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Acetyl-CoA carboxylase and stearoyl-CoA desaturase protein expression in subcutaneous adipose tissue is reduced in pigs selected for decreased backfat thickness at constant intramuscular fat content[1]

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ABSTRACT: The objectives of this study were 1) to determine whether selection toward less subcutaneous fat thickness at constant intramuscular fat content in pigs is related to tissue-specific changes in the expression of lipogenic enzymes acetyl-CoA carboxylase (ACC), stearoyl-CoA desaturase (SCD), and Δ^6-desaturase (Δ6d); and 2) to investigate tissue specific distribution of the porcine ACC, SCD, and Δ6d. The study was conducted on 20 purebred Duroc barrows. Ten animals were from a group selected for decreased subcutaneous fat thickness at constant intramuscular fat content (experimental group). The other 10 animals were from the unselected (control) group. Distribution of ACC, SCD, and Δ6d was investigated in semimembranosus muscle (SM), subcutaneous adipose tissue (SA), liver (L), kidney (K), heart (H), diaphragm (D), rectus capitis muscle (RCM), and abdominal fat (AF). The enzyme expression was studied in 10 animals in the case of SM and SA and in 4 animals in the case of other tissues. The following expression pattern was established for ACC: SM $\leq$ H = K $\leq$ D < RCM < L < AF = SA, whereas the expression patterns for SCD and Δ6d proteins were SM < H < RCM < D < L < K < AF = SA and RCM = SM = D < L $\leq$ H < SA < K < AF, respectively. Expression of ACC and SCD proteins was less in subcutaneous adipose tissue of the experimental animals when compared with the control group ($P < 0.001$). However, no difference ($P > 0.1$) in ACC and SCD protein expression between the control and experimental groups was observed in SM. Expression of Δ6d protein did not differ between the control and experimental groups for SA ($P = 0.47$) or SM ($P = 0.31$). There was a positive relationship between muscle SCD protein expression and intramuscular fat content (r = 0.48, $P < 0.05$). Intramuscular fat content did not correlate with ACC or Δ6d protein expression ($P = 0.23$ and $P = 0.80$, respectively). We conclude that SCD might be an effective potential biomarker for intramuscular fat deposition.

Key words: intramuscular fat, lipogenic enzyme expression, meat quality, pig, subcutaneous fat

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INTRODUCTION

An increase in carcass lean content has been considered a major objective of the pig industry. This has been achieved via selective breeding against subcutaneous fat thickness (McPhee and Trout, 1995; Hermesch et al., 2000). However, selection toward leaner carcasses has also resulted in a reduction in intramuscular fat (**IMF**), which is known to contribute to eating quality of pork (Ellis et al., 1996; Verbeke et al., 1999; Ruiz et al., 2000). Therefore, a challenge is to produce pigs with less subcutaneous fat thickness without reduction of IMF below the level required for optimum eating quality. It has been suggested that fat deposition in different depots might be regulated by different mechanisms (Gardan et al., 2006; Gondret et al., 2008) and that the lipogenic enzyme stearoyl-CoA desaturase (**SCD**) plays the key role in this process (Da Costa et al., 2004; Doran et al., 2006). Contribution of other lipogenic enzymes to the tissue-specific regulation of fat deposition in pigs remains unclear. Moreover, the mechanisms regulating IMF and subcutaneous fat

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deposition are largely unknown. Knowledge of these mechanisms would allow identification of physiological candidate genes that could be used for developing of tests for evaluation of effectiveness of genetic selection or dietary manipulations.

The objectives of the present study were 1) to investigate whether selection toward less backfat thickness at constant IMF content in pigs is related to tissue-specific changes in the expression of the lipogenic enzymes acetyl-CoA carboxylase (**ACC**), SCD, and Δ^6-desaturase (**Δ6d**), and 2) to characterize tissue distribution of ACC, SCD, and Δ6d. This would contribute to our knowledge about possible involvement of different tissues in fat deposition in pigs.

MATERIALS AND METHODS

All the experimental procedures were approved by the Ethics Committee for Animal Experimentation of the University of Lleida.

Animals and Sample Collection

The experiments were conducted on 20 purebred Duroc barrows, which were selected from previously established experimental and control groups (Reixach et al., 2008, 2009). Ten of these animals were randomly chosen from a genetic group selected for decreased backfat thickness at constant BW and IMF content (experimental group). The other 10 animals were randomly chosen from the unselected (control) group. The experimental group differed from the control group in backfat thickness but not in IMF content and composition (Reixach et al., 2008). Mid-parent BLUP breeding values for backfat depth (based on approximately 37,000 records) and IMF content in gluteus medius (based on 3,000 records) were used to separate newborn litters into 2 groups differing in backfat depth but not in IMF content. This 1-generation selection process was repeated in 4 consecutive batches. Pigs in the present manuscript were obtained from the third batch. The groups were constituted according to the mid-parent (litter) breeding values for backfat thickness and BW at 180 d and for IMF content in the gluteus medius muscle adjusted for carcass weight, which were predicted using the model described in Solanes et al. (2009). Litters in the experimental group were selected against backfat thickness while maintaining IMF content and BW to the values most similar to those in the control group. Linear programming was used to select the litters satisfying the constraints above. At the age of 11 wk, 2 barrows per litter were taken at random and moved to a finishing facility until the age of 200 d. During the test period, pigs had ad libitum access to a commercial diet (Esporc, Ruidarenes, Girona, Spain; Table 1). Feed analyses were performed in triplicate. Dry matter was determined by oven-drying at 100 to 102°C for 24 h; ash was determined by muffle-heating at 550°C until constant weight. Crude protein was analyzed by the Kjeldahl method (AOAC, 2000), crude lipid was determined by Soxhlet fat analysis (AOAC, 2000), and crude fiber was analyzed by acid and alkaline digestion with a Dosi-Fiber extractor (Selecta, Barcelona, Spain; AOAC, 2000). Analyses of fatty acid composition were performed as described below for tissue samples after extracting the total lipids by the method of Hanson and Olley (1963).

Table 1. Composition of the diet fed during the finishing period

Item	Amount
DM, g/kg	893.2
CP, g/kg of DM	164.5
Crude lipid, g/kg of DM	66.1
Crude fiber, g/kg of DM	61.6
Ash, g/kg of DM	60.0
Fatty acid composition, mg/g of fatty acids	
C12:0	1.2
C14:0	12.2
C16:0	208.8
C16:1	25.2
C18:0	60.1
C18:1	353.2
C18:2	293.5
C18:3	1.8
C20:0	28.3
C20:1	8.0
C20:2	5.4
C20:4	2.3
SFA	310.6
MUFA	386.4
PUFA	303.0

Pigs were performance-tested at around 180 and 200 d of age. This included the measurement of BW and backfat thickness. The backfat thickness was determined ultrasonically at 5 cm off the midline at the position of the last rib (Piglog 105, SFK-Technology, Herlev, Denmark). Pigs were slaughtered at 203 d in a commercial abattoir. Carcass backfat thickness at 6 cm off the midline between the third and fourth last ribs and carcass lean percentage were measured by an on-line ultrasound automatic scanner (AutoFOM, SFK-Technology, Herlev, Denmark). Carcass lean content was estimated on the basis of 35 measurements of AutoFOM points by using the official approved equation (Decision 2001/775/CE, 2001). The differences that have been observed for backfat thickness in live animals (at 200 d) and for carcasses (at 203 d) are related to the use of different measurement techniques. Samples from 10 pigs from the same slaughtering batch (each originated from a different litter) were collected. Intramuscular fat content was measured as described previously (Bosch et al., 2009).

Immediately after slaughter, samples of semimembranosus muscle (**SM**) and subcutaneous adipose tissue were collected at the level of the third and fourth ribs. In addition, samples of liver, kidney, heart, diaphragm, rectus capitis muscle, and abdominal fat were collected from 4 randomly chosen animals and were used to in-

vestigate tissue distribution of lipogenic enzymes. The animals used in these experiments were the first 4 pigs on the slaughter line. Three of these animals were from the experimental group, and 1 animal was from the control group.

All the tissue samples were snap-frozen and stored at −80°C until analyzed. It has been previously demonstrated that these storage conditions do not affect expression of lipogenic enzymes (Doran et al., 2006).

Fatty Acid Analysis

Once defrosted, SM and subcutaneous adipose tissue samples were freeze-dried and thoroughly homogenized by mixing with sand using a glass stirring rod. Due to the small sample size, DM was calculated as the weight difference before and after freeze-drying. Fatty acid composition analysis was performed in duplicate by quantitative determination of the individual fatty acids by gas chromatography. Fatty acid methyl esters were directly obtained by transesterification using a solution of 20% boron trifluoride in methanol (Rule et al., 1997). Methyl esters were determined by gas chromatography using a capillary column SP2330 (30 m × 0.25 mm, Supelco, Bellefonte, PA) and a flame ionization detector with helium as carrier gas. The analytical column was coated with a 0.20-μm film.

The oven temperature program was increased from 150 to 225°C (by 7°C per min). The injector and detector temperatures were 250°C (Tor et al., 2005). Fatty acid quantification was carried out via normalization of the area under appropriate picks after adding 1,2,3-tripentadecanoylglycerol into each sample as an internal standard. Intramuscular fat content in SM and gluteus muscles was calculated as a sum of individual fatty acids expressed as triglyceride equivalents (AOAC, 2000) on a dry tissue basis (Bosch et al., 2009).

Isolation of Microsomal and Cytosolic Fractions

Expression of SCD and Δ6d proteins was analyzed in microsomal fraction; ACC protein expression was analyzed in cytosolic fraction. Microsomes and cytosol were isolated by differential centrifugation with Ca^{2+} precipitation (Schenkman and Cinti, 1978) with minor modifications. In brief, approximately 10 g of frozen tissue (2 g for liver) was homogenized in 20 mL of cold sucrose buffer (10 m*M* Tris-HCl, 250 m*M* sucrose, pH 7.4) and centrifuged at 12,000 × *g* for 10 min at 4°C. The supernatant (or infranatant in the case of adipose tissue) was collected and mixed with 8 m*M* $CaCl_2$ to facilitate sedimentation of the microsomal fraction. Microsomes were obtained by centrifugation at 25,000 × *g* for 35 min at 4°C. The supernatant (cytosolic fraction) was collected and the remaining microsomal pellet was resuspended in a buffer containing 10 m*M* Tris-HCl, 250 m*M* KCl (pH 7.4), and inhibitors of proteolytic enzymes (1.5 μ*M* antipain, 1.5 μ*M* pepstatin, and 2 μ*M* leupeptin; Sigma, Dorset, UK). Total microsomal and cytosolic protein content was determined by the Bradford method using BSA as the standard (Bradford, 1976).

Protein Expression

Expression of the lipogenic enzymes was analyzed by western blotting. Microsomal and cytosolic proteins (6 μg) were separated by SDS-PAGE and electroblotted onto a nitrocellulose membrane as described previously (Nicolau-Solano et al., 2006). The membrane was incubated with 1 of the following antibodies: goat polyclonal anti-human ACC (Santa Cruz Biotechnology Inc., Santa Cruz, CA), rabbit polyclonal anti-bovine adipose tissue SCD (custom-made at the University of Bristol, Bristol, UK), or rabbit polyclonal anti-Δ6d IgG (Sigma Genosys Ltd., Cambridge, UK). Incubation with primary antibody was followed by incubation with secondary antibody that was horseradish peroxide-linked donkey anti-rabbit IgG for SCD and Δ6d (GE Healthcare, Amersham, Bucks, UK) or donkey anti-goat IgG for ACC (Santa Cruz Biotechonology). Blots were developed using an Enhanced Chemiluminescent Reagent (GE Healthcare) and quantified using the ImageQuant program (Molecular Dynamics, Sunnyvale, CA). A microsomal or cytosolic preparation from 1 particular animal was present on all the blots (the reference sample). Intensity of the reference sample signal was taken as 100 arbitrary units, and the intensities of other samples on the blot were expressed as fractions of the reference sample. In the case of the genetic selection effects study, we have used subcutaneous adipose tissue and SM reference samples for adipose tissue and muscle Western blots, respectively. In the case of between-tissue enzyme expression comparison, the reference sample was prepared from liver and has been continuously used on all the relevant blots. All gels and blots were done in duplicate. The duplicate samples were run on different blots. The average intensity of the duplicates was calculated.

Statistical Analyses

The effect of selection for reduced backfat thickness at restrained BW and IMF content on lipogenic enzyme expression was analyzed by comparing mean values between the control and experimental groups for each tissue using a *t*-test. Regression analysis was used to test the association between fat content and expression of the lipogenic enzymes. A regression × group interaction was included to test if associations differed across the groups. Results were considered statistically significant at $P < 0.05$. Data were analyzed with SAS (SAS Inst. Inc., Cary, NC) using PROC MIXED procedure. No significant diversion from normality according to the Shapiro-Wilk test was found ($P < 0.05$). Between-tissue differences in enzyme expression were

Table 2. Backfat thickness, BW, and carcass characteristics in the control (n = 10) and experimental (n = 10) groups

	Group		
Trait	Control	Experimental[1]	SEM
Live measurement at 180 d			
Age, d	177.5	179.4	
BW, kg	110.6	107.0	3.2
Backfat thickness,[2] mm	19.0[a]	16.1[b]	1.0
Live measurement at 200 d			
Age, d	199.5	201.4	
BW, kg	125.7	121.3	3.3
Backfat thickness,[2] mm	21.9[a]	18.3[b]	1.1
Carcass measurement			
Carcass weight, kg	96.9	94.1	2.9
Backfat thickness,[3] mm	28.8[a]	25.0[b]	1.3
Lean percentage, % of fresh tissue	41.8	44.8	1.3
Intramuscular fat in GM,[4] % of fresh tissue	5.0	5.0	0.4
Intramuscular fat in SM,[4] % of fresh tissue	2.2	1.7	0.3

[a,b]Means within a row with different superscript differ ($P < 0.05$).

[1]Pigs in the experimental group were selected for decreased backfat depth at restrained intramuscular fat content.

[2]Backfat thickness on live animal was measured ultrasonically at 5 cm off the midline at the position of the last rib using the Piglog technology (SFK-Technology, Herlev, Denmark).

[3]Backfat thickness of carcasses was determined at 6 cm off the midline between the third and fourth last ribs using the AutoFOM automatic scanner (SFK-Technology).

[4]GM = gluteus muscle; SM = semimembranosus muscle.

analyzed by 1-way ANOVA with 8 levels and post-hoc comparison of means by the Tukey test.

RESULTS

The results of meat quality traits analysis in pigs from experimental and control groups are shown in Table 2. Backfat thickness in the experimental group was less ($P < 0.05$) at all time points (i.e., in live animals at 180 d, live animals at 200 d, and in carcasses at 203 d by 15.3, 14.4, and 13.2%, respectively), whereas IMF content in the gluteus muscles did not change. Fatty acid composition of the SM and subcutaneous adipose tissue for the control and experimental groups did not differ (Table 3).

Effect of Selection on Expression of Lipogenic Enzymes

To determine whether the reduction in subcutaneous fat thickness in experimental animals is related to inhibition of lipogenic enzyme expression, the expression of ACC, SCD, and Δ6d proteins was analyzed. Figure 1 shows that there was a decrease in the expression of

Table 3. Fatty acid composition of semimembranosus muscle and subcutaneous adipose tissue in the control (n = 10) and experimental (n = 10) groups

	Semimembranosus muscle			Subcutaneous adipose tissue		
Fatty acid, mg/g of DM[1]	Control	Experimental[2]	SEM	Control	Experimental[2]	SEM
Total SFA	28.95	22.20	3.27	240.32	247.09	6.20
C14:0	1.17	0.89	0.14	10.83	10.37	0.25
C16:0	18.75	14.21	2.11	157.81	159.01	3.89
C18:0	8.91	7.02	1.03	70.47	76.44	2.41
C20:0	0.11	0.08	0.02	1.22	1.26	0.05
Total MUFA	37.72	26.17	4.34	340.43	324.67	8.03
C16:1	2.52	1.47	0.30	17.87	16.88	0.81
C18:1	34.65	24.32	4.12	315.91	301.41	7.21
C20:1	0.55	0.38	0.07	6.65	6.38	0.18
Total PUFA	13.26	12.69	0.85	143.68	139.25	4.61
C18:2	10.14	9.56	0.72	125.49	122.13	4.19
C18:3	0.46	0.42	0.06	9.80	9.13	0.32
C20:2	0.41	0.35	0.12	6.62	6.33	0.12
C20:4	2.24	2.35	0.08	1.77	1.67	0.10

[1]DM determined by freeze-drying to constant weight.

[2]Pigs in the experimental group were selected for decreased backfat depth at restrained intramuscular fat content.

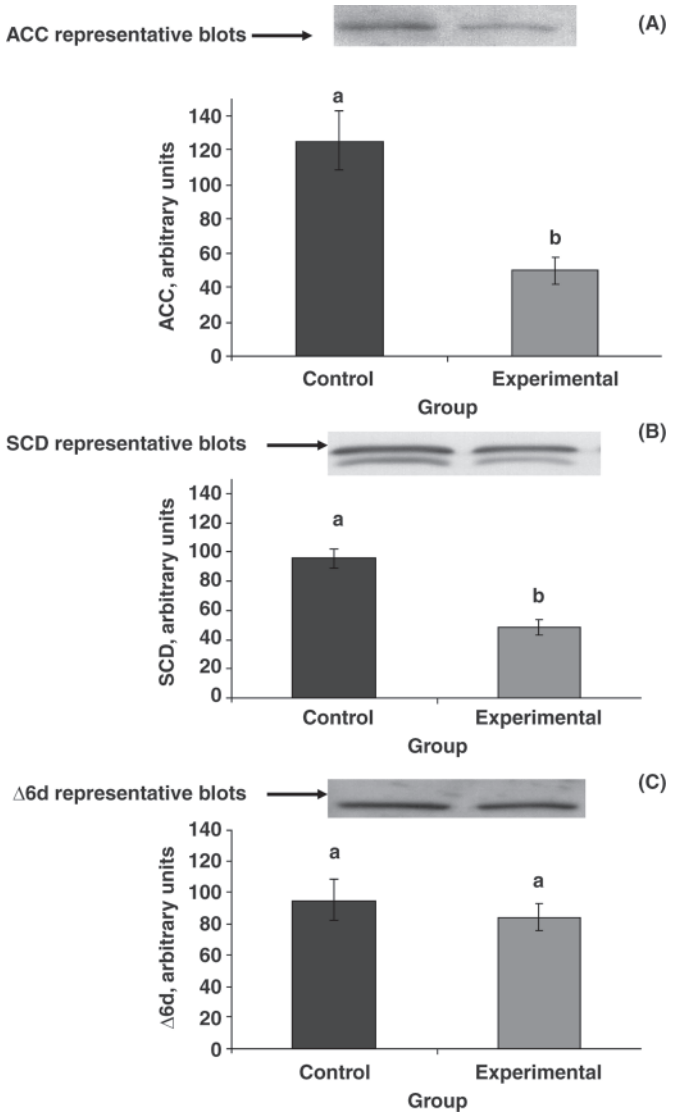

Figure 1. Representative blots and expression of acetyl-CoA carboxylase (ACC), stearoyl-CoA desaturase (SCD), and Δ^6-desaturase (Δ6d) proteins in subcutaneous adipose tissue of pigs in the control and experimental groups. Pigs in the experimental group were selected for decreased backfat depth at restrained intramuscular fat content. Bars represent average of measurements for 10 animals. All measurements were done in duplicate. Error bars represent SEM. [a,b]Means without a common letter differ ($P < 0.001$).

ACC and SCD proteins in the subcutaneous adipose tissue from the experimental group. The ACC protein level was about 60% less ($P < 0.001$; Figure 1A) and SCD expression was about 50% less ($P < 0.0001$, Figure 1B) than the control group. No differences between control and experimental groups were observed in the case of Δ6d protein expression ($P = 0.47$; Figure 1C). There was no relationship between the ACC, SCD, and Δ6d protein expression and the content of SFA, MUFA, and PUFA, respectively, in adipose tissue for the whole set of animals (control plus experimental groups, data not shown). However, a regression × group interaction analysis showed that selection against backfat thickness triggered a change in the association pattern between SCD protein expression and subcutaneous adipose tissue MUFA content. Thus, there was a negative relationship between SCD protein expression and MUFA content in the control ($r = -0.68$, $P < 0.05$), but not in the experimental group ($r = 0.48$, $P = 0.20$; Figure 2).

Results of ACC, SCD, and Δ6d proteins expression analysis in SM are presented in Figure 3. There were no differences ($P > 0.1$) between the control and experimental groups for the enzymes investigated. Moreover, no regression × group interaction of IMF, SFA, MUFA, and PUFA content on ACC, SCD, and Δ6d protein expression was found ($P > 0.05$, data not shown). When the results were analyzed as 1 data set, a positive relationship was found between SCD protein expression and IMF content ($r = 0.48$, $P < 0.05$; Figure 4A). Similar relationship was also found between SCD expression and the product of SCD-catalyzed reaction, MUFA ($r = 0.53$, $P < 0.05$; Figure 4B); and SCD expression and C18:1/C18:0 ratio ($r = 0.61$, $P < 0.01$; Figure 4C). No relationship was observed between IMF content and expression of ACC ($P = 0.23$) or Δ6d ($P = 0.80$) in muscle.

Tissue Distribution of ACC, SCD, and Δ6d Proteins

Although subcutaneous and intramuscular adipose tissues are the most important fat depots in terms of meat quality traits, understanding the mechanism regulating the whole body fat distribution is important for designing strategies for manipulation of fat partitioning. In this study, we have investigated tissue-specific distribution of the key lipogenic enzymes, ACC SCD, and Δ6d, catalyzing the biosynthesis of SFA, MUFA, and PUFA, respectively. Immunoreactive bands for all 3 proteins were detected in the liver, subcutaneous adipose tissue, abdominal fat, rectus capitis muscle, SM, diaphragm, heart, and kidney samples. Expression profiles of ACC, SCD, and Δ6d proteins are presented in Figure 5. Expression of ACC protein was the greatest in subcutaneous adipose tissue and abdominal fat, with a decrease in liver, followed by rectus capitis muscle; the smallest level occurred in SM, heart, and kidney ($P < 0.05$; Figure 5A). Similar to ACC, the greatest SCD expression level was observed in subcutaneous adipose tissue and abdominal fat. In contrast to ACC, the next organ with the greatest SCD expression was kidney, followed by liver, diaphragm, heart, rectus capitis muscle, and SM ($P < 0.05$; Figure 5B). The greatest Δ6d protein expression was observed in abdominal fat, kidney, subcutaneous adipose tissue, liver, and heart. The smallest Δ6d protein level was observed in diaphragm, rectus capitis muscle, and SM ($P < 0.05$; Figure 5C).

DISCUSSION

It has been demonstrated that that reduction in IMF negatively affects meat juiciness, tenderness, and flavor (Fernandez et al., 1999), and therefore increasing IMF content without affecting backfat thickness is one of the challenges of the pig industry. Positive correlation be-

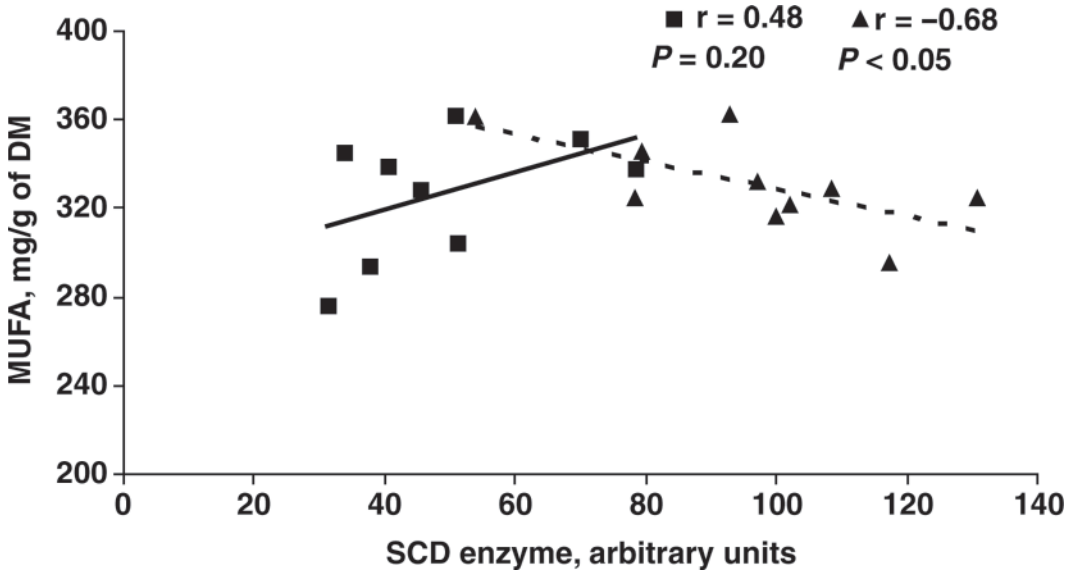

Figure 2. Relationship between stearoyl-CoA desaturase (SCD) protein expression and MUFA content (mg/g of DM) in subcutaneous adipose tissue of pigs in the control (▲, $P < 0.05$) and experimental groups (■, $P = 0.20$). Pigs in the experimental group were selected for decreased backfat depth at restrained intramuscular fat content.

tween IMF and pork eating quality has been found in a range of IMF from 0.7 to 4.5% (Goransson et al., 1992; Eikelenboom et al., 1996). In spite of a large number of reports supporting the positive impact of IMF on eating quality of pork, some reported a lack of correlation between IMF and perceived juiciness, which might be related to the age and eating habits of the particular group of consumers involved in this study (Ventanas et al., 2007).

In the present study, maximum differences in the backfat thickness between the control and experimental groups were observed at 180 d. This is consistent with the results of our previous larger study (188 and 172 pigs for control and experimental groups, respectively), which demonstrated that the difference in backfat depth between the 2 groups was already significant at 120 d, reached the maximum at around 180 d, and remained relatively stable, or even decreased, by 210 d (Reixach et al., 2009).

The present study demonstrated that selection for reduced subcutaneous fat in pigs with constant IMF is accompanied by significant decreases of ACC and SCD protein expression in subcutaneous adipose tissue but not in muscle. Stearoyl-CoA desaturase is the enzyme involved in the biosynthesis of MUFA from SFA (Enoch et al., 1976), whereas ACC catalyzes the first step in the SFA biosynthesis. Acetyl-CoA carboxylase is considered to be a rate-limiting enzyme of lipogenesis in animal tissues, and in pig tissues in particular (Scott et al., 1981). Tissue-specific responses of porcine ACC and SCD have been previously reported in dietary trials. Thus, Doran et al. (2006) established that a reduced protein diet increases the expression of SCD protein (and to a less extent ACC protein) in pig LM, but not in subcutaneous adipose tissue. The reasons for tissue-specific changes in lipogenic enzyme expression are not clear. This could be related, at least in part, to variations in the level of transcription factors regulating the enzyme expression. It is known that IMF fat is a later-maturing tissue when compared with subcutaneous fat (Gardan et al., 2006), and there are significant morphological and metabolic differences between these 2 depots, including differences in transcription factors level (Gardan et al., 2006; Gondret et al., 2008).

The other possible reason for tissue-specific responses of the porcine ACC and SCD could be tissue-specific expression of ACC and SCD isoforms. It is known that more than 1 ACC and SCD isoforms exist in mice, rats, and some other species (Thiede et al., 1986; Miyazaki and Ntambi, 2003; Miyazaki et al., 2003). It has also been demonstrated that SCD isoforms are tissue-specific, distributed with SCD1 being the predominant isoforms in liver and adipose tissue (Ntambi et al., 1988; Kim et al., 2002), whereas SCD2, SCD3, and SCD4 have been found in brain, skin, and heart, respectively (Kaestner et al., 1989; Zheng et al., 2001; Miyazaki et al., 2003). So far, 2 SCD isoforms have been reported in pigs: SCD1, which is preferentially expressed in subcutaneous adipose tissue (Ren et al., 2004); and SCD5, which has been recently reported to be expressed at very high levels in pig brain (Lengi and Corl, 2008). The SCD isoform spectrum in pig muscles and other tissues remains unknown.

In the present study, a decrease in the expression of adipose tissue SCD in the experimental group was not accompanied by changes in the amount of the product of SCD catalyzed reaction, namely C16:1 and C18:1, although there was a trend toward decrease in the amount of these fatty acids. A lack of differences might be related to large between-individual variations (especially in the case of C18:1) and to a relatively small number of animals.

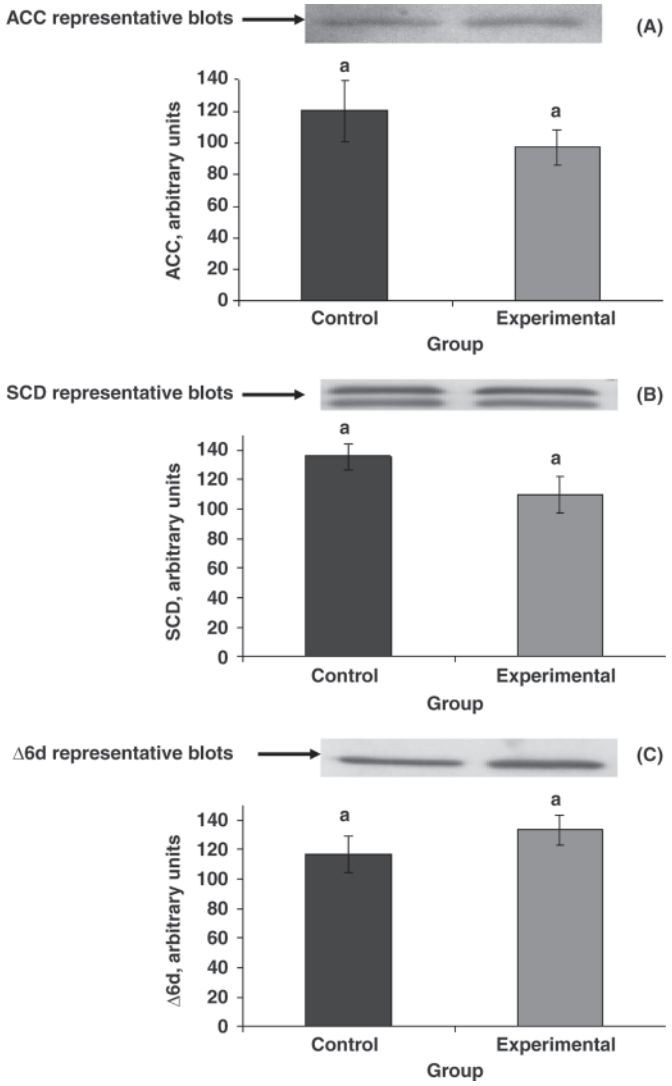

Figure 3. Representative blots and expression of acetyl-CoA carboxylase (ACC), stearoyl-CoA desaturase (SCD), and Δ^6-desaturase (Δ6d) proteins in semimembranosus muscle of pigs from control and experimental groups. Pigs in the experimental group were selected for decreased backfat depth at restrained intramuscular fat content. Bars represent average of measurements for 10 animals. All measurements were done in duplicate. Error bars represent SEM. [a]Means without a common letter differ ($P < 0.05$).

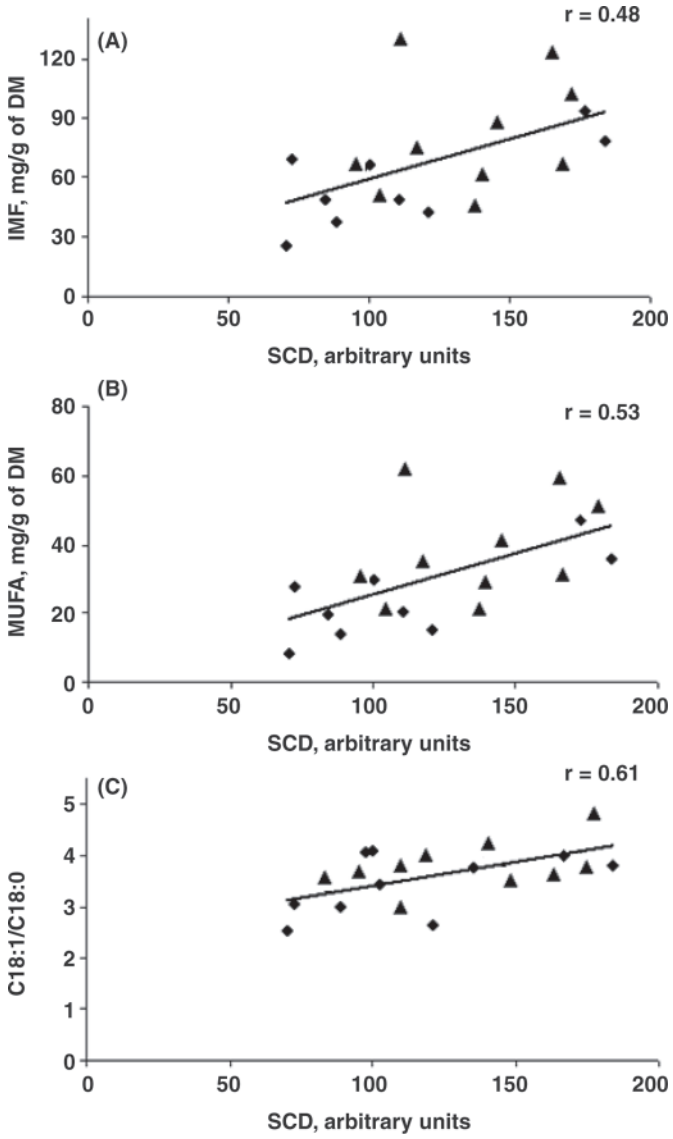

Figure 4. Relationship between stearoyl-CoA desaturase (SCD) protein expression and intramuscular fat (IMF) content (A), MUFA content (B), and C18:1/C18:0 ratio (C) in semimembranosus muscle of pigs of the control (▲) and experiment (◆) groups.

In the present study, the selection for reduced backfat thickness with constant IMF was not accompanied by any change in the expression of muscle or subcutaneous fat Δ6d protein. Delta-6 desaturase is one of the enzymes that catalyzes the conversion of the essential fatty acids (linoleate and α-linolenate) into long-chain PUFA in animal tissues (Cho et al., 1999). The fact that we did not observe any changes in Δ6d in this study indicates that biosynthesis of PUFA might have less input in the regulation of fat partitioning in pig when compared with the biosynthesis of MUFA and SFA.

Fatty acid composition and fat content depend on not only the rate of de novo lipogenesis in a particular tissue, but also on several other factors, including the rate of fatty acid transport from other lipogenic sites. In most mammals, lipogenesis occurs predominantly in the liver and adipose tissue (Girard et al., 1994). In pigs, the major site of fatty acid metabolism is subcutaneous adipose tissue, which has the greatest expression and activity of the key lipogenic enzymes (O'Hea and Leveille, 1969). The input of other tissues in regulation of fat deposition in pigs remains unclear, and tissue-specific distribution of lipogenic enzymes is unknown. In this study, we have characterized the distribution of ACC, SCD, and Δ6d proteins in 8 tissues from organs with diverse physiological functions. Immunoreactive bands corresponding to all 3 enzymes investigated were detected in liver, subcutaneous adipose tissue, abdominal fat, rectus capitis muscle, SM, diaphragm, heart, and kidney. The greatest expression of the lipogenic enzymes was found in subcutaneous adipose tissue and abdominal fat, which is consistent with the key role of these enzymes in lipid biosynthesis and other processes

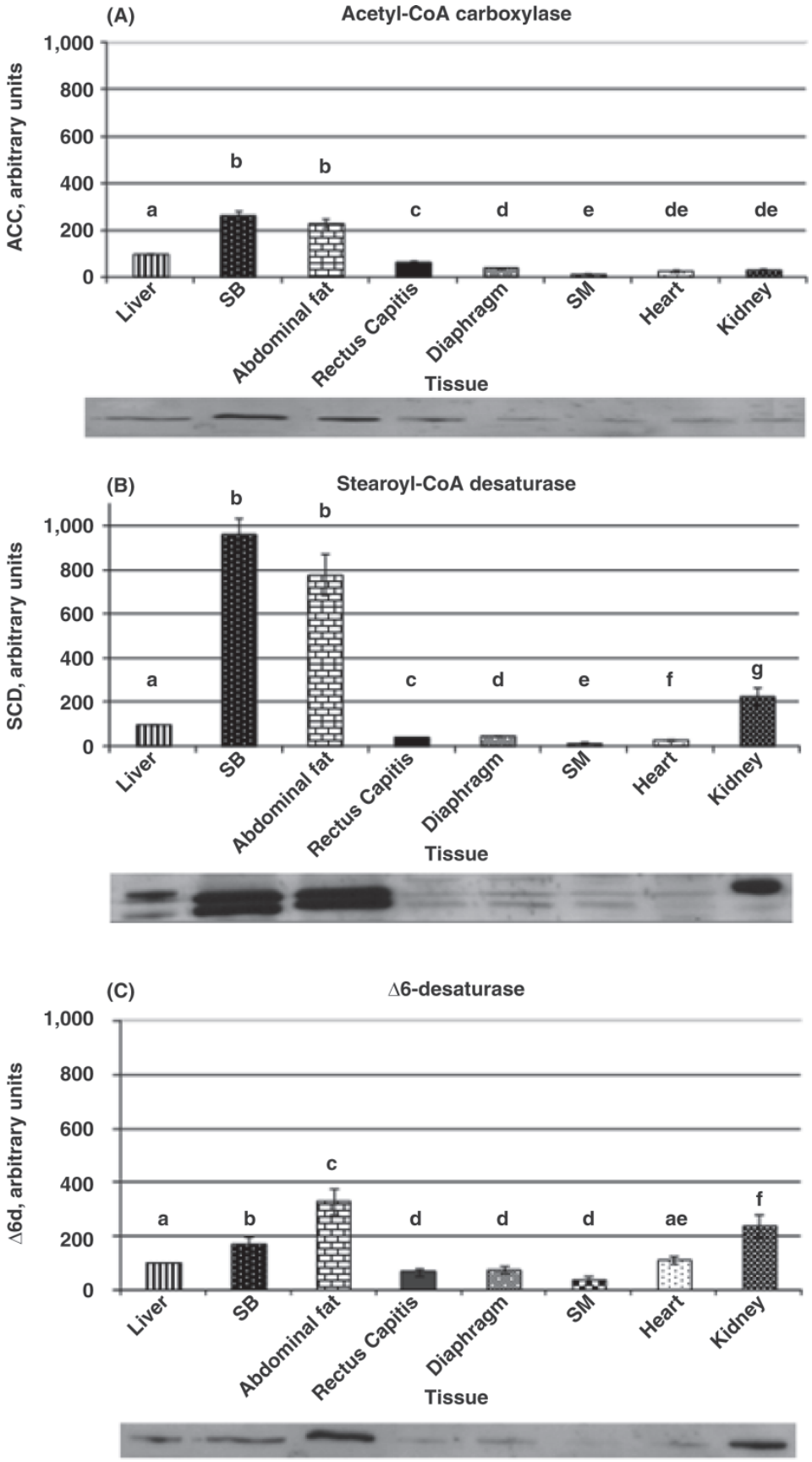

Figure 5. Expression profile of acetyl-CoA carboxylase (ACC), stearoyl-CoA desaturase (SCD), and Δ^6-desaturase (Δ6d) proteins in pig tissues. Each bar represents means of 4 pigs. All measurements were done in duplicate. Error bars represent SEM. [a–g]Means without a common letter differ ($P < 0.05$). Preparations from liver were used as reference samples in all the cases. The intensity of the signal of the reference sample for each enzyme was taken as 100 arbitrary units. The intensity of the signals of other samples on the same blot has been calculated as a fraction of the reference sample. Representative blots of tissue-specific distribution of ACC, SCD, and Δ6d proteins are given under corresponding graphs. SB = subcutaneous adipose tissue; SM = semimembranosus muscle.

that take place in adipose tissue (Ntambi and Miyazaki, 2004). Interestingly, we have also observed greater levels of SCD and Δ6d proteins in kidney (when compared with the liver, rectus capitis muscle, diaphragm, SM, and heart). Moreover, a greater level of ACC, SCD, and Δ6d proteins was also observed in the liver (when compared with the diaphragm, rectus capitis muscle, SM, and heart). Tissue-specific distribution of lipogenic enzymes is well known in other species and might be related to tissue-specific distribution of particular transcription factors and gene-specific promoter signals (Kim and Tae, 1994; Raclot and Oudart, 1999). Results of this study contribute to our understanding of the mechanisms regulating whole body fatty acid metabolism and partitioning in pigs.

Favorable scenarios for IMF content increase during genetic selection are expected as long as predicted breeding values based on IMF records are available (Solanes et al., 2009). At the present time, IMF evaluation in live animals is mainly conducted by ultrasound, which is expensive and not very accurate. Evaluation of IMF in carcasses can be performed by gas chromatography or similar techniques that are time-consuming and also expensive. Several DNA polymorphisms have been considered for developing of genetic tests, but the known polymorphisms only explain a small percentage of variations in IMF. Therefore, it would be beneficial to identify reliable biomarkers for rapid IMF evaluation. From the results of the present study, we conclude that SCD might be effective potential biomarker for fat deposition and partition in pigs. Further validation of the strength of the relationship between the lipogenic enzyme expression and fat content in a larger pig population is required.

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