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Comparison of Sarcoplasmic Proteomes between Two Groups of Pig Muscles Selected for Shear Force of Cooked Meat

ELISABETH LAVILLE,*,† THIERRY SAYD,† CLAUDIA TERLOUW,†
CHRISTOPHE CHAMBON,† MARIE DAMON,‡ CATHERINE LARZUL,§ PASCALE LEROY,||
JÉRÔME GLÉNISSON,# AND PIERRE CHÉREL#

Unité Recherches Qualité des Produits Animaux, Institut National de la Recherche Agronomique, 63122 St-Genès-Champanelle, France, Unité Mixte de Recherches Systèmes d'Elevage Nutrition Animale et Humaine, Institut National de la Recherche Agronomique, 35590 Saint Gilles, France, Unité Génétique Quantitative et Appliquée, Institut National de la Recherche Agronomique, 78352 Jouy en Josas, France, Unité Mixte de Recherches Génétique Animale, Institut National de la Recherche Agronomique, 35000 Rennes, France, and France Hybrides, 45808 St Jean de Braye, France

Two-dimensional electrophoresis was used to compare *Longissimus* sarcoplasmic protein abundance between two groups (tough meat and tender meat), defined on the basis of extreme Warner−Bratzler shear force values measured on cooked pork. Fourteen protein spots differed in quantity ($P < 0.05$) between the two groups and were identified. Adypocyte fatty acid binding protein and acyl-CoA binding protein involved in lipid traffic and in the control of gene expression regulating cell proliferation and differentiation, and *Enoyl-CoA hydratas*e, aldose reductase and *triosephosphate isomerase* indirectly related to lipid metabolism were overrepresented in the tender group. The tender group was further characterized by increased levels of proteins involved in protein folding and polymerization (initiation factor elf-3β, chaperonin subunit 2, profilin II). The results suggest that the lower post-cooking shear force could at least in part be related to muscle adipogenetic and/or myogenetic status of which the possible underlying mechanisms are discussed.

KEYWORDS: Proteome analysis; pig muscle; cooked meat; *Longissimus lomborum*; Warner−Bratzler shear force; adenylate kinase; four and a half LIM domain protein 3

INTRODUCTION

An significant amount of pork meat is consumed fresh. Texture and juiciness are major sensory traits determining acceptability of pork meat by consumers (*1*). Cooked meat texture can be evaluated by both instrumental and sensory methods. The Warner−Bratzler shear device is the most widely used and usually shows good relationships with sensory panel scores for meat tenderness (*2*). Meat texture is a complex trait depending on technological factors and different biological mechanisms (for a review, *3*). Among these mechanisms, *post mortem* glycolysis and proteolysis have a major influence. Structural and chemical characteristics of the connective tissue network and intra muscular fat deposition are also greatly involved in meat texture, especially in cooked meat. Despite extensive studies, models for predicting texture are still insufficient. Proteins are key elements in the determination of meat texture. Texture depends on *post mortem* chemical and structural changes of proteins (ruptures, denaturation) and on biochemical reactions in which proteins may be involved as enzymes or inhibitors, for example. Therefore, proteins are, on one hand, targets, but, on the other, mediators. Post-genomics tools (transcriptomics, proteomics) provide important molecular information related metabolic pathways and help to propose mechanisms of meat quality development (*4*). Proteomics have successfully described *post mortem* modifications of pig muscle proteins (*5, 6*) or characterized in a detailed way PSE (pale, soft, exsudative) zones in *Semimembranosus* muscle (*7*). Other proteomic studies have investigated correlations between proteins and fragments abundance with meat quality attributes such as Warner−Bratzler shear force (*8*) or L^* value and drip loss (*9, 10*).

The objective of the present work was to improve our understanding of mechanisms underlying cooked meat texture variability using a proteomic approach on pig *longissimus* muscles. The study focuses on the sarcoplasmic fraction to

* Corresponding author. Telephone: +33 (0)4 73 62 48 34. Fax: +33 (0)4 73 62 42 68. E-mail elaville@clermont.inra.fr.
† Unité Recherches Qualité des Produits Animaux, Institut National de la Recherche Agronomique.
‡ Unité Mixte de Recherches Systèmes d'Elevage Nutrition Animale et Humaine, Institut National de la Recherche Agronomique.
§ Unité Génétique Quantitative et Appliquée, Institut National de la Recherche Agronomique.
|| Unité Mixte de Recherches Génétique Animale, Institut National de la Recherche Agronomique.
France Hybrides.

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increase our knowledge of proteins involved in metabolic processes underlying high or low shear force values measured on cooked meat. This cellular fraction, which represents only 30% of the total proteome of the cell, contains most of the proteins involved in metabolism and signal transduction pathways, with exception of those involved in *post-mortem* proteolysis. The sarcoplasmic fraction excludes proteins of myofibrils, membranes, and connective tissue but increases the gel resolution of the studied fraction and thus the number of proteins that can be quantified in this fraction.

MATERIALS AND METHODS

Animals and Carcass Sampling. Muscles were sampled from a population of 1000 pigs generated as an F2 intercross between two production sire lines: FH016 (Pietrain type, France Hybrides SA, St Jean de Braye, France) and FH019 (synthetic line, from Duroc, Hampshire and Large White founders, France Hybrides SA, St Jean de Braye, France). Pigs were slaughtered at an average carcass weight of 92.7 kg. A 5 g sample of longissimus lomborum (LL) muscle was collected on all pigs 20 min after stunning at the last rib position. From this sample, 1 g of muscle was used for pH measurement and the remaining fraction was immediately snap frozen in liquid nitrogen and subsequently stored at −80 °C for proteomic analysis. The half-carcasses were chilled at 12 °C for 4 h post mortem and then stored at 3 °C. The next day, meat pH was recorded 24 h post mortem (pH_u) in the loin directly on the carcass. At 36 h post mortem loins were rigidified, by keeping them for 30 min at −10 °C, to be sliced using an automated industrial cutter, irrespective of anatomical ribs boundaries. Two slices of 4 cm width (326 g ± 65 g) including 11th and 12th ribs were kept. One slice was used for color measurements and chemical analyses. The remaining part was kept at 4 °C in a closed plastic bag for raw meat shear force determination 48 h later. The second slice was weighed, kept at 4 °C in a closed plastic bag, and used for drip loss and cooked meat shear force measurements 48 h later.

pH Measurements. A 1 g muscle sample collected 20 min after death was incubated at 39 °C for 25 min and then dispersed (ultra turrax, Ika-Werke, Staufen, Germany) in 9 mL of 5 mM soduim iodoacetate buffer, pH 7.0. The pH 45 min *post mortem* (pH_{45}) was then measured on this solution within 4 h after dispersion using a glass/KCl electrode (N1041A, Schott AG, Mainz, Germany) and a Knick 766 bench top pH meter (Knick Elektronische Messgeräte GmbH & Co., Berlin, Germany). Meat pH_u was recorded on the *longissimus* muscle on the level of the last rib 24 h *post mortem* using an Ingold Xerolyt penetration gel electrode (Mettler Toledo SA, Viroflay, France) and a portable Knick 911 pH meter (Knick Elektronische Messgeräte GmbH & Co., Berlin, Germany).

Drip and Cooking Losses. Chops stored at +4 °C for 48 h in a plastic bag were weighed before and after storage. Drip loss (DL) was defined as % losses during storage, calculated as 100 × (1 − weight after storage/weight before storage). Chops were grill-cooked at 240 °C in a dry oven for 30 min and allowed to cool at room temperature before meat shear force measurements. The same chops were weighed again after cooking and cooking loss (CL) was defined as % losses during cooking, calculated as 100 × (1 − weight after cooking/weight before cooking).

Warner—Bratzler Shear Force (WBSF) Measurement. For both raw and cooked meat shear force measurements 84 h *post mortem*, ten cylinders of 1 cm diameter and 3 to 4 cm long were sampled from each chop, with the main cylinder axis parallel to the muscle fibers direction and sections dispersed across the whole muscle area. Each of the cylinders was then sheared across its main axis in a Warner−Bratzler cell, using a reverse V (60 °C) shaped blade with smooth edges, at a 100 mm/mn speed, mounted in an Instron 4442 tester (Instron France S.A.S., Guyancourt, France) equipped with a 500 N cell. Force records during shearing were continuously collected and processed using Merlin IX software (Instron France S.A.S., Guyancourt, France) software, from which maximal force during shearing (e.g., at rupture time), was used as a shear force value. Shear force measurements on raw and cooked meat were defined as the arithmetical mean of the 10 cylinder maximum forces, after discarding records differing from the 10-records mean by more than 2 standard deviations. All shear force measurements, for both cooked and raw meat, were performed without prior freezing of meat.

Loin Color. Color was recorded on three 10 mm diameter spots from each eye rib surface, within 2 h following loin slicing (36 h *post mortem*). Indicators of lightness (L^*), redness (a^*), and yellowness (b^*) were recorded using a Minolta CR300 colorimeter, illuminant C (Konica Minolta Sensing Europe BV, Roissy Charles de Gaulle, France), and the average value of the three spots was used.

Chemical analyses were performed on loin samples collected 36 h *post mortem* as a single chop and subsequently stored at −20° until analysis.

Intramuscular Fat (IMF). Lipids were extracted from freeze-dried muscles using a 17-fold dilution of tissue in 2:1 chloroform/methanol (vol:vol) according to the method outlined by Folch (*11*). Lipid content of fresh tissue (g/100 g) was obtained by taking into account the DM content determined from the weight of minced tissues before and after freeze-drying.

Glycolytic potential (GP) was determined according to Monin and Sellier (*12*), as GP = 2([glycogen] + [glucose] + [glucose-6-phosphate]) + [lactate]. After homogenization of 1 g of muscle into 10 mL of perchloric acid 0.55 M, glucose and glucose-6-phosphate were determined altogether using an enzymatic method (glucose HK, ABX Diagnostics kit, 34187 Montpellier, France). Lactate was determined using an enzymatic method (Biomerieux kits, 69280 Marcy l'Etoile, France). These analyses were performed on an automatic spectrophotometric analyzer (Cobas Mira Roche, Basel, Switzerland). Muscle glycogen content was determined from glucose determination (see above) after hydrolysis by amiloglucosidase, as described by Talmant (*13*). Lactate, free glucose and glucose-6-phosphate, and glucose derived from glycogen hydrolysis were expressed as μmol/g of wet tissue. GP was expressed as μmol of lactate equivalent/g of wet tissue.

Sarcoplasmic Protein Extraction and Electrophoresis. From the 1000 loin shear force values, two groups of 12 each were chosen from the extremes to create a tough (TO) and tender (TE) group. Selection of the 2 groups of 12 extreme WBSF values was based on their high or low residual value of shear force on cooked loin (cooked WBSF), after correction for a slaughter batch fixed effect and chop weight regression (GLM procedure), and selecting one sample with high and one with low shear force from each of 12 half sib families. The corresponding samples, previously frozen at 20 min *post mortem*, were then used for proteomic analysis. The 2DE analysis was carried out on 1 g of muscle as previously described (*14*). Briefly, muscle was homogenized, using a glass bead agitator MM2 (Retsch, Haan, Germany), in 40 mM Tris (pH 8), 2 mM EDTA and a protease inhibitors cocktail (SIGMA) at 4 °C, at the ratio of 1:4 (w/v). The homogenate was centrifuged at 4 °C for 10 min at 10000*g*. Supernatant, referred to as the sarcoplasmic extract, was stored at −80 °C. Protein concentration was determined by the Bradford assay (BioRad, Hercules, CA). 900μg of sarcoplamic proteins were included in a buffer containing 7M Urea, 2M Thiourea, 2% (w/v) CHAPS 0.4% (v/v) carrier ampholyte and bromophenol blue. Samples were loaded onto immobilized pH-gradient strips (pH 5−8, 17 cm, BioRad) and isoelectric focusing was performed using a Protean IEF cell system (BioRad). Gels were passively rehydrated for 16h. Rapid voltage ramping was subsequently applied to reach a total of 85 kVh. In the second dimension, proteins were resolved on 12% SDS-PAGE gels using Protean II XL system (BioRad). Gels were Coomassie Blue (colloidal blue) stained as previously described (*15*). Three gels were produced per sample. Technical variabilitity was assessed (variation coefficient of 24.3%) and found to lower than biological variability.

Image Analysis and Protein Identification by Mass Spectrometry. Gel images were acquired using a GS-800 imaging densitometer (BioRad) and analyzed using the two dimension electrophoresis (2DE) image analysis software PDQuest (BioRad). Image analysis was performed as previously described (*14*). The intensity-dependent distribution of spots was assessed according to the technique of Meunier (*16*) and found to be normal. Proteins were identified as previously described (*14*). Spots of interest were excised and the proteins were subjected to in-gel tryptic digestion and peptide extraction. Peptides

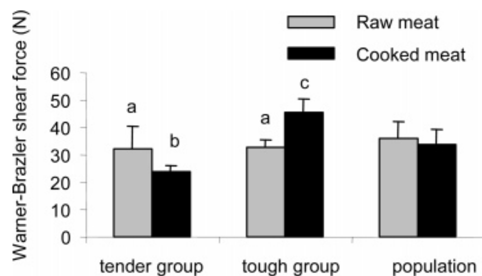

Figure 1. Warner–Bratzler shear force of muscles before and after cooking. Parts **a**, **b**, and **c** differences are significant, $P < 0.01$.

were analyzed using a MALDI−TOF mass spectrometer Voyager DE-Pro model (PerSeptive BioSystems, Framingham, MA). Proteins were identified from their peptide mass fingerprinting, searching the NCBI (National Center for Biotechnology Information) protein sequence databases (http://www.ncbi.nlm.nih.gov/Database/) using the MASCOT software [http://www.matrixscience.com (last accessed December, 2006)]. The initial search parameters allowed a single trypsin missed cleavage, carbamidomethylation of cystein, partial oxidation of methionine and an m/z error of 25 ppm. Protein spots, which could not be identified by MALDI−TOF−MS, were subsequently analyzed by MS/MS using a LCQ Ion Trap mass spectrometer equipped with a nanoelectrospray source (Thermo Scientific, Courtaboeuf, France). The nanoelectrospray capillaries (Protana/MDS Proteomics, Odense, Danemark) were loaded with 6 μL of peptide mixture in 50% acetonitile in water containing 0.5% formic acid. Peptides were directly analyzed by infusion. Data acquisition was performed in manual mode and the collision-induced dissociation was preferentially performed on detected masses by MALDI−TOF. The MS/MS data were searched using the NCBI data base with the search engine SEQUEST (LCQ-Deca software package).

Statistical Analysis. Statistical analyses were performed using SAS software (SAS Inst., Inc; Cary, NC). The GLM procedure (samples nested within group) was used to test significance of the effect of cooking on WBSF and the effect of group on raw and cooked WBSF. Where significant interactions between group and effect of cooking were found, the SNK option was used to locate differences. The ANOVA procedure was used to test significance of the fixed effect of group on meat characteristics (IMF, GP, pHu, pH_{45}, L^*, DL, CL), as well on averaged detected and matched spot quantities. Finally, to obtain a visual representation (dendrogram) of relationships between proteins of interest and meat quality traits, the Hierarchical Ascendant Classification was applied to spots differentially expressed and to meat quality characteristics. The Ward's dissimilarity aggregation procedure based on Euclidian distances from XLSTAT 2007 software (Addinsoft, Paris, France) was used.

RESULTS

Meat Characteristics. Meat quality characteristics are presented in **Figure 1** and **Table 1**. Average WBSF values were 23.8 ± 2.1 and 45.4 ± 5.3 N, in the groups that will be referred to as TE (tender meat, low WBSF cooked values) and TO (tough meat, high WBSF cooked values), respectively. The TO group and TE group differed in WBSF value by 4 standard deviations. The WBSF measured on raw meat was not different between the two groups. Cooking reduced WBSF in the TE and increased WBSF in the TO group (**Figure 1**). IMF and pHu were higher, while GP, L^*, DL, and CL were lower in the TE group (**Table 1**). The differences between population and TO and TE groups expressed proportionally to the standard deviation of the population (**Table 1**) showed that meat characteristics of TO group were relatively similar to those of the population, except WBSF of cooked meat and CL. Conversely, meat characteristics of the TE group were rather different than those of the population.

Proteomic Analysis. On 2-D gels, out of 310 matched spots, 14 spots showed a significant differential abundance between the two groups. They were successfully identified using peptide mass fingerprinting. **Figure 2** shows a representative gel of the soluble muscle proteins with the position of the 14 spots. The identification and information related to the validity of search results are shown in **Table 2**. Position of all spots in the gel corresponded well with their theoretical molecular weight, suggesting that spots are entire proteins. **Figure 3** shows the representation of the 14 proteins in the two groups and the muscle characteristics. The two main clusters naturally separate proteins over-represented in the TO group and over-represented in the TE group. Concerning muscle characteristics, WBSF of cooked meat and of raw meat, DL, CL, L^*, and GP were grouped with proteins overrepresented in TO group. These indicators were opposed to pH_{45}, pHu, and IMF, which were related to the proteins overrepresented in TE group.

Proteins Overrepresented in the TE Group. Eight proteins were overrepresented in the TE group. Adipocyte fatty acid binding protein (A-FABP) is exclusively expressed in adipocytes. Fatty acids (FA) are bound by FABP at the cell membrane and transported to sites of FA oxidation (peroxisome and mitochondria), to sites of FA esterification into triacylglycerols or phospholipids, and to the nucleus for regulation of gene expression (*17*). In addition, A-FABP transfers FA back to the cell membrane after lipolysis (*18*). Enoyl-CoA hydratase is involved in the β-oxidation of fatty acid in mitochondria and peroxysome, leading to the formation of acetyl-CoA which enters the Krebs cycle or is reutilized for FA synthesis. Aldose reductase, chain D (AR) catalyzes the NADPH-dependent reduction of various carbonyl compounds (aldose sugars, aldehydes and their glutathione conjugates). AR has broad substrate specificity and is best known for its role in the polyol

Table 1. Mean and Standard Deviation of Meat Characteristics and Group Effect

meat characteristics[a]	population $N = 1000$	TE group[a] $N = 12$	TO group[a] $N = 12$	Pr > F[b]	group differences[c]	TE vs pop.[d]	TO vs pop.[d]
WBSF raw	35.9 ± 6.5	32.2 ± 8.4	32.6 ± 3.2	0.998	0.06	−0.57	−0.51
WBSF cooked	34 ± 5.4	23.8 ± 2.1	45.4 ± 5.3	<0.0001	4	−1.89	2.11
IMF	2.2 ± 0.7	2.6 ± 0.8	1.9 ± 0.6	0.035	−1	0.6	−0.43
GP	162.4 ± 25.1	127.4 ± 39.6	157 ± 25.0	0.035	1.18	−1.39	−0.21
pH_u	5.7 ± 0.2	6.0 ± 0.3	5.6 ± 0.1	0.008	−2	1.5	−0.5
pH_{45}	6.5 ± 0.2	6.6 ± 0.1	6.4 ± 0.3	0.063	−1	0.5	−0.5
L^*	49.6 ± 3.3	45.4 ± 2.6	50.7 ± 3.2	0.0001	1.6	−1.27	0.33
DL	1.6 ± 1	0.94 ± 0.7	2 ± 0.7	0.002	1.06	−0.66	0.4
CL	25.9 ± 4.6	21.8 ± 3.6	29.1 ± 3.5	0.0001	1.6	−0.89	0.69

[a] WBSF, Warner-Bratzler shear force; IMF, intramuscular fat; GP, glycolytic potential; pH_u, ultimate pH; pH_{45}, pH at 45 min; L^*, luminence; DL, drip loss; CL, cooking loss; TE, tender group; TO, tough group. [b] $P < 0.05$: differences are significant. [c] To facilitate comparisons, differences between TO and TE groups are expressed proportionally to the standard deviation of the population (difference between TO and TE means/population SD) [d] To facilitate comparisons, differences between TE or TO groups, and population are expressed proportionally to the standard deviation of the population (difference between TE or TO and population means/population SD)

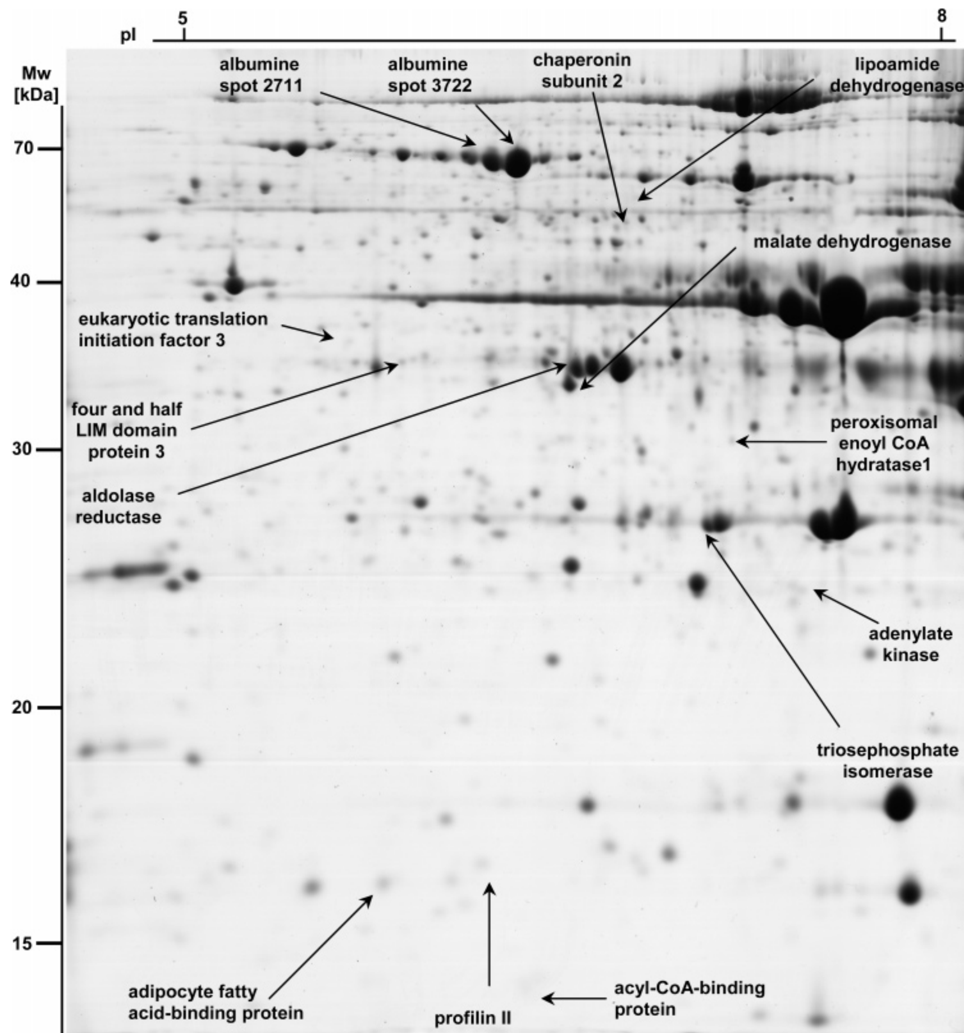

Figure 2. 2DE gel of *longissimus lomborum* of pig sarcoplasmic proteins. Arrows indicate the identified proteins that were differentially represented between tough and tender groups.

Table 2. Proteins Differentially Represented between Tough and Tender Groups in Pig *Longissimus Lomborum*

spot no.	protein ID[a]	accession number[a]	Mowse score[b]	sequence coverage[c]	MP[d]	M_w (kDa)/pI theoretical[e]	M_w (kDa)/pI observed[e]	relative abundance[f]	spot p value[g]
2012	adipocyte fatty acid-binding protein	gi|4160392	83	53%	7	14.6/6.29	16.2/5.55	2.9	<0.05
1210	Eukaryotic translation initiation factor 3	gi|62752021	74	45%	12	36.4/5.38	35.6/5.4	2.8	<0.05
4508	chaperonin subunit 2 (beta)	gi|13938629	80	41%	17	54.4/5.97	55.3/6.45	1.8	<0.05
4614	lipoamide dehydrogenase	gi|47522940	72	28%	14	54.1/7.59	61.9/6.5	1.8	<0.01
2007	profilin II	gi|1172624	145	68%	14	11.9/8.9	16.6/5.86	1.6	<0.05
6103	triosephosphate isomerase 1	gi|80971510	167	56%	13	26.7/7.01	27.4/6.83	1.5	<0.05
3214	aldose reductase (chain D)	gi|515113	89	31%	10	35.6/5.7	34.5/6.24	1.4	<0.01
6109	peroxisomal enoyl CoA hydratase 1	gi|73746067	72	30%	6	36.1/7.6	31.1/6.95	1.3	<0.05
3004	acyl-CoA-binding protein (ACBP)	gi|118277	41[h]	48%	5	9.8/7.8	14.3/6.03	–3	<0.001
2202	four and half LIM domain protein 3	gi|27716129	119	39%	11	31.7/5.67	34.5/5.56	–1.9	<0.05
7008	adenylate Kinase	gi|230801	77	59%	12	21.7/8.37	24.5/7.28	–1.7	<0.05
2711	albumin	gi|833798	236	45%	24	69.3/5.9	68.4/5.85	–1.2	<0.001
3212	malate dehydrogenase	gi|164543	86	45%	11	31.6/6.15	33.7/6.21	–1.2	<0.05
3722	albumin	gi|833798	186	45%	26	69.0/5.92	69.3/5.92	–1.1	<0.05

[a] Protein name and accession numbers were derived from NCBI database [b] The MASCOT baseline significant score is 68. [c] % of coverage of the entire amino acid sequence [d] Number of matched peptides in the database search. [e] MW and pI, theoretical (recorded in NCBI database) and observed (calculated from the spot position on the gel). [f] Relative abundance: greatest average quantity/lowest average quantity. A minus sign indicates that the relative abundance of the protein is lower in the tender group. [g] P value from the ANOVA analysis (effect of WBSF group) [h] 41 is a nonsignificant score: validation by MS/MS. Name: Acyl-CoA-binding protein (ACBP). Accession number: gi|118277. Score Sequest: 3.27 (Sequest baseline significant score is 2.2 for doubly charged ions). Sequence coverage: 19%. Match peptide: 1. Peptide Sequence: –TKPADDEMLFIYSHYK–.

pathway in relation to diabetic complications (*19*). Triose phosphate isomerase (TPI) is involved in the glycolysis pathway transforming dihydroxyacetone phosphate (DHAP) to glyceraldehyde 3-phosphate in a reversible reaction. DHAP is produced by fructose-1,6-bisphophate hydrolysis in the glycolysis pathway or by glycerol hydrolysis. Lipoamide dehydrogenase

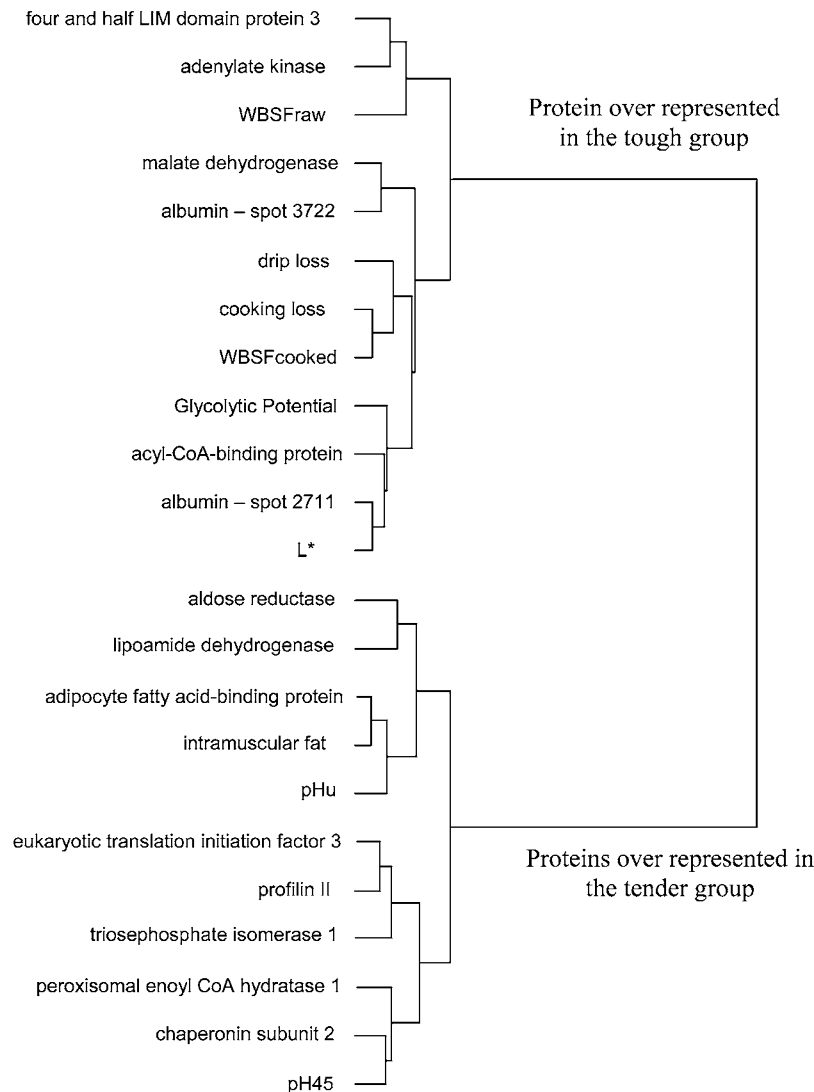

Figure 3. Clustering representation of the 14 significant spots and the meat characteristics.

(E3) is an enzymatic subunit common to different enzymatic complexes: the pyruvate dehydrogenase complex (PDHc), the α-ketoglutarate complex (KGDHc) and the branched-chain ketoacid dehydrogenase complex (BCKDHc). PDHc transforms pyruvate to acetyl-CoA. KGDHc catalyzes the decarboxylation of α-ketoglutarate to succinyl-CoA in the TCA cycle and the BCKDc catalyzes the oxidative decarboxylation of protein debranched-chain α-ketoacids (BCKAs) (*20*). The elF-3 beta (elf-3*β*) subunit is one of the 10 subunits involved in the composition of the translation initiation factor elf-3 complex which plays an important role in the initiation during protein synthesis (*21*). Elf-3*β* contains a tryptophan aspartic acid (WD) repeat element which could interact with other proteins (*22*). The chaperonin subunit 2 (TCP-1 beta) is an element of the ring complex TCP-1 (TRiC or CCT). TRiC is involved in *in vivo* folding of *de novo* synthesized cytoskeletal proteins actin and tubulin and in folding of the motor domain of myosin heavy chain improving the actin binding site (*23*).This complex plays also a role in folding non cytoskeletal proteins containing WD repeat domains (*24*). Finally, profilin II was also overrepresented in the TE group. This protein is an actin monomer-binding protein, which regulates actin polymerization. In the presence of ATP, it promotes actin filament assembly at the barbed end (*25*). Isoform II of profilin has a lower affinity to actin monomer than isoform I and may have a regulatory role in the transmission of signals for cell growth and differentiation due to its affinity to phosphatidylinositol 4,5-biphosphate (PIP_2), (*25, 26*).

Proteins Overrepresented in the TO Group. Six proteins were overrepresented in the TO group. Malate dehydrogenase is involved in the TCA cycle. It converts malate to oxaloacetate producing an equivalent amount of NADH. Adenylate kinase (AK1) involved in the adenine nucleotides ratios and in energy metabolism, catalyzes the reversible reaction of 2 ADP molecules into ATP + AMP. The activity and mRNA level of AK1 are more abundant in glycolytic muscle and consequently contribute to glycolytic ATP production (*27*). In skeletal muscle, AK1 functions as a high-energy phosphoryl transfer system regulating ATP generation in correspondence with its consumption by specific cellular processes. The four and Half LIM domain protein 3 (FHL3) is a protein of the LIM protein family which is involved in a wide range of protein–protein interactions. Presence of FHL3 is confined to the Z-line of mature skeletal muscle. Via its four and a half LIM domains, FHL3 combines with different proteins to regulate the function of cytoskeletal proteins and to connect the cytoskeleton and contractile elements to the extracellular matrix. It binds actin, inhibiting α-actinin–actin bundling activity (*28*), and thus participates in the remodeling of myofilaments after physical effort and stress. It interacts with a_7b_1 integrin receptors thus playing a role in mechanical stabilization of muscle cells (*29*).

The best studied member of the FHL family is FHL2, highly expressed in cardiac muscle where it serves similar functions as FHL3 in skeletal muscle. Lange (*30*) showed its binding ability with several metabolic enzymes and notably with adenylate kinase suggesting that it acts as a specific adaptor to couple metabolic enzymes to high-energy consumption sites of the sarcomere. Acyl-CoA binding protein (ACBP) specifically binds long-chain acyl-CoA esters, the metabolic active form of fatty acids used for β-oxidation and glycerolipid synthesis which are also regulators of various intracellular signaling functions for enzyme activities, ion channels, and gene expression (*31*). Finally, two spots of the plasmatic transporter albumin were also overrepresented in the TO group.

DISCUSSION

The present work compared protein abundance between two classes of pig LL muscles selected on the basis of their extreme WBSF measured on cooked meat, in order to improve our understanding of protein related metabolic pathways underlying meat tenderness, other than proteolysis.

The key finding of the study was the overexpression of the fatty acid carrier A-FABP and a higher IMF level in the TE group. Increased levels of A-FABP, or increased A-FABP mRNA expression are related to increases of triacylglycerol deposits, the major component of intra muscular fat (*32, 33*). As reported in Essen-Gustavsson (*34*) and Gondret (*35*), more than 80% of IMF is stored in adipocytes interspersed in the perimysium and less than 20% located within cytoplasm of myofibers. In Duroc crossbreds, A-FABP protein content was mainly related to the number of intra muscular adipocytes (*32*) and probably reflected an increase of adipocyte hyperplasia. In the current study, only the adipocyte form of the protein (A-FABP) showed differential expression, the heart form of the protein (H-FABP), also found in skeletal muscle, was not increased. The lower WBSF of the TE group was probably at least in part due to increased intra muscular adipocyte fat deposition. The increased A-FABP level was further accompanied by higher average pH_u, and lower GP. Higher pH_u may have contributed to the lower WBSF of the TE group, but explains only part of the variability (*3*). Higher pH_u may be the result of a more lipid-oriented energy metabolism in the TE compared to the TO group. At first sight, the general profile of enzymes representation does not support this idea, with only malate dehydrogenase overrepresented in the TO group. Similarly, Damon (*32*) did not find any relationship between IMF content and expression of genes that are commonly reported to be involved in muscle lipogenesis (acetyl-CoA carboxylase or glucose-6-phosphate dehydrogenase), in FA oxidation (β-hydroxyacyl-CoA dehydrogenase, peroxisome proliferators activated receptor delta), or in energy expenditure. In the current study, we found, however, several proteins of which expression may be indirectly related to lipid metabolism. Thus, ACBP was present at a very low level in the TE group. In the cluster representation, this protein was very close to WBSF of cooked meat. Helledie (*31*) showed that ACBP regulates the availability of acyl-CoA esters for various metabolic and regulatory purposes and particularly, key adipogenic transcription factors such as peroxisome proliferators activated receptor γ (PPARγ), involved in adipocyte differentiation. Helledie (*36*) suggested that ACBP expressed at high levels may act as negative regulator of PPAR activation. Enoyl-CoA hydratase is involved in the β-oxidation of fatty acid in the peroxysome. Its overexpression in the TE group suggests a relative increase in the use of fatty acids for substrate in oxidation. The AR protein, also overrepresented in the TE group, catalyzes the NADPH-dependent reduction of alhehydes which are the end products of lipid peroxidation (*19*). Srivastava (*19*) suggested that AR may impact signaling mechanisms or gene transcription (PPAR) by its action on oxidized phospholipids. The overrepresentation of TPI in the TE group is in accordance with Lametsch (*8*) who found it to be highly correlated with WBSF force of cooked pork meat. In the glycolytic pathway TPI transforms glyceraldehyde 3-phosphate to DHAP in a reversible reaction. DHAP, produced during glycolysis, is the precursor for triacylglycerol synthesis in adipose tissue. Thus, overexpression of TPI, may be related to higher IMF because of its effect on DHAP production. In addition, elf-3β, chaperonin subunit 2 and profilin II, all involved in the folding and polymerization of *de novo* synthesized proteins specifically actin and myosin, are overrepresented in the TE group, suggesting increased protein synthesis and protein turnover in this group.

Interestingly, shear force of raw meat was similar, and the effect of cooking on WBSF was opposite between the two groups. Cooking decreased WBSF and reduced WBSF variability within the TE group, while it increased WBSF in the TO group. This observation suggests that physical and chemical mechanisms underlying shear force in cooked meat differ between the two groups. As indicated above, higher A-FABP levels in the TE group probably express higher adipocyte levels. Adipocyte levels may influence the effect of cooking on shear force in two ways. First, adipocytes are known to spread apart the strands of connective tissue between the muscle fibers, thereby providing a looser structure that facilitates heat penetration and consequently the solubilization of these connective tissue strands (*37*). Second, cooking losses, mainly constituted by water, were lower in TE group. One suggestion could be that fat of adipocytes distributed around myofibers and fiber bundles contribute to keep water within muscle tissue, thereby avoiding increase of shear force values due to cooking looses. Early work already suggested a higher water-holding capacity in cooked meat related to the presence of fat (*3, 38*). An alternative hypothesis is that a higher level of intramuscular fat may be related to higher content of specific collagen type such as type XII or type XIV, as suggested for bovine muscle (*39*). These collagen types have a higher extractability (Listrat, pers.com.) and may become more soluble during cooking. Further studies are needed to elucidate relationships between collagen type, solubility, and tenderness development during cooking.

Other mechanisms may have contributed to the higher tenderness of the TE, compared to the TO group. Two proteins involved in the structure and contractile function of myofibers (AK1 and FHL3) were more abundantly expressed in the TO group. Localized at the Z-line, the FHL3 protein has an important role of anchoring contractile elements to the sarcolemma and to the extracellular matrix via $\alpha_7\beta_1$ integrin receptor suggesting a higher contractile activity. In the cardiac muscle, FLH2 binds to AK1 (*28, 29*). The concomitant decreased abundance of FHL3 and AK1 found in the TE group suggests that the two proteins are also related in skeletal muscle. Lower levels of FLH3 and AK1 in TE group may explain part of their lower WBSF, as this may reflect reduced efficiency in energy mobilization leading to a slower pH decline, a higher ultimate pH. Both features are known to reduce WBSF, due to the less extended formation of the actomyosin complex and the increased water holding capacity (review in *3*). Faster pH decline and lower ultimate pH explain the higher L^* values of the TO group compared to the TE group. It is noteworthy that the TO group

had lower ultimate pH and higher L^* compared to the TE group, but hardly compared to the whole population. The changes in FLH3 and AK1 levels described above, and the accompanying increase in rate or extent of pH decline, may explain the higher WBSF in the TO compared to the TE group, but not compared to the whole population. Further studies are therefore warranted.

In conclusion, depending on the selected group, TE or TO, cooking reduced or increased shear force. The TE group was characterized by the overabundance of adipocyte-specific fatty binding proteins, suggesting higher levels of adipocytes. This protein and ACBP are also involved in the control of gene expression regulating cell proliferation and differentiation, probably related to the higher levels of adipocytes. The group was further characterized by proteins involved in protein folding and polymerization, also suggesting increased protein synthesis. The cooking-induced decrease in shear force of muscle of the TE group may be explained by a better water holding capacity, due to higher ultimate pH and the increased presence of adipocytes, and possibly to a better collagen solubilization. The cooking-induced increase in shear force of muscle of the TO group is related to increased cooking losses. Higher levels of AK1 and FHL3 suggest a higher efficiency in energy mobilization and explain the faster pH decline and lower ultimate pH of the muscles, of which the negative effects of water holding capacity and shear force are well-known. To improve eating quality of pork, it is of utmost importance to find markers of quality traits. Among proteins evidenced in this study, some are potential marker candidates to predict post-cooking meat tenderness.

ABBREVIATIONS USED

LL, *longissimus lomborum*; DL, drip loss; CL, cooking loss; pH_{45}, pH 45′ *post mortem*; pH_u, ultimate pH; WBSF, Warner–Bratzler shear force; IMF, intramuscular fat; GP, glycolytic potential; TE, tender group; TO, tough group; A-FABP, adipocyte fatty acid binding protein; FA, fatty acid; AR, aldose reductase, chain D; TPI, triose phosphate isomerase; DHAP, dihydroxyacetone phosphate; elF-3β, elF-3 beta subunit; AK, adenylate kinase; FHL3, four and a half LIM domain protein 3; ACBP, acyl-CoA binding protein; PPARγ, peroxisome proliferators activated receptor γ.

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