN) muscles from Limousin, Angus, or Japanese Black × Angus steers finished during 6 mo (study 1). Results are means ± SEM. bl or s, or BL or S = a significant effect of breed plus location or anatomical site ($P < 0.05$ or 0.001, respectively); and bl × s or BL × S = a significant interaction ($P < 0.02$ or $P < 0.001$). [a–d]Means without a common superscript letter differ ($P < 0.05$). †Means from Angus or Japanese Black × Angus tend to differ ($0.05 < P < 0.1$) from that from Limousin steers.

Limousin steers (Figure 4). No difference between breed plus location was observed for FAS, G3PDH, or LPL activities nor for ACC, FAS, LPL, or PPARγ2 mRNA levels.

Activities of Lipogenic Enzymes in s.c. Adipose Tissue from 28-Mo-Old J. Black Cross and Angus Steers

From data presented in Table 2 and Figures 1 and 3, we did an additional analysis to compare steers of the same age and reared in the same country. Despite their 1.4-fold greater ($P = 0.023$) rib fat thickness, Angus steers were characterized by less (–29 to –38%) MD ($P = 0.017$), G6PDH ($P = 0.021$), FAS ($P = 0.009$), and G3PDH ($P = 0.008$) activities in s.c. adipose tissue compared with J. Black cross steers. Activity of LPL was not significantly different between these steers.

DISCUSSION

Devising methods to manipulate the different adipose tissue depots in order to improve carcass or meat quality requires an understanding of the metabolic pathways involved in the variability of these adipose tissue masses. In order to induce a wide range of carcass and muscular adiposity, we compared a lean breed reared in France to 2 fat ones reared in Australia. This allowed us to identify adiposity-related specific levels of expression for some of the enzymes and proteins involved in the main lipid synthesis and deposition pathways. The main finding of this work is that G6PDH activity and

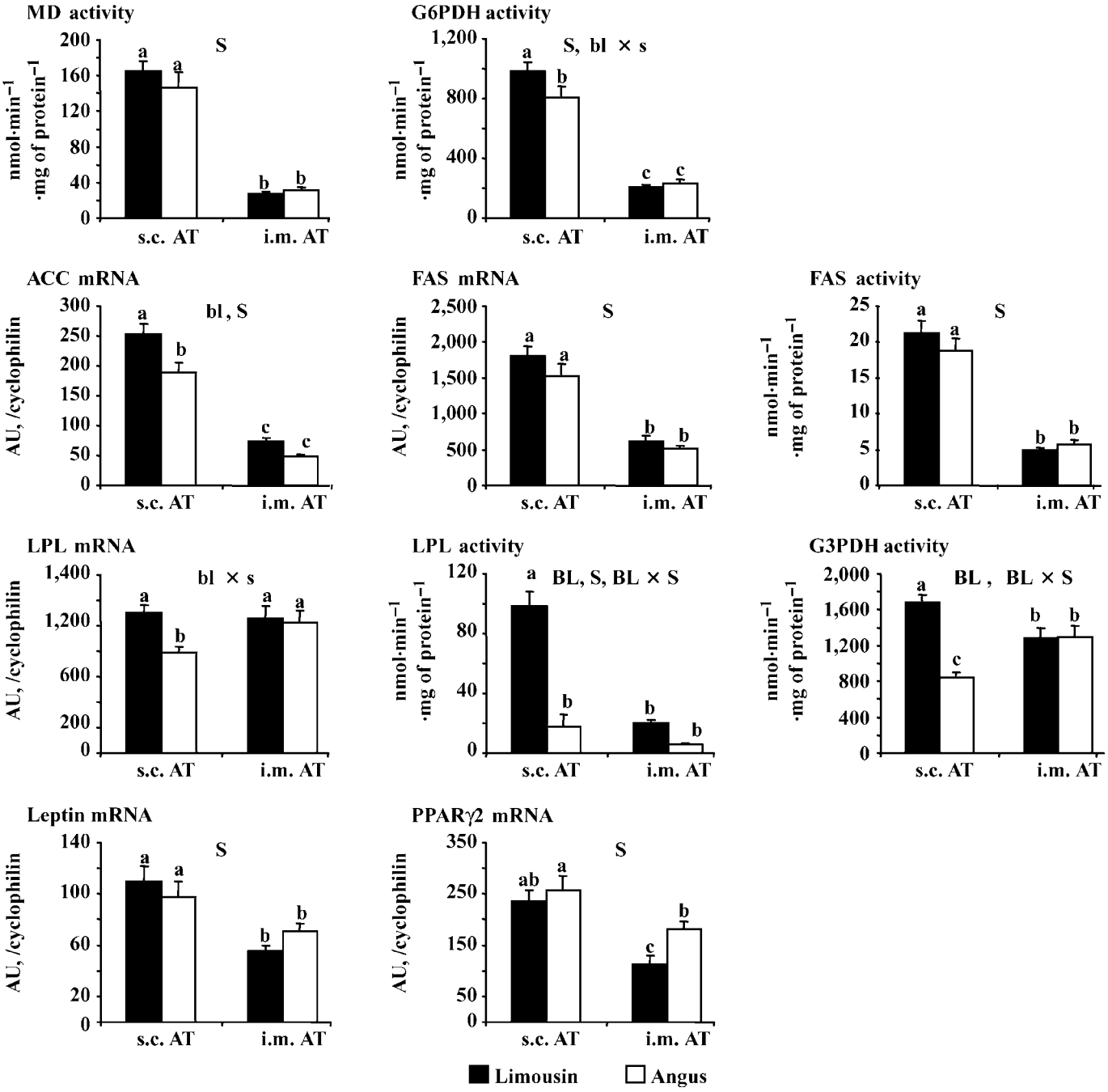

Figure 3. Activities (nmol·min^{-1}·mg of protein^{-1}), mRNA levels [related to cyclophilin mRNA level, arbitrary unit (AU)], or both, of malic enzyme (MD), glucose-6-phosphate dehydrogenase (G6PDH), acetyl-coA carboxylase (ACC), fatty acid synthase (FAS), lipoprotein lipase (LPL) and glycerol-3-phosphate dehydrogenase (G3PDH), leptin, and peroxysome proliferator-activated receptorγ2 (PPARγ2) in subcutaneous (s.c.) and intramuscular (i.m.) adipose tissues (AT) from Limousin and Angus steers finished during 6 or 10 mo, respectively (study 2). Results are means ± SEM. bl or s, or BL or S = a significant effect of breed plus location or anatomical site ($P < 0.05$ or $P < 0.001$, respectively); bl × s or BL × S = a significant interaction ($P < 0.05$ or $P < 0.001$). [a–c]Means without a common superscript letter differ ($P < 0.05$).

leptin mRNA level were greater in muscles with the greater TAG content (from Angus and J. Black cross) whatever the final BW and age, suggesting that leptin and G6PDH are related to the development of i.m. adipose tissue in beef cattle.

Carcass Adiposity and Lipogenic Activities in s.c. Adipose Tissue

We report here evidence that some metabolic pathways involved in lipid deposition (i.e., de novo lipogen-

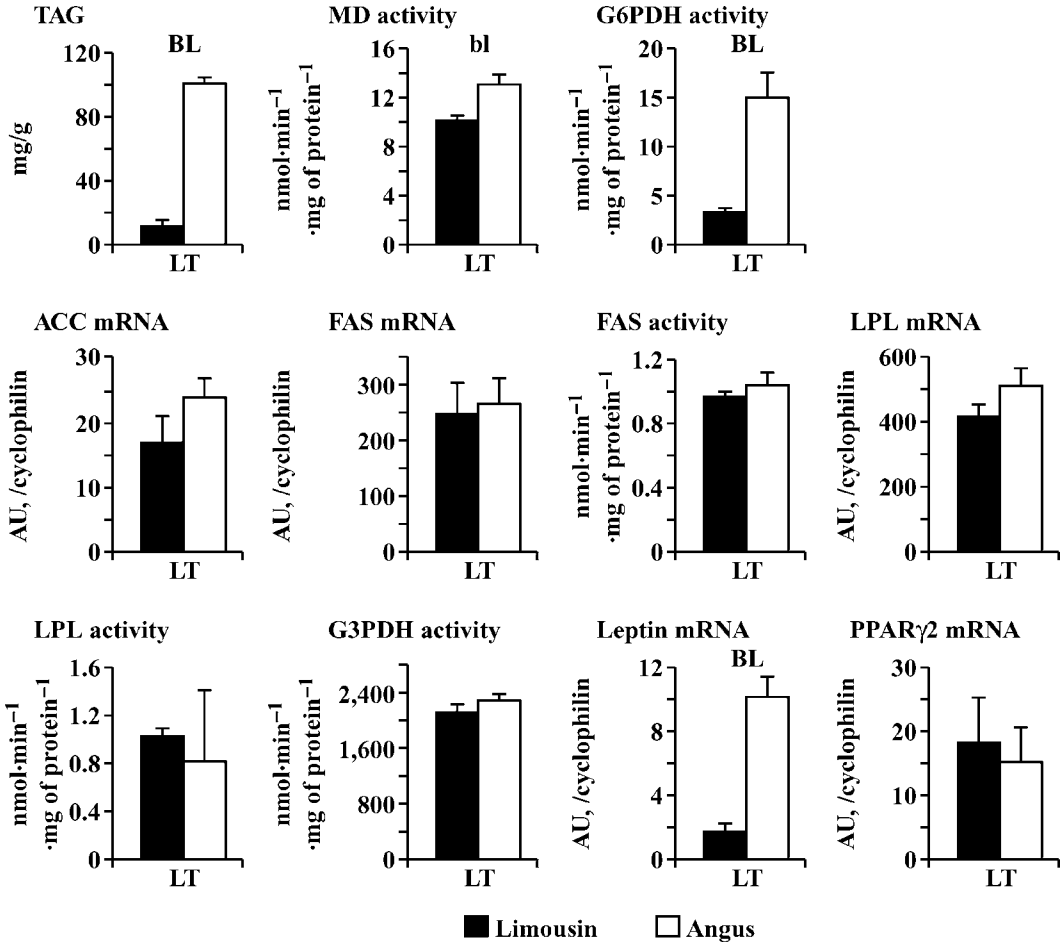

Figure 4. Triacylglycerol (TAG) content (mg·g^{-1}), activities (nmol·min^{-1}·mg of protein^{-1}) and/or mRNA levels [related to cyclophilin mRNA level, arbitrary unit (AU)] of malic enzyme (MD), glucose-6-phosphate dehydrogenase (G6PDH), acetyl-coA carboxylase (ACC), fatty acid synthase (FAS), lipoprotein lipase (LPL) and glycerol-3-phosphate dehydrogenase (G3PDH), leptin and peroxysome proliferator-activated receptor γ2 (PPARγ2) in longissimus thoracis (LT) from Limousin or Angus steers finished during 6 or 10 mo, respectively (study 2). Results are means ± SEM. bl or BL = a significant effect of breed plus location ($P < 0.05$ or $P < 0.001$, respectively).

esis, fatty acid uptake and esterification, leptin and PPARγ2 synthesis) differ according to the range of carcass adiposity of the studied Limousin, Angus, and J. Black cross steers. Indeed, less carcass adiposity, estimated by rib fat thickness, was concomitant with less FAS and G6PDH activities in the s.c. adipose tissue from Limousin compared with Angus of the same age (study 1, Figure 5A). Similar links between carcass adiposity and FAS and G6PDH, involved in de novo lipogenesis, were already observed in previous breed comparisons [e.g., Holstein compared with Hereford × Angus (Hood and Allen, 1975), Santa Gertrudis compared with Angus (Miller et al., 1991), Angus compared with Wagyu crossbred (May et al., 1994), and in Asturiana compared with Morucha (Mendizabal et al., 1999)]. Moreover, it is possible that age could affect the relationship between de novo lipogenesis and carcass adiposity because it was less apparent when the same Limousin were compared with the 5 mo older J. Black cross steers. Indeed, lipogenic activities increased with age until 7 to 13 mo of age depending on the breed and the plane of nutrition (Hood and Allen, 1975; Whitehurst et al., 1981; Smith et al., 1984) and then decreased. Elsewhere, the greater LPL activity in Limousin s.c. adipose tissue may indicate a lack of relationship between its potential for circulating fatty acid uptake and carcass adiposity (Figure 5A). In agreement with our results, similar LPL mRNA levels were reported in Charolais and Holstein steers, despite the less body fat content of Charolais (Ren et al., 2002). Likewise, the greater G3PDH activity in s.c. adipose tissue from Limousin and Angus than from J. Black cross steers in our study does not match with the differences in carcass adiposity and could be the result of a greater rate of esterification linked to greater level either of de novo lipogenesis in Angus or of LPL activity in Limousin (Figure 5A). This hypothesis was reinforced by positive correlations between the activities of G3PDH and either FAS ($r = 0.79$, $P < 0.01$) or G6PDH ($r = 0.68$, $P < 0.05$) in s.c. adipose tissue from Angus

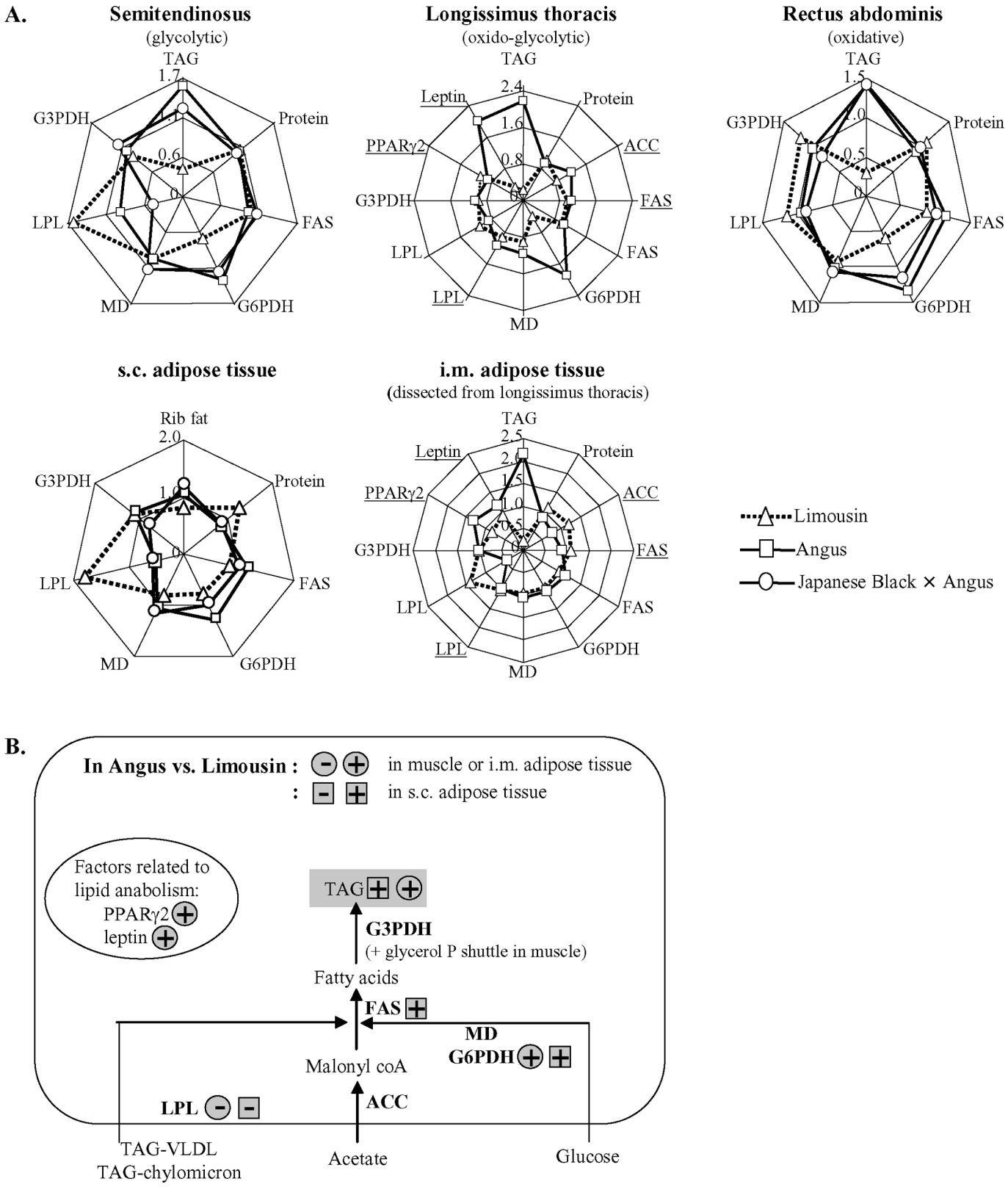

Figure 5. A: Similarities or differences between the relative (general mean among breeds defined as 1.0) level of carcass (rib fat thickness) or muscular [triacylglycerol (TAG)] adiposity and the relative activities, mRNA levels (underlined), or both of malic enzyme (MD), glucose-6-phosphate dehydrogenase (G6PDH), acetyl-coA carboxylase (ACC), fatty acid synthase (FAS), lipoprotein lipase (LPL) and glycerol-3-phosphate dehydrogenase (G3PDH), leptin and peroxysome proliferator-activated receptor γ2 (PPARγ2) in muscles, s.c., and i.m. adipose tissues. B: Synthetic metabolic scheme that underlines the metabolic pathways related positively or negatively to carcass or muscular adiposity. VLDL = very low density lipoprotein.

and between activities of G3PDH and LPL (r = 0.75, $P < 0.01$) in s.c. adipose tissue from Limousin. Our results supplement data from Mendizabal et al. (1999) reporting correlations between FAS and G3PDH activities in some meat-producing Spanish breeds. In the s.c. adipose tissue from steers used in the current study, we did not observe any breed plus location differences either for MD, in agreement with some authors (May et al., 1994; Eguinoa et al., 2003) or in contrast to other findings reporting greater activity associated with greater adiposity (Miller et al., 1991; Mendizabal et al., 1999). Such a variable relationship between MD activity and carcass adiposity could be related to its low activity in ruminants, which in general is not rate limiting for de novo lipogenesis (Vernon, 1980).

When the differences in carcass adiposity were maximized by comparing the same Limousin group to older and fatter Angus steers, slaughtered at a similar BW after a 10-mo finishing period (study 2), rib fat thickness did not match any more with the activities of de novo lipogenesis, but was always negatively related to LPL activity. In this study, greatest G6PDH and G3PDH activities as well as ACC mRNA level were observed in s.c. adipose tissue from Limousin steers, whereas they were expected to be the greatest in the Angus. Indeed, the rate of fatty acid synthesis was reported to be greater in 470-kg small-frame steers (such as Angus) compared with large-frame steers (such as Limousin) of similar BW (Hood and Allen, 1975). This surprising lack of relationship between adiposity and lipogenic activities did not result from a difference in age or location between Limousin and Angus steers. Indeed a lack of relationship was also observed when we compared 28-mo-old J. Black cross with these very fat Angus of the same age and reared in the same country. Thus, this lack of relationship resulted more likely from the excessive fatness of the Angus steers. This could also have contributed to the decrease in G6PDH and G3PDH activities between 23 and 28 mo of age in the Angus steers. In agreement with the hypothesis of an inhibition of lipogenic activities by excessive fatness, Hood and Allen (1975) reported that the positive relationship between lipogenic activity and adiposity (adipocyte size) disappeared after a critical cell size was reached. Moreover, Chilliard and Robelin (1985) reported a decrease in s.c. adipose tissue LPL activity, whereas the weight of body lipids and the size of s.c. adipocytes increased in very fat compared with medium fat dry cows. Similar variations for de novo fatty acid biosynthesis and the size of s.c. adipocytes were reported more recently in corn-fed Wagyu steers (Chung et al., 2007). Moreover, Eguinoa et al. (2003) reported that smaller s.c. adipocytes had greater lipogenic activities per cell than larger ones. Thus, it could be hypothesized that the similar or lower lipogenic activities in adipose tissues from the very fat Angus compared with Limousin steers was the result of an inhibition by adipocyte hypertrophy rather than its cause. To our knowledge, such downregulation of lipogenic gene expression by adipocyte hypertrophy is poorly documented in ruminant species, whereas it has been reported for numerous genes involved in lipid anabolism in obese mice compared with lean ones (Lan et al., 2003).

In summary, our results highlight that FAS and G6PDH could be related to carcass adiposity until a threshold of adiposity was reached, independently of the age or the location where the steers were reared. The links between metabolic pathways and adiposity are thus complex because they could be modified depending on cellular and molecular regulations that avoid excessive cell hypertrophy.

Muscular Adiposity and Lipid Anabolism Pathways in Muscles and i.m. Adipose Tissue

We report evidence that some metabolic pathways involved in lipid deposition differ according to the range of muscular adiposity in Limousin, Angus, and J. Black cross. Compared with Limousin steers, Angus of similar age or J. Black cross 5 mo older had greater muscular TAG content, both in oxidative RA and glycolytic STN muscles, in agreement with previous data comparing lean and fat breeds (Zembayashi, 1994). Concomitantly, in Angus or J. Black cross steers, we observed a greater activity of some enzymes involved in de novo lipogenesis: G6PDH in RA and STN plus FAS and MD in RA (Figure 5A). This suggests that these enzymes, especially G6PDH, could be involved in the determination of muscular adiposity. Indeed, significant correlations ($P < 0.01$, n = 64 observations from RA and ST muscle from the 3 breeds) were found between muscular TAG content and the activities of MD (r = 0.42), FAS (r = 0.47), and more importantly G6PDH (r = 0.81). To our knowledge, very little data are available concerning the relation between adiposity and lipogenic pathways in muscles. However, in agreement with our results, Belk et al. (1997) reported a putative involvement of G6PDH activity in the marbling of the oxido-glycolytic LT from Wagyu and Angus steers. Conversely, our results for G3PDH and LPL activities suggest that these enzymes are not involved in the ability to accumulate i.m. adipose tissue (Figure 5A). A lack of correlation was similarly observed in bovine pars costalis diaphragmatis muscle between lipid content and diacylglycerol acyltransferase activity, which is also involved in esterification (Middleton et al., 1998). Thus, surprisingly, no clear relationship appeared between the enzymes of esterification and the TAG content of muscles, either because some enzymes are involved in other metabolic pathways, such as the glycerol phosphate shuttle for G3PDH (Estabrook and Sacktor, 1958), or because their activity is not limiting for the fatty acids esterification rate. Taken all together, our results suggest that in muscles, especially in the oxidative RA, some de novo lipogenic pathway enzymes rather than the uptake of circulating TAG and esterification could be involved in muscular adiposity (Figure 5A). This hypothesis was confirmed by measuring the same lipogenic activities

plus the mRNA level of other enzymes or proteins involved in lipid anabolism in the oxido-glycolytic LT from Limousin and Angus steers in which the marbling difference was maximized (study 2). Indeed, the greater TAG content of LT from Angus was concomitant to and correlated (n = 20) with greater MD (r = 0.68, $P < 0.01$) and, more closely, G6PDH (r = 0.82, $P < 0.01$) activities. Among the additional pathways studied, we did not observe breed plus location-differences for ACC and PPARγ2 mRNA levels, but we did observe a greater mRNA level for leptin in LT from Angus than from Limousin steers (Figure 5A). To our knowledge, breed-related leptin gene expression in muscles has never been reported before. Because leptin mRNA level in bovine is high in adipose tissue but barely detectable in muscle (Chelikani et al., 2003), and most of the lipogenic enzymes are more strongly expressed in adipose tissues than in muscles of ruminants (Howarth et al., 1968; Vernon et al., 1987; Belk et al., 1997), the greater G6PDH and MD activities and leptin mRNA level in LT from Angus vs. Limousin steers may result from a greater number of muscular adipocytes, from greater lipogenic activities of these cells, or both. To address this question, we assayed the same variables in the i.m. adipose tissue dissected from the LT. In favor of a greater number of muscular adipocytes, we observed 1) a lower protein content per gram of i.m. adipose tissue in LT from Angus than from Limousin steers, which is likely related to a greater lipid content, and 2) no differences between breeds plus location in i.m. adipose tissue for the gene expression of the studied lipogenic enzymes (Figure 5A). This is in agreement with the link between the fat cell number and the marbling score already described in adult cattle by a histological study of LT sections (Moody and Cassens, 1968) and cellularity measurements in i.m. adipose tissue (Cianzio et al., 1985).

Elsewhere, similar lipogenic activities between i.m. adipose tissue from Angus and Limousin steers contrast with published data showing greater lipogenic activities in breeds with the greater marbling score (Miller et al., 1991; May et al., 1994). However, as discussed for s.c. adipose tissue, it could be hypothesized that a lack of relationship between lipogenic activity and adiposity in the high range of fatness also occurred in i.m. adipose tissue from the very fat Angus steers. Compared with Limousin, in Angus steers, excessive i.m. adiposity together with a greater number of adipocytes per gram of muscle would be consistent with the greater mRNA level of PPARγ2 observed in i.m. adipose tissue (Figure 5A). Such greater level of PPARγ2 mRNA was not observed when assayed in LT, probably due to a low expression in muscle assuming that in bovine, like in monogastric species (Moller and Berger, 2003), the expression of PPARγ2 is restricted to adipose tissues. The greater PPARγ2 gene expression in i.m. adipose tissue of Angus steers could promote the differentiation of preadipocytes into new small adipocytes. Such cellular adaptations have been described in murine models of insulin resistance where an activation of PPARγ2 induced adipocyte differentiation and thus the appearance of new small adipocytes (Yamauchi et al., 2001). Assuming that the accumulation of TAG in LT of Angus is associated with insulin resistance as in human and rodents (Moller and Berger, 2003), the induced expression of PPARγ2 could be a compensatory mechanism to help to maintain normal insulin sensitivity, at least partly by increasing adipocyte differentiation. Although the molecular mechanisms remain unknown, the appearance of new adipocytes in i.m adipose tissue was reported in steers as a function of age (Cianzio et al., 1985) or diet composition (Schoonmaker et al., 2004).

In summary, our results on muscles highlight breed plus location-related differences for activities or mRNA levels for leptin and enzymes involved in de novo synthesis (FAS, MD) and esterification (G3PDH) of fatty acids, as well as circulating TAG uptake (LPL). Malic enzyme, G3PDH, and LPL were also dependent on the muscle type and the duration of the finishing period and did not match with the muscular TAG content. In contrast, G6PDH activity and the leptin mRNA level were found to be greater in muscles showing more marbling, whatever the final BW and age (Figure 5B). However, the cause-to-effect relationship between marbling and leptin expression remains unclear, as well as the role of G6PDH in the expression of marbling.

Differences Between Anatomical Sites for Lipid Anabolism

Whatever the breed plus location, the greater MD, G6PDH, FAS, and LPL activities and the greater leptin, FAS, ACC, PPARγ2 mRNA levels in s.c. than i.m. adipose tissue are in agreement and supplement the data reported for enzymes involved in de novo lipogenesis in different breeds of growing meat cattle (Chakrabarty and Romans, 1972; Whitehurst et al., 1981; Smith and Crouse, 1984; Miller et al., 1991). These greater activities probably contribute to the greater lipid deposition in s.c. adipose tissue, as suggested by its lower protein content, and confirm the repeatedly observed later maturity of i.m. adipose tissue by comparison with the s.c. one (Hood, 1982). In RA muscle, the greater FAS, MD, G6PDH, and LPL activities probably contribute to its greater marbling compared with glycolytic STN. Conversely, G3PDH activity was greater in the glycolytic STN than in the oxidative RA. To our knowledge, very little data are available concerning the lipogenic activities of muscles depending on their metabolic types. However, one study reported a 2-fold greater G3PDH activity in skeletal muscles than in s.c. adipose tissue in sheep (Vernon et al., 1987), which was also observed in our study. From these results, we conclude that in muscles from steers, G3PDH is not only involved in TAG synthesis but probably also in the glycerol phosphate shuttle

(Estabrook and Sacktor, 1958). This last metabolic pathway transfers the cytosolic NADH into the mitochondria during ATP synthesis from glucose. This could explain the greater G3PDH activity in glycolytic than in oxidative muscle that we report here.

In conclusion, our results highlight that leptin and G6PDH are closely related to the deposition of i.m. adipose tissue in beef cattle, and thus could be good indexes of marbling, which remains to be confirmed in a greater marbling range among different breeds and throughout each breed. Once confirmed, it would be necessary to develop simple, rapid, and low cost assays (enzymatic or immunologic) for G6PDH activity and leptin protein content in order to predict, from a muscular biopsy, if an animal could develop an adequate marbling score before slaughter.

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