Postnatal Expression Pattern of Adipose Type Fatty Acid Binding Protein in Different Adipose Tissues of Porcine*

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ABSTRACT : Adipocyte fatty acid-binding protein (A-FABP), which belongs to the FABP family, plays an essential role in long-chain fatty acid uptake and metabolic homeostasis, especially in adipose tissue. The pattern of A-FABP gene mRNA expression in different growth stages and its relation to intramuscular fat (IMF) accretion in pigs was studied. Fifteen female Duroc × Landrace × Yorkshire pigs in five groups of three pigs each, weighing 1, 30, 50, 70 and 90 kg were used to study developmental gene mRNA expression of A-FABP in various adipose tissues by means of semi-quantitative RT-PCR. Results showed that A-FABP mRNA levels in subcutaneous and ventral adipose tissues first increased from 1 to 50 kg, then gradually declined from 50 to 90 kg. Moreover, the rank order of A-FABP mRNA levels determined in three adipose tissues was as follows: subcutaneous adipose>ventral adipose>mesenteric adipose. A-FABP mRNA expression in mesenteric adipose tissue was constant during development. In addition, a positive correlation from 1 to 50 kg BW pigs and a negative correlation from 50 to 90 kg BW between A-FABP mRNA levels in subcutaneous and ventral adipose and IMF content were found. (Key Words : A-FABP, Intramuscular Fat, Gene Expression, Fat Deposition, Pigs)

INTRODUCTION

Fatty acid binding proteins (FABPs), a multigene family of 14-15-kDa proteins of which at least nine distinct types, with tissue-specific distribution, have been identified (Ribarik and Bernlohr, 1998). The primary role of all the FABP family members is regulation of fatty acid uptakes and intracellular transport (Veerkamp and Maatman, 1995). FABPs are generally believed to function in the solubilization, transport, and/or metabolism of FA in cells. Given their cell-specific expression, abundance, and high (nanomolar) affinity for FA (Richieri et al., 1994), individual FABPs may perform distinct roles within various tissues; however, specific functions have not yet been established for any FABP. In addition, FABP may regulate lipid metabolism and other cellular processes such as gene transcription, cellular signaling, growth, and differentiation. The adipocytes fatty acid-binding protein (A-FABP) belongs to the family of small intracellular proteins that noncovalently bind hydrophobic ligands, principally unesterified fatty acids (FA). A-FABP, also known as aP2 and adipocyte lipid-binding protein, is expressed only in differentiated adipocytes, where it constitutes 1-6% of the total cytosolic protein in the mature adipocyte, such that the majority of intracellular FA are most likely bound to A-FABP. A Primary function of A-FABP is to carry fatty acids away from intracellular lipid droplets after triglyceride hydrolysis (Vogel-Hertzel and Bernlohr, 2000).

Meat quality is attracting increasingly more attention in pig breeding. One of the main factors affecting sensory meat quality is IMF content, which has been positively correlated with meat tenderness, juiciness, and taste (De Vol et al., 1988; Wood et al., 1988). Juiciness increases with the increase of IMF (Okumura et al., 2007). IMF content is a major determinant of the eating quality of pork and is highly heritable (Hovenier et al., 1993). However, improving IMF content by selective breeding is difficult because this trait is measured on the carcass. Marker or gene-assisted selection is a promising strategy for genetic improvement of such carcass traits (Meuwissen and Goddard, 1996). In the 5'-flanking region of lipoprotein

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lipase (LPL) gene, the loci d and e significantly affect thickness of subcutaneous fat, abdominal fat weight and subcutaneous fat, while in the coding region, synonymous mutation in exon 8 is significantly associated with IMF width of chicken (Liu et al., 2006). The Melanocortin-4 Receptor (MC4R) and Insulin-like Growth Factor (IGF-2) are two important candidate genes related to fat deposition and carcass traits (Chen et al., 2005). The porcine A-FABP was mapped to chromosome 4 and the marker order was defined as S0001-[9.9 cM]-A-FABP-[10.1 cM]-S0217 (Gerbens et al., 1998; Gerbens et al., 2000). In that region a QTL for IMF was suggested (De Koning et al., 1999; Rattink et al., 2000). Significant associations between genetic variation at the adipocyte fatty acid-binding protein loci and IMF content in purebred Duroc pigs have been identified (Gerbens et al., 1998).

The objective of this research was to investigate the pattern of AFABP gene mRNA expression in various adipose tissues of different growth stages Duroc×Landrace×Yorkshire pigs and explore its relation to IMF content and fat deposition in order to obtain information for regulation pork meat quality.

**MATERIALS AND METHODS**

**Animals**

This experiment was approved by the Institutional Animal Care and Use Committee at Zhejiang University and was conducted in accordance with the National Institutes of Health guidelines for the care and use of experimental animals. A total of fifteen female Duroc×Landrace×Yorkshire pigs in five groups, each group containing three pigs weighing 1, 30, 50, 70, 90 kg were euthanized under anaesthesia and exsanguinated for sampling after a 12 h fast and ad libitum access to water.

**Meat quality data**

Subcutaneous, ventral and mesentery adipose tissues were quickly collected and frozen in liquid nitrogen, then stored at -70°C until RNA analysis. Left-half carcasses were weighed after the head, hooves, tail, viscera (except the kidney) were removed, subcutaneous, ventral and mesentery adipose tissues in left-half carcasses were dissected and weighed, and fat deposition rates were calculated. Twenty-four hours after slaughter, a slice of the longissimus lumborum muscle was isolated from the right carcass half at the third lumbar vertebra to assess IMF content. A muscle sample was taken from this slice, carefully avoiding IMF depots surrounding the muscle. The IMF content was determined using Soxhlet petroleum-ether extraction and expressed as the weight percentage of wet muscle tissue.

**RNA extraction**

Total RNA was isolated from the subcutaneous, ventral and mesentery adipose tissues by using TRIzol (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's manual. After pulverization and homogenization of the tissues, the homogenate was extracted with chloroform and then precipitated by isopropanol. The resulting pellets of total RNA were dissolved in ultra-pure water; the purity and concentration of total RNA were measured by a spectrophotometer at 260 nm and 280 nm.

**Semi-quantitative RT-PCR**

RT-PCR was performed in a thermocycler (Gene Amp PCR system 9600). 2 µg of total RNA were converted in cDNA; 2 µg of total RNA and 2 µl of Random primers (500 µg/ml; Promega Corp., Madison, WI) were denatured at 70°C for 5 min, and cooled to 25°C. The following components were added to give a total reaction volume of 25 µl: 5 µl M-MLV 5x reaction buffer (250 mM Tris.HCl (pH 8.3), 375 mM KCl, 15 mM MgCl2, and 50 mM dithiohreitol), 2 µl dNTPs mix (10 mM each of dATP, dCTP, dGTP, and dTTP), and 1 µl M-MLV reverse transcriptase (200 units/µl; 0.5 µl RNasin ribonuclease inhibitor and nuclease-free water (Promega Corp.)). It was mixed gently by flicking the tube, and the reaction mixture was incubated at 37°C for 60 min.

All PCRs were performed with 1 µl of each resulting cDNA in a 50-µl reaction volume containing 0.2 mM deoxynucleoside triphosphates, 1.5 mM Mg2+ (A-FABP, 18S rRNA), 0.4 µM each sense and antisense primer, and 1 U Taq DNA polymerase (Promega). Primer sequences for A-FABP and 18S rRNA were designed by using the Prime program of the Winconsin Sequence Analysis Package (Genetics Computer Group, inc., Madison, Wis.) based on known sequences deposited in GenBank. The primers used and the resultant cDNA sizes were as follows: A-FABP sense, 5’-TAC TGA GAT TGC CTT CAA ATT GGG-3’; A-FABP antisense, 5’-TCT GGT AGC CGT GAC ACC TTTC-3’ (207 bp); 18S rRNA sense, 5’-GAC CAG AGC GAA AGC ATT-3’; 18S rRNA antisense, 5’-TCC ACC AAC TAA GAA CGG-3’ (375 bp). Specific PCR products for A-FABP and 18S rRNA were obtained by using these primers and confirmed by direct sequencing. The optimum PCR reaction cycle numbers, Mg2+ concentration and annealing temperature to give a linear amplification of each transcript were determined by a preliminary experiment (Data not shown). The PCR profiles for A-FABP and 18S rRNA included denaturation at 94°C for 2 min, followed by 29 cycles (A-FABP) or 23 cycles (18S rRNA) of denaturation at 94°C for 50 seconds, annealing at 55°C (A-FABP) or 52.5°C (18S rRNA) for 50 seconds, and...
extension at 72°C for 1 min, and a final extension at 72°C for 10 min.

A 5-µl portion of each PCR products was subjected to electrophoresis on a 1% agarose gel with ethidium bromide. PCR products were normalized according to the amount of 18S rRNA detected in the same cDNA sample, and A-FABP/18S rRNA ratios were calculated. The expression level of A-FABP gene in different adipose tissues were compared on the basis of A-FABP-to-18S rRNA ratio.

Data analysis

Electrophoresis band intensities of the PCR products were quantified using Image Master VDS software (Amersham Pharmacia Biotech, Uppsala, Sweden). Mean A-FABP mRNA expression levels normalized against 18S rRNA levels from each treatment group were presented in absolute integrated optical density. All the data were analysed statistically according to the ANOVA procedure (SAS Institute, 1989) and the treatment means were separated by Duncan’s multiple range test. Statistical significance was at p<0.05 for all statistical test.

RESULTS

Developmental pattern of A-FABP gene mRNA expression in different adipose tissues

The developmental patterns of A-FABP gene mRNA expression in subcutaneous, ventral and mesentery adipose tissues from pigs with live weight of 1, 30, 50, 70 and 90 kg were evaluated using semi-quantitative RT-PCR, which allows the assessment of relative A-FABP mRNA levels in pigs at different growth stages. The results of electrophoresis of PCR products of the three pigs from each group are shown in Figure 1. Electrophoresis band intensities of PCR products were quantified and analyzed for statistical difference. The current results (see Figure 2) showed that The relative A-FABP mRNA levels (A-FABP/18S rRNA) first steadily increased, then gradually declined as the pigs grew, the highest expression of A-FABP mRNA was observed at 50 kg body weight in porcine subcutaneous and ventral adipose tissues. However, A-FABP mRNA was observed at 50 kg body weight in porcine subcutaneous and ventral adipose tissues. However, A-FABP mRNA was observed at 50 kg body weight in porcine subcutaneous and ventral adipose tissues. However, A-FABP mRNA was observed at 50 kg body weight in porcine subcutaneous and ventral adipose tissues.
FABP mRNA expression in mesentery adipose tissue was constant during development. The results also showed that A-FABP mRNA levels in subcutaneous, ventral and mesentery adipose tissues of pigs with live weight of 1, 30, 50, 70 and 90 kg were as follows: subcutaneous adipose>ventral adipose>mesenteric adipose.

### Developmental pattern of fat deposition

Table 1 shows that the fat (subcutaneous, ventral and mesentery adipose tissues) deposition rates were 7.94, 11.48, 14.99, 16.75, and 20.73% at the growth stages of pigs with 1, 30, 50, 70, and 90 kg live weight, respectively. The fat deposition rate increased significantly at all stages studied (p<0.05). The weight of the subcutaneous, ventral and mesenteric adipose tissues at different growth stages increased significantly between all stages studied (p<0.05), with the subcutaneous adipose tissues accounting the highest percentage (Table 1). The IMF content steadily increased from 1.21% to 2.04% as the pigs grew (p<0.05).

### The relationship between A-FABP gene mRNA level, fat deposition and intramuscular fat content

A-FABP mRNA levels in subcutaneous were negatively correlated with IMF content (r = -0.998, p<0.05) during growth from 50 to 90 kg body weight. A-FABP mRNA levels in ventral adipose tissue was positively correlated with IMF content (r = 0.993, p<0.05) during growth from 1 to 50 kg body weight, and negatively correlated with IMF content (r = -0.994, p<0.05) during growth from 50 to 90 kg body weight. Correlation analysis also showed that there was a significantly positive correlation between fat deposition rate and IMF content during growth from 1 to 90 kg (r = 0.950, p<0.05).

### DISCUSSION

Adipose tissue plays the central role in regulating fatty acid metabolism and its release of fatty acids is subject to regional variation. To our best knowledge, this is the first report of A-FABP gene mRNA expression pattern in different adipose tissues of porcine during development. The results demonstrate markedly different developmental patterns of A-FABP in porcine subcutaneous, ventral and mesentery adipose tissues at the range from 1 to 90 kg body weight. Adipocyte lipid binding protein (ALBP, the human homologue of the mouse protein aP2) is the major FABP in adipose tissue. Adipocytes from aP2-/- mice exhibit diminished lipolysis (Ribarik CoeN et al., 1999; Scheja et al., 1999) and the -/- animals fed a high fat diet fail to develop the insulin resistance normally associated with the ensuing obesity (Hotamisligil et al., 1996). These data suggest that ALBP could be of importance for non-hormonal regulation of adipose tissue lipolysis and insulin resistance. Subcutaneous adipose tissue constitutes about 80% of all fat tissue in man, but the metabolism of adipose tissue from various depots within the body has been found to differ. Omental (visceral) adipose tissue is more sensitive to the stimulation of lipolysis by catecholamines whereas subcutaneous adipose tissue is more sensitive to the antilipolytic effect of insulin (Kissebah and Krakower, 1994; Arner, 1995). Mechanisms linked to the actions of lipolysis regulating hormones can explain only in part the metabolic differences between adipose tissue depots. Non-hormonal mechanisms, such as those involving FABPs are important for regional variations in lipolysis. In the obese subjects, ALBP protein and RNA expression was higher in subcutaneous compared with omental adipose tissue (Fisher et al., 2001). The current results also showed that the A-FABP mRNA levels exhibited different patterns in different adipose tissues: subcutaneous adipose>ventral adipose>mesenteric adipose. There are potentially important consequences of altering the relative expression in adipose tissue of A-FABP. The biological importance of the present findings should be considered. Because the amount of adipose tissue available was extremely limited and was barely sufficient for protein and mRNA determinations, no mechanistic studies could be done. However, in view of the putative roles of FABPs in fatty acid trafficking within the adipocyte, we can speculate on some mechanisms. It is possible that variation in FABP content between visceral, mesenteric and subcutaneous fat cells determines both fatty acid release and intracellular accumulation. This could influence peripheral and “portal” fatty acid concentrations as well as induce differential effects on gene expression in the different fat depots due to the regulation of gene transcription by fatty acids.

The weight of subcutaneous, ventral and mesentery adipose tissues increased significantly during growth from 1

### Table 1. Developmental adiposition rate (mean±SD)

<table>
<thead>
<tr>
<th>Liveweight (kg)</th>
<th>Subcutaneous adipose weight (kg)</th>
<th>Ventral adipose weight (kg)</th>
<th>Mesenteric adipose weight (kg)</th>
<th>Fat deposition rate (%)</th>
<th>Intramuscular fat content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.031±0.017</td>
<td>0.003±0.001</td>
<td>0.028±0.002</td>
<td>7.94±0.75</td>
<td>1.21±0.04</td>
</tr>
<tr>
<td>30</td>
<td>1.236±0.036</td>
<td>0.042±0.002</td>
<td>0.395±0.043</td>
<td>11.48±0.23</td>
<td>1.24±0.09</td>
</tr>
<tr>
<td>50</td>
<td>3.890±0.053</td>
<td>0.254±0.124</td>
<td>0.605±0.099</td>
<td>14.99±0.43</td>
<td>1.54±0.11</td>
</tr>
<tr>
<td>70</td>
<td>6.661±0.536</td>
<td>0.344±0.068</td>
<td>1.029±0.059</td>
<td>16.75±1.13</td>
<td>1.91±0.17</td>
</tr>
<tr>
<td>90</td>
<td>11.12±3.08</td>
<td>0.920±0.076</td>
<td>1.798±0.226</td>
<td>20.72±0.57</td>
<td>2.04±0.10</td>
</tr>
</tbody>
</table>

Means within a column with different superscripts are significantly different (p<0.05).

to 90 kg body weight (p<0.05). This results indicated that the capacity of adipose deposition in pigs increased as the pigs grew. Wang and Shao (1989) reported that the fat percentage increased significantly as the weight of commercial lean pigs increased. The study from Souza et al. (2004) showed that the fat content of the carcass was significantly correlated (p<0.001) with animal age; the fat percent in the carcass, shoulder, loin, belly and ham primal cuts significantly increased (p<0.05) from 16 to 25 weeks of age. The backfat depth along the carcass midline at the fore, middle and hind sites significantly increased (p<0.001) in pigs from 16 to 25 weeks of age.

Previous studies have demonstrated associations between polymorphisms in the A-FABP and IMF content in pigs (Gerbens et al., 1998, 1999). Further analysis of pigs from a crossbred Meishan×Dutch White pig population supports involvement of H-FABP but not A-FABP in IMF accretion (Gerbens et al., 2000). For Hinfl, HaeIII and Hinfl* H-FABP RFLP, significant contrasts of 0.78%, -0.69% and 0.72% are detected in the least square means of IMF content between the homozygous genotype HH and hh, DD and dd, BB and bb classes, respectively. The results indicate that the HHddBB genotype has the highest IMF content in experimental pig populations (Zeng et al., 2005). However, these analyses do not exclude other closely linked genes from being responsible for these effects on IMF content. Both MC4R and IGF-2 genes are significantly associated with fat deposition and carcass traits in about 300 pigs (Chen et al., 2005). Regulation of the lipid metabolism leads to energy homeostasis and involves many control systems, which must be precisely coordinated. Response to signals triggers activation of specific transcription factors and Fat can act as such signaling molecules. It has been revealed that the increased FA concentration triggers the process of preadipocyte differentiation and the expression of terminal differentiation-related genes (Amri et al., 1994), such as A-FABP (Distel et al., 1992). A-FABP has been considered as candidate gene for pig fatness traits. The genome scanning studies revealed that it is located within the regions containing QTLs for these traits. Initially the direct effect of the A-FABP microsatellite traits was postulated by Gerbens et al. (1998), but other studies did not confirm these results (Gerbens et al., 2000; Nechteleberger et al., 2001; Chmurzynska et al., 2004). A significant relationship between A-FABP mRNA but not protein expression levels and IMF content was found (Gerbens et al., 2001). The current determined results suggested that A-FABP mRNA levels in subcutaneous and ventral adipose tissues were positively correlated with IMF content during growth from 1 to 50 kg body weight, and negatively correlated with IMF content during growth from 50 to 90 kg body weight. One of the possible explanations of the inconsistent effects observed in the different populations is the linkage between an unknown QTL affecting fatness traits and some of the A-FABP alleles. It could be due to the pig population under investigation. The sensitivity of the semi-quantitative RT-PCR assays may also affect the outcome of the study. It needs to be stressed that in all mentioned experiments, the analyzed material was completely different and therefore comparison of the results is rather problematic. Furthermore, the moment of sampling of the pigs may also have been inappropriate. Namely, IMF content and fat deposition rate are traits that result from fat accretion over the lifetime, whereas the A-FABP mRNA levels, IMF and fat deposition are measured at several single moments during the life of the pig. The different fat depots in pig develop in a specific gradient from subcutaneous to inter-to IMF (Lee and Kaufman, 1974) and this latter fat depot increases with age surely beyond the common slaughter age of 180d (Catchpole and Lawrie, 1972). Unfortunately, it is not known which period during the lifetime of a growing pig is responsible for genetic variation in IMF content and hence the most appropriate moment to assess FABP mRNA levels.

CONCLUSION

This study showed the developmental patterns of A-FABP gene expression in different adipose tissues in different growth stages. The A-FABP mRNA levels in subcutaneous and ventral adipose tissues exhibited positive relation with adipose deposition and IMF content at previous growth stage, and negative relation with adipose deposition and IMF content at later growth stage in pigs. The research on the developmental pattern of FABP gene expression and the relationship between their expression and fat deposition could open the door for novel approaches to regulate fat deposition and improve pork meat quality.

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