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Metabolic and histochemical characteristics of fat and muscle tissues in homozygous or heterozygous pigs for the body composition QTL located on chromosome 7

Julie Demars,[1] Juliette Riquet,[1] Marie-Pierre Sanchez,[2] Yvon Billon,[3] Jean-François Hocquette,[4] Bénédicte Lebret,[5] Nathalie Iannuccelli,[1] Jean-Pierre Bidanel,[2] Denis Milan,[1] and Florence Gondret[5]

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Demars J, Riquet J, Sanchez M-P, Billon Y, Hocquette J-F, Lebret B, Iannuccelli N, Bidanel J-P, Milan D, Gondret F. Metabolic and histochemical characteristics of fat and muscle tissues in homozygous or heterozygous pigs for the body composition QTL located on chromosome 7. *Physiol Genomics* 30: 232–241, 2007. First published April 10, 2007; doi:10.1152/physiolgenomics.00270.2006.—Quantitative trait loci (QTL) influencing many traits including backfat thickness and carcass composition have been detected on porcine chromosome 7 (SSC7) in an F2 cross between Large White (LW) and Meishan (MS) pigs. However, the genes and controlled pathways underlying the QTL effects on body phenotype remain unknown. This study aimed at investigating the tissue characteristics at metabolic and cellular levels in pigs that were either homozygous or heterozygous for a body composition SSC7 QTL. A backcross pig (BC3) was first progeny tested to confirm its heterozygoty for the SSC7 QTL; results on all offspring (n = 80) confirmed the QTL effects on body fatness. This boar was then mated with three sows known to be heterozygous for this QTL. In the subset of pigs per genotype, we found that heterozygous LW_{QTL7}/MS_{QTL7} pigs had smaller adipocytes in backfat, together with a lower basal rate of glucose incorporation into lipids and lower activities of selected lipogenic enzymes in backfat isolated cells, compared with homozygous LW_{QTL7}/LW_{QTL7} pigs. A higher number of adipocytes was also estimated in backfat of LW_{QTL7}/MS_{QTL7} animals compared with LW_{QTL7}/LW_{QTL7} pigs. The SSC7 QTL did not influence oxidative and glycolytic metabolisms of longissimus and trapezius muscles, as estimated by the activities of specific energy metabolism enzymes, or the myofiber type properties. Altogether, this study provides new evidence for an altered adipocyte cellularity in backfat of pigs carrying at least one MS allele for the SSC7 QTL. Some candidate genes known for their functions on adipocyte growth and differentiation are suggested.

quantitative trait locus; porcine chromosome 7; energy metabolism; lipogenesis; adipocytes

THE IDENTIFICATION OF GENES underlying body composition is of major interest for the improvement of livestock animal production and for a better knowledge of mammalian physiology, including the human obesity-related phenotype. One strategy to achieve that is to identify porcine genomic regions containing quantitative trait loci (QTL) for economically important traits, such as carcass composition. In pigs, a QTL with marked effects on body fatness has been detected on chromosome 7 (SSC7), using an F2 cross between European Large White (LW) and Chinese Meishan (MS) divergent founder breeds (4, 30, 33, 39). Pure MS pig populations deposit more fat than conventional Western breeds of pigs (34). However, alleles originating from the MS breed in the SSC7 QTL region, which are partially dominant over the LW alleles (33), are surprisingly associated with a lower fat percentage and a higher lean meat content of the carcass in all studies (11, 40).

In early life, adipose tissue expansion results from the development of adipocytes originating from precursor cells (adipogenesis), under the influence of numerous transcription factors (6). Then, the deposition of fat into differentiated adipose cells mainly results from the balance of absorption of fatty acids circulating in blood, de novo lipogenesis, esterification, lipolysis, and in situ lipid oxidation. Previous studies have shown that pig breeds with a greater propensity to fatten have larger adipocytes and consequently fewer cells per gram of adipose tissue than lean pigs (20, 41). Both elevated lipogenic enzyme activities and in vitro lipogenic rate have also been associated with the variation of adiposity between fat and lean pig breeds (20, 31, 41). Since skeletal muscle is the primary site of insulin-stimulated glucose disposal and whole body lipid oxidation, close relationships between the potential of muscle to use carbohydrates or lipid fuels and the development or maintenance of body fat mass have also been described recently in various species. In particular, reduced activity levels of the oxidative enzymes citrate synthase (CS), β-hydroxyacyl-CoA dehydrogenase (HAD), and/or cytochrome-*c* oxidase (COX) and elevated phosphofructokinase (PFK) activity as an index of the glycolytic capacity have been found in skeletal muscle of obese humans (23, 42). However, compared with lean LW pigs, the MS pigs exhibit a more oxidative and a less glycolytic muscle metabolism, as well as a higher capacity to use lipids as an energetic substrate (27).

Conservation of the syntenies between porcine SSC7 and human chromosomes 6, 14, and 15 has been demonstrated (44), although some microrearrangements occur in the swine QTL region (9, 10). About 120 genes are localized in the SSC7 interval (9.7 cM) close to the swine leukocyte antigen complex between SW1856 and NFY microsatellites markers (11). Some of these genes have known or suggested biological roles in adipose cell growth and differentiation, storage of fatty acids,

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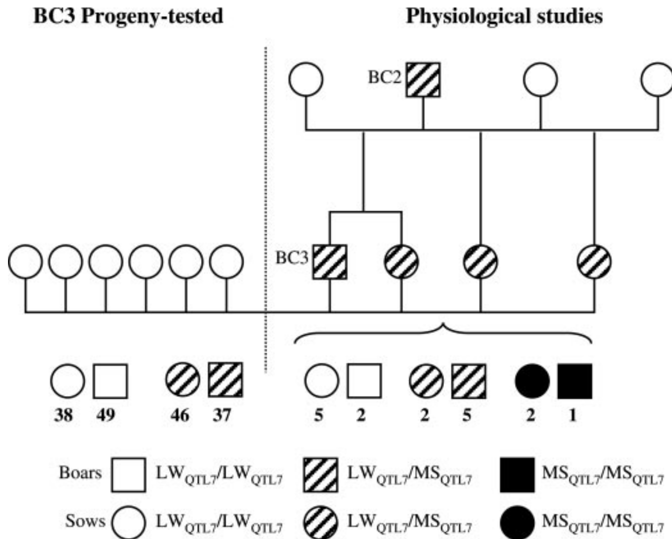

Fig. 1. Pedigrees of animals used for metabolic and physiological studies of the porcine chromosome 7 (SSC7) quantitative trait locus (QTL). LW$_{QTL7}$/LW$_{QTL7}$, LW$_{QTL7}$/MS$_{QTL7}$, and MS$_{QTL7}$/MS$_{QTL7}$: animals that are homozygous Large White LW/LW, homozygous Meishan MS/MS, and heterozygous LW/MS in the mapping interval of the SSC7 body composition QTL, respectively, and have a 15/16 similar LW genetic background. The boar that was progeny tested belonged to a third-generation backcross (BC3) and was shown to be heterozygous for the SSC7 QTL (*experiment 1*). This boar was mated to 3 sows with a LW$_{QTL7}$/MS$_{QTL7}$ genotype (*experiment 2*) to produce the 3 possible QTL genotypes (LW$_{QTL7}$/LW$_{QTL7}$, LW$_{QTL7}$/MS$_{QTL7}$, and MS$_{QTL7}$/MS$_{QTL7}$).

downstream events implicated in the adipogenic action of insulin, oxidation, or muscle contractile apparatus. The present study aimed to investigate the main cellular and metabolic pathways related to lipid accumulation or energy expenditure in adipose tissue and skeletal muscles of pigs with either the LW/LW or LW/MS genotype for the SSC7 QTL but having the same genetic background. This may give some indication of the candidate genes at the SSC7 QTL responsible for the genetic effects on body fatness variation in pigs.

MATERIALS AND METHODS

Genetic Analyses

Experimental animals. Animals were reared and slaughtered in accordance with French standard guidelines for human care and use of animals in research (certificate of authorization to experiment on living animal no. 35-22 delivered by the French Department of Agriculture to F. Gondret).

A recombinant backcross design was developed for the fine mapping of a carcass composition QTL located on chromosome 7 as described in Demeure et al. (11). Offspring originating from a second-generation backcross (BC2) boar found to be heterozygous at the QTL through progeny testing were produced, among which one male (BC3) and three females were kept for breeding. These four animals all presented an identical-by-descent MS segment inherited from a single female MS ancestor. Their pedigree is presented in Fig. 1. They were used to produce two different experimental crosses.

Experiment 1. The BC3 boar was mated to 14 LW sows and was subsequently progeny tested to identify its genotype for the SSC7 QTL. Offspring (n = 80) were performance tested and slaughtered in a commercial slaughterhouse (Fleury-les-Aubrais, France) at ~150 days of age. Several traits related to carcass composition were assessed, as explained in RESULTS.

Experiment 2. To produce pigs with the same genetic background and differing only for the SSC7 QTL region, the BC3 boar was mated to its three heterozygous LW/MS half-sister sows. BC3 was then the father of all pigs used for metabolic and cellular studies. Three different genotypes were thus obtained: homozygous LW/LW (LW$_{QTL7}$/LW$_{QTL7}$), homozygous MS/MS (MS$_{QTL7}$/MS$_{QTL7}$), and heterozygous LW/MS (LW$_{QTL7}$/MS$_{QTL7}$) in the SSC7 QTL region. All of these animals have a 15/16 similar LW genetic background. Pigs were fed ad libitum with conventional diets for weaning, post-weaning, and growing-finishing periods. At ~150 days of age, seven LW$_{QTL7}$/LW$_{QTL7}$ (n = 5 females, and n = 2 barrows), three MS$_{QTL7}$/MS$_{QTL7}$ (n = 2 females, and n = 1 barrow), and seven LW$_{QTL7}$/MS$_{QTL7}$ (n = 2 females, and n = 5 barrows) pigs were weighed individually and transferred to the INRA experimental slaughterhouse of Saint-Gilles, France (transport duration, 3 h). They were slaughtered after overnight fasting by electrical stunning and exsanguination in compliance with present national regulations applied in slaughterhouses. Body composition, tissue sampling, and physiological characteristics of selected tissues were then assessed as explained below.

Molecular Analyses

All offspring resulting from the progeny test (*experiment 1*) and their parents and grandparents were typed for seven microsatellites markers covering the SSC7 QTL region (from 4 to 105 cM). The names and locations of microsatellites markers are indicated in Fig. 2. DNA was extracted from tail samples. Amplifications were performed on ABI 9700 PCR machines (Applied Biosystems, Foster City, CA), and genotyping was carried out on an ABI 3730 automatic sequencer (Applied Biosystems). Genotypes were then determined using the

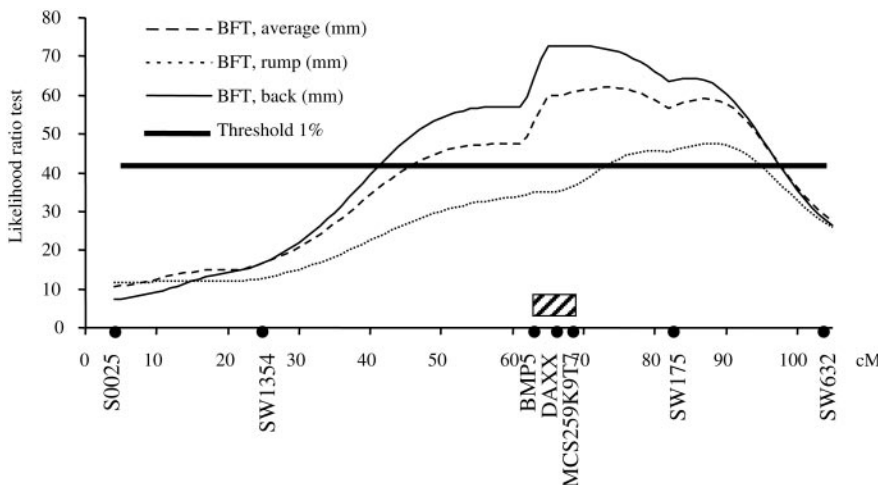

Fig. 2. Likelihood ratio curves on swine chromosome 7 for single-trait, single-QTL mapping. Results are of QTL analysis for backfat thickness (BFT) measured after slaughter. The different markers used and their locations are represented by black circles. The box represents the interval of localization of the QTL.

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Genescan and Genotyper softwares (Applied Biosystems). Results of genotyping were checked, validated, and stored in the GEMMA database (21).

Carcass Measurements

Carcass weight and length were recorded shortly after slaughter. Perirenal fat (leaf fat) was weighed at the same moment. Subcutaneous fat thickness (backfat thickness; BFT) was measured using a Fat-O-Meater probe (SFK, Herlev, Denmark) at the levels of the back [means of measurements taken at the 3rd/4th lumbar vertebra (G1) and 3rd/4th last rib (G2) levels], the neck (means of 2 measures), and/or the rump (means of 2 measures). Muscle depth was measured at the 3rd/4th last rib levels (loin eye depth, M2). Lean meat content (LMC) was estimated using an equation combining G1, G2, and M2 measurements (8).

The carcass was then chilled at 4°C for one night. The day after, the right half-carcass was divided into retail cuts (ham, loin, belly, shoulder, backfat) that were individually weighed. Chilled perirenal fat and the entire longissimus and trapezius muscles excised from the loin and shoulder cuts, respectively, were individually weighed.

Tissue Sampling

For adipocyte isolation purposes, fresh portions of subcutaneous backfat (at the 5th lumbar vertebra level) were excised from the left carcass side, just after pig exsanguination. Minced tissues were placed in warm Krebs-Ringer bicarbonate buffer (Sigma, St. Quentin Fallavier, France) and immediately processed. For histological, biochemical, and enzyme analyses, samples of backfat (last rib level) and longissimus (last rib level, i.e., a white fast-twitch glycolytic muscle) and trapezius (middle portion, a red slow-twitch oxidative muscle) muscles were excised from the left carcass side within 30 min after exsanguination. Visible intermuscular adipose tissue was carefully removed from muscles. Then, backfat adipose tissue and skeletal muscles oriented following the myofiber longitudinal axis were put on flat sticks, frozen in liquid nitrogen, and stored at −75°C until analyses.

Isolation of Adipocytes

Adipocytes were collected after a collagenase (Roche Applied Science, Meylan, France; 0.22 U/mg) treatment of backfat, as described previously (13) but with some adaptations (29). Briefly, samples of minced backfat were shaken (40 rpm) for 60 min at 37°C in sterile polypropylene flasks in Krebs-Ringer bicarbonate buffer (3 ml/g tissue) containing 3% bovine serum albumin (BSA), 10 mM glucose, 1.3 mg/ml collagenase A, and antibiotics. The digested material was then filtered through 200-μm sterile nylon mesh filters. Adipocytes were allowed to float; they were then rinsed three times with DMEM (5.5 mM glucose; Invitrogen, Cergy-Pontoise, France) by removing the infranatant using a plastic catheter attached to a syringe. Cells were then maintained in the same medium at 37°C in an atmosphere of O_2-CO_2 (95:5%). An aliquot (10^3 cells) of isolated adipocytes was placed on a heated (37°C) glass plate and immediately digitized using a photomicroscopy system. Individual diameters were measured using an image analysis system (Optimas 6.5; Media Cybernetics, Silver Spring, MD). Cell diameters were categorized into classes (every 10 μm), and the relative frequency of adipocytes in each class was then calculated. Because of problems during the isolation procedure, only four LW_{QTL7}/MS_{QTL7} pigs and four LW_{QTL7}/LW_{QTL7} pigs could be finally considered.

Glucose Incorporation into Lipids

The lipogenic rate was estimated by quantifying the conversion of D-[U-^{14}C]glucose (GE Healthcare, Orsay, France) into lipids on *day 1* after cell isolation, as previously described (15). Briefly, cells (~2 × 10^5 cells) were incubated with 0.4 μCi of D-[U-^{14}C]glucose at 37°C, either in the absence (basal rate) or in the presence of insulin (17 nM) under an O_2-CO_2 (95:5%) atmosphere. After 4 h of incubation, the medium was removed. Dole's reagent was immediately added, and lipids were extracted (12). Incorporated radioactivity was measured by liquid scintillation counting. The number of adipocytes in the suspension was determined as previously described (29) from the measurement of lipid content in the aliquot of suspension (12), and the mean adipocyte volume was determined as described above, under the assumption that the cells were spherical. Glucose incorporation into lipids was expressed as nanomoles of glucose incorporated per 10^7 cells per 4 h.

Tissue Lipid Content

Lipid content in backfat and muscles was estimated using the total lipid extraction procedure outlined by Folch and et al. (14) and was expressed as percentage per gram of fresh tissue. Total amounts of lipids in tissue cuts were calculated considering chilled weights of tissues of the right carcass side.

Histochemistry

For adipocyte measurements, three cross sections of frozen backfat (10-μm thick, 50-μm intervals) were obtained from each sample, using a cryostat at −30°C. Tissue sections were fixed for 10 min in 0.1 M phosphate buffer containing 2.5% (vol/vol) glutaraldehyde (25% aqueous solution; Sigma, St. Louis, MO). They were stained for 4 min in isopropanol containing 0.5% oil red O (wt/vol), and membranes of adipocytes and myofibers were then counterstained in an aqueous solution of crystal violet indicator (17). Visible adipocytes in three randomly chosen fields per section were carefully reproduced on transparent plastic sheets using a projection microscope (Visopan, Reichert, Vienna), and individual cell areas were measured using a programmable planimeter (Hitachi Siko, Tokyo, Japan). Mean diameter (μm) of adipocytes was then calculated. The number of adipocytes per gram of backfat was estimated by dividing the total lipid content expressed per gram of backfat by the adipocyte mean volume (26), considering that triglyceride density is ~0.96.

For myofiber histochemistry, five serial transverse cross sections (10-μm thick) were cut in each muscle using the cryostat at −20°C. One section was stained in red with azorubin for reference. Cross-sectional areas of the myofibers (CSA, μm^2) were measured using a macro-program developed on the Optimas image analysis system, on three randomly digitized fields. Three other sections were processed for actomyosin ATPase activity after acidic preincubation at pH 4.25, 4.35, and 4.45 (7) to identify slow-twitch type I, fast-twitch type IIA, and fast-twitch type IIB/X fibers. The last section was stained for succinate dehydrogenase (SDH) activity (37) to assess the aerobic oxidative capacity of the fibers. Relative percentages of type I, IIA, or IIB/X or SDH-positive myofibers of each muscle sample were determined on the basis of three selected fields of 300 fibers each per sample, using the projection microscope.

Lipogenic Enzyme Activities

A weighed quantity of backfat (about 0.6 g) was homogenized in 2 ml of ice-cold 0.25 M sucrose containing 1 mM dithiothreitol and 1 mM EDTA. The mixture was then centrifuged at 100,000 *g* for 1 h at 4°C, and the cytosolic fraction was collected and stored at −75°C. Activities of enzymes representing a key step in the formation of palmitic acid (fatty acid synthase; FAS) or providing a reduced equivalent for fatty acid synthesis (malic enzyme; ME) were measured according to the method described by Bazin and Ferré (3). Enzyme activities were assayed spectrophotometrically at 340-nm absorbance following the disappearance (FAS) or the production (ME) of NADPH. Substrate quantities were optimized (50–300 μl) to ensure linearity of the reactions. Lipogenic enzyme activities were expressed as nanomoles of NADPH per minute and per gram of tissue.

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Enzyme data were also expressed in units per cell, by dividing the activity per gram of tissue by the number of adipocytes per gram of backfat as calculated above. Finally, protein content of the cytosolic fraction was determined using the bicinchoninic acid assay with BSA as standard. Enzyme activities were then also expressed as nanomoles of NADPH per minute and per milligram of proteins.

Catabolic Energy Enzyme Activities

A frozen portion of muscles (~0.2 g) was homogenized in 50 vol (wt/vol) of ice-chilled 0.1 M phosphate buffer (pH 7.5) containing 2 mM EDTA and sonicated. After centrifugation at 1,700 *g* for 15 min at 4°C, the supernatant fraction (soluble enzymes and mitochondrial material) was collected and used for further analyses. The maximal activities of mitochondria oxidative markers, reflecting either fatty acid β-oxidation (HAD), mitochondrial density (CS), and/or phosphorylative oxidation (COX), were determined spectrophotometrically according to methods described previously (2, 43). In addition, the activities of PFK and of lactate dehydrogenase (LDH) as markers of anaerobic glycolysis were determined in the same samples (46). Enzyme activities were expressed as micromoles of substrate degraded per minute and per gram of fresh muscle. Total enzyme activities in the muscles were calculated by multiplying the activities per gram of muscle by the weights of individual muscles. Protein content of the total fraction was determined as described above. Enzyme activities were also expressed as nanomoles per minute and per milligram of proteins.

Statistical Analyses

Statistical analysis software (SAS; SAS Institute, Cary, NC) was used for data analyses.

In *experiment 1*, progeny test analyses were conducted similarly to those described by Sanchez et al. (40). Data were first adjusted for environmental effects using the GLM procedure of SAS (1999) and corrected for the fixed effects of batch and sex. Some traits were additionally adjusted to a constant weight by adding slaughter weight as a covariate in the model. A half/full-sibling model was used, which assumes that the sire progeny is distributed into several independent full-sibling families. For each centimorgan along a chromosome, the hypothesis of one QTL (H1) linked to the set of markers considered was compared with the hypothesis of no QTL (H0) at the same location. Under the H1 hypothesis, a single-QTL model with a gene substitution effect for the sire and for each dam was fitted to the data. Likelihoods were then maximized under each hypothesis, and the statistical test was computed as the ratio of likelihoods (L ratio). Average substitution effects were estimated for the sire and each dam family at the location with the highest L ratio (28). Significance thresholds were determined, assuming a polygenic infinitesimal model and a normal distribution of phenotypes (28).

In *experiment 2*, final live weight measurements were compared using a fixed linear model with the QTL genotype and sex as main effects. The same model, including pig weight assessed just before transportation as a covariate, was used for data analysis of carcass traits and metabolic and cellular characteristics of backfat and skeletal muscles. The effect of interaction between QTL genotype and sex was tested in preliminary analyses but never reached significance ($P > 0.10$); consequently, it was removed from the final model. The analyses were performed using the GLM procedure of SAS software (SAS Institute, 1999). Least square mean values for QTL genotype were compared using the PDIFF statement of the GLM procedure. Differences were considered to be significant at $P \leq 0.10$.

RESULTS

Experiment 1: Genetic Analyses

Results of QTL effects on body composition traits in the backcross family are presented in Table 1. Significant effects were obtained in the region of the SSC7 QTL for most carcass composition traits, especially backfat traits (weight and thickness at G1 and G2 levels, $P < 0.1$), estimated LMC ($P = 0.01$), and belly weight ($P < 0.1$). Likelihood ratio curves are presented in Fig. 2 for BFT. It clearly shows that the BC3 progeny tested boar, which had a LW/MS haplotype between BMP5 and MCS259K9T7 microsatellites markers, was heterozygous for the QTL of interest in the SSC7 region.

Table 1. *Carcass composition traits affected by swine chromosome 7 QTL segregation in the backcross Large White family*

Traits	No. of Animals	*P* Value	Position, cM	Estimated Effect
BFT, average (mm)	79	†	74	2.728
BFT, rump (mm)	79	†	88	2.673
BFT, back (mm)	80	†	69	3.764
BFT, neck (mm)	80	NS	67	1.662
LMC (%)	80	§	65	−0.754
G1 (mm)	80	*	74	1.662
G2 (mm)	80	†	65	1.783
M2 (mm)	80	NS	41	2.797
Carcass length (mm)	80	†	67	−22.114
Ham (kg)	80	‡	29	0.132
Belly (kg)	80	*	65	0.134
Backfat (kg)	80	†	65	0.189
Loin (kg)	80	NS	24	0.139

QTL, quantitative trait locus; NS, not significant; BFT, backfat thickness; LMC, lean mean content; G1 and G2, fat measurements at the levels of 3rd to 4th lumbar vertebrae (G1) and 3rd to 4th last ribs (G2); M2, lean depth of longissimus at 3rd to 4th last ribs level. *P* values: 0.05 (‡) and 0.01 (§) chromosome-wide significance levels and 0.10 (*) and 0.05 (†) experiment-wide significance levels. "Estimated effect" is the estimate of the allele substitution effect (Large White-Meishan alleles) in trait units.

Experiment 2: Physiological Phenotyping

Only results obtained for homozygous LW_{QTL7}/LW_{QTL7} and heterozygous LW_{QTL7}/MS_{QTL7} pigs are presented. The number of homozygous MS_{QTL7}/MS_{QTL7} animals still alive at final age (only 3 pigs) was too low to estimate genotypic means with a sufficient accuracy. Moreover, principal component analysis further showed that two of these pigs markedly deviated for all measurements performed (data not shown).

Pigs were the same age at the time of slaughter, and final live weight and hot and cold carcass weights did not significantly differ between homozygous LW_{QTL7}/LW_{QTL7} and heterozygous LW_{QTL7}/MS_{QTL7} pigs (Table 2). Whatever their genotype at the SSC7 QTL, females were lighter ($P = 0.01$) than barrows at slaughter. The LW_{QTL7}/LW_{QTL7} pigs exhibited higher perirenal fat weight ($P < 0.05$) and elevated proportions of belly (+18%) and fatty cuts (+17% for the sum of backfat and belly) in the carcass than LW_{QTL7}/MS_{QTL7} pigs. Although backfat thickness was 3.8 mm higher at the G1 level ($P = 0.07$) for LW_{QTL7}/LW_{QTL7} than LW_{QTL7}/MS_{QTL7} pigs (Table 2), no haplotype-related differences were observed for backfat weight (data not shown) and the relative proportion of backfat in the carcass. Relative proportions of loin, ham, and shoulder and of the sum of lean cuts (loin + ham + shoulder) in the carcass were not significantly different between genotypes, although they were close to significance ($P = 0.11$) for the proportion of ham. There were no sex-related differences in carcass compo-

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Table 2. *Body weight and composition at slaughter for LW_{QTL7}/LW_{QTL7} and LW_{QTL7}/MS_{QTL7} pigs*

Traits	LW_{QTL7}/LW_{QTL7}	LW_{QTL7}/MS_{QTL7}	SE	*P* Value	
				Genotype	Sex
Live weight, kg	89.0	95.3	9.3	0.28	0.01
Hot carcass weight, kg	69.8	70.6	0.9	0.18	0.84
Cold carcass weight, kg	68.0	68.7	0.9	0.23	0.86
Perirenal fat, g	1,130	566	261	0.006	0.21
Backfat thickness, mm					
G1	18.1	14.3	3.0	0.07	0.5
G2	14.8	12.3	3.4	0.23	0.1
Average	16.4	13.3	3.0	0.13	0.23
*Carcass composition, %**					
Backfat	7.0	5.9	2.0	0.38	0.37
Belly	13.7	11.6	0.8	0.002	0.97
Fatty retail cuts†	19.4	16.5	2.1	0.05	0.39
Loin	27.6	27.1	1.5	0.58	0.11
Ham	23.6	24.9	1.2	0.11	0.71
Shoulder	25.5	26.2	0.7	0.17	0.04
Lean retail cuts‡	76.7	78.2	1.9	0.24	0.51

Homozygous LW_{QTL7}/LW_{QTL7} ($n = 7$) or heterozygous LW_{QTL7}/MS_{QTL7} ($n = 7$) pigs for porcine chromosome 7 (SSC7) QTL. LW, Large White; MS, Meishan. *Weight percentage of the carcass. †Sum of backfat and belly weights. ‡Sum of loin, ham, and shoulder weights. *P* values are levels of significance for the effect of the genotype group and sex.

sition, except a slightly lower proportion of shoulder in females than in barrows.

Lipid-Related Traits

The relative content and the total amount of lipids in backfat and in muscles did not differ between LW_{QTL7}/LW_{QTL7} pigs and LW_{QTL7}/MS_{QTL7} pigs (Table 3). Mean diameter of backfat adipocytes estimated from tissue slices was 18% smaller in LW_{QTL7}/MS_{QTL7} pigs than in LW_{QTL7}/LW_{QTL7} animals; consequently, adipocytes in backfat were calculated to be far numerous in LW_{QTL7}/MS_{QTL7} than LW_{QTL7}/LW_{QTL7} pigs (Table 3). Results from adipocyte isolation confirmed the reduction in cell diameter for LW_{QTL7}/MS_{QTL7} compared with LW_{QTL7}/LW_{QTL7} pigs (−13%, $P = 0.05$, data not shown). The diagrams of the distribution of cell diameters show that differences between genotypes are in particular related to a lower proportion of large adipocytes (diameter >60 μm) in LW_{QTL7}/MS_{QTL7} compared with LW_{QTL7}/LW_{QTL7} pigs. However, the sharp cut-off in the distribution pattern at the lower ends also suggests that cells exhibiting a diameter lower than 20 μm could not really be considered in our experimental conditions. Therefore, a difference in the proportion of small adipocytes (<20 μm) between genotypes could not be excluded.

The basal rate of glucose incorporation into lipids was markedly lower in backfat cells of LW_{QTL7}/MS_{QTL7} pigs than in those of LW_{QTL7}/LW_{QTL7} pigs (Table 3). In both genotypes, insulin addition in the medium resulted in a dramatic increase in the lipogenic rate. However, differences in insulin-stimulated rate of lipogenesis between genotypes did not reach statistical significance ($P = 0.18$).

Activities of FAS and ME as selected enzymes involved in lipogenesis were twofold lower in LW_{QTL7}/MS_{QTL7} pigs than in LW_{QTL7}/LW_{QTL7} animals when expressed per adipose cells. When expressed per gram of wet tissue or per milligram of proteins, the data of enzyme activities did not differ between both genotypes.

Cellular and Metabolic Muscle Characteristics

The weights of longissimus (1,937 and 1,767 g in LW_{QTL7}/LW_{QTL7} and LW_{QTL7}/MS_{QTL7} pigs, respectively) and trapezius (97 and 91 g in LW_{QTL7}/LW_{QTL7} and LW_{QTL7}/MS_{QTL7} pigs, respectively) muscles did not differ between the two genotypes. In addition, there was no difference in the mean cross-sectional areas of the myofibers between genotypes for both muscles (Tables 4 and 5). No significant genotype-related differences were observed for the activities of the oxidative enzymes or glycolytic enzymes in both longissimus (Table 4) and trapezius (Table 5) muscles when expressed per gram of tissue, per milligram of proteins, or per wet tissue. The ratio of LDH (glycolysis) to CS (oxidation) activities tended to be higher in the longissimus of LW_{QTL7}/MS_{QTL7} pigs compared with LW_{QTL7}/LW_{QTL7} pigs (270 vs. 226, $P = 0.10$) but did not differ between genotypes in the trapezius (data not shown). Finally, in both muscles, the proportions of fiber types were similar between LW_{QTL7}/LW_{QTL7} pigs and LW_{QTL7}/MS_{QTL7} pigs (Tables 4 and 5).

DISCUSSION

The effects of the SSC7 QTL on carcass measurements have been largely documented in the literature (4, 30, 33, 39). However, the present approach is the first one aiming to characterize at a tissue level the metabolic and cellular effects of the genotype at SSC7 QTL. To analyze these aspects, pigs with a similar 15/16 Large White genetic background and either homozygous LW/LW or MS/MS or heterozygous LW/MS for SSC7 QTL were produced. Only results of LW_{QTL7}/LW_{QTL7} and LW_{QTL7}/MS_{QTL7} were presented because the strength of MS_{QTL7}/MS_{QTL7} was too small, and pigs were very heterogeneous. Progeny testing of the BC3 boar used as a sire allowed us to confirm the QTL effects on carcass traits previously described in the second-generation backcross pigs of the INRA PorQTL program (11, 40). Indeed, a decreased fat thickness at the back and rump levels and a lower

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Table 3. *Lipid-related traits at slaughter in backfat and muscles from LW_{QTL7}/LW_{QTL7} and LW_{QTL7}/MS_{QTL7} pigs*

Variables	LW_{QTL7}/LW_{QTL7}	LW_{QTL7}/MS_{QTL7}	SE	*P* Value	
				Genotype	Sex
Backfat					
Lipid content, g per 100 g wet tissue	54.1	54.3	0.07	0.97	0.34
Total amount of lipids in tissue, g*	1,239	1,125	451	0.70	0.52
Adipocyte diameter, μm	61.4	50.0	6.0	0.01	0.20
Adipocyte no., per g of tissue ($\times 10^9$)	5.1	8.8	2.1	0.02	0.11
Total no. of adipocytes in tissue ($\times 10^{12}$)	10.9	15.8	3.7	0.06	0.62
Glucose incorporation into lipids, nmol/4 h and per 10^7 cells†					
Basal rate	2.0	1.1	0.6	0.01	0.77
With insulin (17 nM)	6.3	5.0	4.0	0.19	0.85
Lipogenic enzymes activities, nmol/min and per 10^7 cells‡					
FAS	0.6	0.3	0.2	0.03	0.40
ME	7.4	3.9	2.0	0.02	0.24
Lipogenic enzymes activities, nmol/min and per g tissue‡					
FAS	276	299	156	0.81	0.48
ME	3,301	3,510	858	0.70	0.50
Lipogenic enzymes activities, nmol/min and per mg proteins‡					
FAS	42.7	37.0	20.7	0.67	0.77
ME	509	468	169	0.70	0.96
Longissimus muscle					
Lipid content, g per 100 g wet tissue	1.3	1.4	0.01	0.76	0.18
Total amount of lipids in tissue, g	24.5	24.4	6.6	0.98	0.27
Trapezius muscle					
Lipid content, g per 100 g fresh tissue	5.4	5.0	0.02	0.78	0.23
Total amount of lipids in tissue, g	5.4	4.8	2.4	0.70	0.28

Homozygous LW_{QTL7}/LW_{QTL7} ($n = 7$) or heterozygous LW_{QTL7}/MS_{QTL7} ($n = 7$) pigs for SSC7 QTL. *Total amount of lipids in the cut obtained in the right carcass. †Capacity of [^{14}C]glucose incorporation in backfat adipose tissue was determined in the absence (basal rate) or presence (17 nM) of insulin ($n = 4$ in each group). ‡Activities of malic enzyme (ME) and fatty acid synthase (FAS) were expressed as nanomoles of NADH produced or catabolized per minute. *P* values are levels of significance for the effects of genotype group and sex.

backfat weight (*experiment 1*, 80 descendants), together with a lower perirenal fat weight and a lower proportion of fatty cuts in the carcass (*experiment 2*, 14 descendants), were shown in pigs carrying at least one MS allele at the SSC7 QTL level. Although backfat thickness at the G1 level tended to be lower in LW_{QTL7}/MS_{QTL7} pigs compared with homozygous LW_{QTL7}/LW_{QTL7} pigs, no significant QTL effects were observed on mean backfat thickness and backfat weight in *experiment 2*. Since SSC7 QTL explains ~12–14% of the phenotypic variance in carcass fatness traits on F2 animals (33), the lack of significant differences in backfat weight (−16%) between LW_{QTL7}/MS_{QTL7} pigs and LW_{QTL7}/LW_{QTL7} pigs is likely to be due to the low number of animals in the experimental design and the heterogeneity of body weight at slaughter. When a limited number of pigs is considered, present results suggest that perirenal fat weight (leaf fat) would be the best indicator of the SSC7 QTL genotype status of tested boars.

Since backfat represents the main site of lipid storage in pigs, the cellular and metabolic characteristics of backfat were investigated in the present study. Despite the lack of any significant difference on quantitative backfat traits, present results provide new evidence for a lower diameter of adipocytes in heterozygous LW_{QTL7}/MS_{QTL7} pigs compared with homozygous LW_{QTL7}/LW_{QTL7} pigs. Additionally, MS_{QTL7}/MS_{QTL7} pigs exhibited numerically smaller (−25%) adipocytes in backfat than LW_{QTL7}/MS_{QTL7} animals (data not shown); this contrast was similar to that observed between LW_{QTL7}/MS_{QTL7} and LW_{QTL7}/LW_{QTL7} pigs. These results strongly suggest that the difference in backfat adipocyte diameter between genotypes might be associated with the presence of the MS allele at the SSC7 QTL. This is somewhat surprising, since pure MS breed pigs display enlarged adipocytes in backfat compared with pure LW breed pigs when slaughtered at the same live weight (5). Nevertheless, this observation seems in accordance with the effect of the SSC7 QTL; the MS allele is associated with a lower fat percentage (4, 33). Adipocyte volume is largely dependent on the accumulation of triacylglycerols, and de novo lipogenesis in the tissue is one possible mechanism resulting in accumulation of triacylglycerols (45), especially in pigs fed a high-starch diet (20). In accordance, we reported a lower basal ability to convert glucose into lipids in isolated adipocytes from LW_{QTL7}/MS_{QTL7} than from LW_{QTL7}/LW_{QTL7} pigs, together with depressed lipogenic enzyme activities per backfat cell in pigs carrying at least one MS allele. The choice of unit for enzyme activity expression is very important in the interpretation of data from adipose tissue containing cells of varying size. The cellular basis has been shown to be the preferred method of expression (20). When expressed on a wet tissue basis or on a soluble protein basis, no differences were observed between genotypes for enzyme data, since the reference units failed to take into account adipose cell size (20). Altogether, our results are in close connection with other experiments indicating that differences in adiposity between pig breeds are mainly related to differences in the lipogenic rate and enzyme activities per cells

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Table 4. *Catabolic energy metabolism and myofiber type composition in longissimus muscle from LW_{QTL7}/LW_{QTL7} and LW_{QTL7}/MS_{QTL7} pigs*

Variables	LW_{QTL7}/LW_{QTL7}	LW_{QTL7}/MS_{QTL7}	SE	*P* Value	
				Genotype	Sex
*Enzyme activities, μmol/min and per g tissue**					
HAD	4.2	3.5	0.7	0.18	0.52
CS	5.7	4.9	1.1	0.25	0.39
COX	6.1	6.3	2.2	0.86	0.38
PFK	28.6	36.1	20.0	0.56	0.65
LDH	1,877	1,897	267	0.90	0.13
*Enzyme activities, nmol/min and per mg proteins**					
HAD	20.0	16.3	3.5	0.13	0.92
CS	28.8	26.4	4.6	0.44	0.11
COX	30.6	33.9	10.1	0.61	0.18
PFK	148	198	128	0.54	0.86
LDH	6,453	6,886	535	0.22	0.43
*Total enzyme activities, mmol/min in wet muscle**					
HAD	8.1	6.3	1.6	0.11	0.97
CS	11.0	8.7	2.5	0.15	0.88
COX	11.8	11.4	4.9	0.90	0.68
PFK	55.2	65.4	44.7	0.72	0.59
LDH	364	338	69	0.56	0.65
Myofiber properties					
Mean cross-sectional area, μm^2	6,701	7,124	1,421	0.64	0.83
Type percentage, %					
I	10.1	10.1	4.7	0.99	0.79
IIA	9.0	6.5	2.7	0.16	0.40
IIB/X	80.9	83.4	4.3	0.36	0.81
SDH positive†	28.4	25.8	3.8	0.30	0.28

Homozygous LW_{QTL7}/LW_{QTL7} ($n = 7$) or heterozygous LW_{QTL7}/MS_{QTL7} ($n = 7$) pigs for the SSC7 QTL. *Activities of lactate dehydrogenase (LDH), citrate synthase (CS), and beta-hydroxy-acyl-CoA-dehydrogenase (HAD) were expressed as micromoles per minutes per gram of fresh muscle; cytochrome *C* oxidase (COX) was expressed as units per gram of tissue wet weight. †Succinate dehydrogenase (SDH) activity. PFK, phosphofructokinase. *P* values are levels of significance for the effects of genotype group and sex.

(20, 41). However, none of the lipogenic enzymes investigated here could be retained as a candidate for the SSC7 QTL effects, since ME and FAS loci are mapped on SSC1 (38) and SSC12 (35), respectively. Furthermore, insulin addition stimulated in vitro glucose conversion into lipids in a similar manner for both genotypes under experiment. This might be an argument to exclude genes coding for downstream events implicated in the lipogenic action of insulin from the SSC7 QTL effects.

Interestingly, FAS and ME enzymes have also been described as late markers of differentiation during the adipogenic process (6). Then, the lower lipogenic activities in adipocytes from LW_{QTL7}/MS_{QTL7} pigs compared with LW_{QTL7}/LW_{QTL7} pigs might be a consequence rather than a cause of the smaller adipocyte diameter of the heterozygous pigs at the QTL. This might indicate a lower physiological maturity of adipose cells of LW_{QTL7}/MS_{QTL7} pigs compared with LW_{QTL7}/LW_{QTL7} pigs when compared at the same age. In accordance, a delayed maturity of adipose tissue has been suggested in the pure MS breed compared with the lean Pietrain breed during animal growth (19). Because backfat lipid content was similar in both genotype groups, it can be concluded that the MS allele at SSC7 QTL was associated with an increased number of adipocytes, on a wet tissue weight basis as per backfat depot. Again, MS_{QTL7}/MS_{QTL7} pigs also displayed more numerous adipocytes (+44%) than LW_{QTL7}/MS_{QTL7} animals (data not shown). It is clear that the procedure for estimating cell numbers may also underestimate the true value, because it does not really account for cells <20 μm in diameter. However, a higher rate of proliferation of preadipocytes in MS animals compared with pure LW animals has been demonstrated previously in primary cultures (16). This feature closely agrees with present results indicating a higher adipocyte number in backfat of pigs carrying at least one MS allele. Altogether, the higher number and lower diameter of adipocytes associated with the lower basal ability to convert glucose into lipids in backfat cells of LW_{QTL7}/MS_{QTL7} pigs compared with LW_{QTL7}/LW_{QTL7} pigs indicate that further work could focus on SSC7 genes with known or suggested functions in the extent and kinetics of adipogenesis. In accordance, a slow differentiation or a later commitment of precursors to adipocyte differentiation has been suggested previously in an MS pure population compared with a Western pig breed (19).

Interestingly, this porcine SSC7 QTL region (9, 10) contains genes with critical roles in adipose cell growth and differentiation, such as mitogen-activated protein kinase [MAPK14/p38 (1)], high-motility group AT-hook 1 [HMGA1 (32)], and peroxisome proliferator-activated receptor-δ (18). In particular, HMGA1 polymorphism was previously found to be associated with fat deposition in European commercial lines (24, 25). Another gene of importance for the adipogenic process in the SSC7 QTL interval might be the cyclin-dependent kinase inhibitor (CDKN1A) known to regulate adipocyte number in mammals (36). The precise functions of one or a cascade of these SSC7 QTL genes in the regulation of adipogenesis and

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Table 5. *Catabolic energy metabolism and myofiber type composition in trapezius muscle from LW_{QTL7}/LW_{QTL7} and LW_{QTL7}/MS_{QTL7} pigs*

Variables	LW_{QTL7}/LW_{QTL7}	LW_{QTL7}/MS_{QTL7}	SE	*P* Value	
				Genotype	Sex
*Enzyme activities, μmol/min and per g tissue**					
HAD	12.4	11.7	1.4	0.42	0.38
CS	18.1	16.9	2.1	0.40	0.86
COX	22.6	23.5	6.4	0.83	0.46
PFK	15.3	21.6	6.6	0.16	0.54
LDH	609	502	130	0.22	0.73
*Enzyme activities, nmol/min and per mg proteins**					
HAD	494	457	84	0.49	0.60
CS	721	658	114	0.28	0.40
COX	144	147	41	0.89	0.51
PFK	98	134	39	0.18	0.56
LDH	23.9	19.2	4.4	0.12	0.39
*Enzyme activities, mmol/min in wet muscle**					
HAD	1.2	1.1	0.3	0.35	0.40
CS	1.8	1.5	0.4	0.34	0.53
COX	2.2	2.2	0.8	0.96	0.48
PFK	1.5	1.9	0.7	0.29	0.82
LDH	58.6	45.4	16.4	0.22	0.53
Myofibers properties					
Mean cross-sectional area, μm^2	3,802	4,145	423	0.22	0.01
Type percentage, %					
I	45	43	5	0.49	0.40
IIA	21	22	3	0.86	0.76
IIB/X	34	36	4	0.54	0.25

Homozygous LW_{QTL7}/LW_{QTL7} (n = 7) or heterozygous LW_{QTL7}/MS_{QTL7} (n = 7) pigs for the SSC7 QTL. *Activities of HAD, CS, COX, PFK, and LDH. *P* values are levels of significance for the effects of genotype group and sex.

more generally their effects on the characteristics of porcine adipose cells deserve further study.

The second major finding of this study is that muscle characteristics are rather similar in carriers and noncarriers of the MS allele. By contrast, significant differences in myofiber size and muscle oxidative metabolism have been found between MS and LW breeds (27). In the present study, skeletal muscles of LW_{QTL7}/MS_{QTL7} and LW_{QTL7}/LW_{QTL7} pigs had the same weights and similar myofiber mean cross-sectional areas and type distribution. In addition, activities of citrate synthase and cytochrome-*c* oxidase enzymes, which are considered convenient and fairly reliable markers of mitochondrial content and function, respectively (22), did not markedly differ between pig carriers or noncarriers of the MS allele at the SSC7 QTL. Only the metabolic differentiation of muscle, defined as the glycolytic-to-oxidative enzyme activity ratio, tended to be higher in the longissimus muscle of LW_{QTL7}/MS_{QTL7} pigs compared with LW_{QTL7}/LW_{QTL7} pigs. Several genes related to the cytochrome *c* (CYCSL 1 for cytochrome *c* somatic-like 1, LOC285849 for similar to cytochrome-*c* oxidase subunit VIa polypeptide 1 precursor, COX6A1P2 for cytochrome-*c* oxidase subunit VIa polypeptide 1 pseudogene 2) or muscle contractile apparatus (LOC442204 for similar to myosin subunit regulatory light chain) are present in the mapping interval of the SSC7 body composition QTL (9, 10). Contrary to studies in humans showing reduced oxidative enzyme activities in skeletal muscle of obese individuals (e.g., Refs. 23, 42), present results demonstrate that skeletal muscles are not the target organs of the SSC7 QTL genes. The present results do not support the association of positional candidate genes related to substrate oxidation with SSC7 QTL effects on pig body composition.

In conclusion, the limited number of progeny used in this experiment was large enough to detect the very strong effects of the SSC7 QTL chromosomal region on adipocyte cellularity and metabolism. One aim of this study was to identify a fine phenotypic measure for use in future progeny tests, eventually resulting in the production of fewer animals to determine homozygoty or heterozygoty at the SSC7 QTL. Significant results obtained on adipocyte cellularity suggest that this measure could be used. Additional progeny testing is necessary to evaluate its efficacy as a fine phenotypic measurement. This phenotypic study using the pig QTL region provides new information on the biological basis of fat mass variation. Results indicate that further studies aiming to understand SSC7 QTL effects may focus on a few candidate genes involved in the adipogenic process rather than genes involved in catabolic oxidative pathways. Additional studies of polymorphisms for these candidate genes in various pig breeds chosen for their divergence in body composition phenotypes, together with siRNA analyses of these few candidate genes using porcine preadipocytes in culture, are now required.

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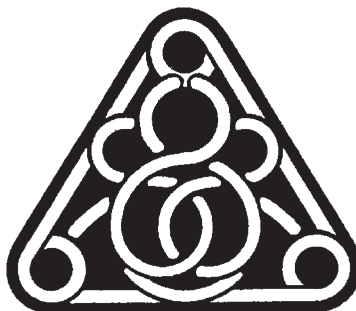

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