Selective transport of long-chain fatty acids by FAT/CD36 in skeletal muscle of broilers

J. Guo1*, G. Shu1*, L. Zhou1*, X. Zhu1, W. Liao1, S. Wang1, J. Yang2, G. Zhou1, Q. Xi1, P. Gao1, Y. Zhang1, S. Zhang1, L. Yuan3 and Q. Jiang1

1College of Animal Science, South China Agricultural University, Guangzhou 510640, P.R. China; 2Department of Human Nutrition, Food and Animal Science, University of Hawaii at Manoa, Honolulu, HI 97822, USA; 3Key Laboratory of the Ministry of Education for Cell Biology and Tumor Cell Engineering, School of Life Sciences, Xiamen University, Xiamen 361005, P.R. China

(Received 24 November 2011; Accepted 25 June 2012; First published online 16 August 2012)

Fatty acid translocase (FAT/CD36) is a membrane receptor that facilitates long-chain fatty acid uptake. To investigate its role in the regulation of long-chain fatty acid composition in muscle tissue, we studied and compared FAT/CD36 gene expression in muscle tissues of commercial broiler chickens and Chinese local Silky fowls. The results from gas chromatography–mass spectrometry analysis of muscle samples demonstrated that Chinese local Silky fowls had significantly higher (P < 0.05) proportions of linoleic acid (LA) and palmitic acid, lower proportions (P < 0.05) of arachidonic acid (AA) and oleic acid than the commercial broiler chickens.

The mRNA expression levels of fatty acid (FA) transporters (FA transport protein-1, membrane FA-binding protein, FAT/CD36 and caveolin-1) in the m. ipsilateral pectoralis and biceps femoris were analyzed by Q-PCR, and FAT/CD36 expression levels showed significant differences between these types of chickens (P < 0.01). Interestingly, the levels of FAT/CD36 expression are positively correlated with LA content (r = 0.567, P < 0.01) but negatively correlated with palmitic acid content (r = -0.568, P < 0.01). Further experiments in the stably transfected Chinese hamster oocytes cells with chicken FAT/CD36 cDNA demonstrated that overexpression of FAT/CD36 improves total FA uptake with a significant increase in the proportion of LA and AA, and a decreased proportion of palmitic acid. These results suggest that chicken FAT/CD36 may selectively transport LA and AA, which may lead to the higher LA deposition in muscle tissue.

Keywords: broiler, fatty acid composition, FAT/CD36, transport, linoleic acid

Implications
The findings aid in understanding the function of fatty acid transporters and provide some novel candidate markers for breeding or nutrition regulation, which could be used to improve meat quality.

Introduction
Recently, consumers have become increasingly concerned about the nutritional quality of meat (Scollan et al., 2001). The optimum intramuscular fat content and the fatty acid (FA) composition of muscle greatly contribute to certain aspects of meat quality, such as marbling, juiciness and flavor (Chizzolini et al., 1999; Cameron et al., 2000). For example, polyunsaturated FAs (PUFA) have been reported to decrease the plasma cholesterol, whereas saturated FA (SFA) seem to increase it, which may be related to the potential health risks of obesity and other metabolic disturbance (Monteiro et al., 2006; De Luis et al., 2009; Heinen et al., 2009). Therefore, the regulation of FA composition has been a research focus of improving meat quality.

It has been reported that the FA composition of chicken varies among breeds and anatomical location, primarily because of the interaction of heredity and myofiber type (Shu et al., 2001; Cortinas et al., 2004). In avian models, the liver, but not adipose tissues, is the main site of de novo FA synthesis (Griffin et al., 1992). Therefore, FAs are obtained chiefly from the intestine and liver in the form of chylomicrons and very-low-density lipoproteins, respectively (Mossab et al., 2002). The free FAs are released by lipoprotein lipase and then transported by several membrane proteins, such as FA transport protein-1 (FATP-1), membrane FA-binding protein (mFABP), FA translocase (FAT/CD36) and caveolin-1 (Berk et al., 1990; Trigatti et al., 1991; Harmon and Abumrad, 1993; Schaffer and Lodish, 1994). In the...
context of such a multiprotein-mediated process, whether these transporters selectively transport various FA across the cell membrane remains to be determined. Certain reports have demonstrated that mice exhibited a spontaneous preference for linoleic acid (LA), but FAT/CD36-null mice did not exhibit such a preference (Laugerette et al., 2005). The incorporation of arachidonic acid (AA) in brain decreased if FABP was ablated (Murphy et al., 2005). These clues suggest the preferences of FA transporters, but direct evidence is lacking.

To investigate the correlation of FA profile and FATP function, gas chromatography–mass spectrometry and Q-PCR were used to analyze the FA composition and the expression of FA transporters (FATP-1, mFABP, FAT/CD36 and caveolin-1) in two anatomical locations (m. biceps femoris and m. ipsilateral pectoralis) of Avian farm broiler breeder and local Silky fowl breeder. Overexpressing cell models were then used to confirm the FA preference of the transporter by dynamic absorption detection at the cellular level.

### Material and methods

The animals in the experiments were reared and slaughtered with the approval of the College of Animal Science, South China Agricultural University; the experiments were also conducted in accordance with ‘the instructive notions with respect to caring for laboratory animals’ issued by the Ministry of Science and Technology of the People’s Republic of China. Chinese hamster ovary (CHO) cell line was donated from Ming Liao (College of Veterinary Medicine, South China Agricultural University, Guangzhou 510640, P.R. China).

**Animals and sampling**

Ten 100-day-old Avian farm broiler breeder (♂) and local Silky fowl breeder (♀), raised in the same standard conditions and fed *ad libitum* with identical diets were selected at random from among the population at Wen’s Group Breeding Farms. According to Chinese animal welfare rules, the animals were slaughtered to excise the *m. biceps femoris* and *m. ipsilateral pectoralis* and then frozen in liquid nitrogen and stored at −80°C for subsequent use.

**FA composition of muscle samples and of CHO cells**

The total FA content of each sample was extracted (Cortinas et al., 2004) and methyl-esterified (Morrison and Smith, 1964); the FA composition was detected by GC–MS (Trace DSQ, Thermo, CA, USA) using a fused silica capillary column DB5 (30 m × 0.25 mm). The temperature was initially set at 50°C for 3 min and then increased up to 180°C at a rate of 10°C/min; after 20 min, the temperature was increased to 250°C and maintained for 2 min. The injected volume was 1 μl, and the flow rate of helium was 30 : 1.

**mRNA expression of FA transporters**

The total RNA of each sample was extracted with TRIZOL Reagent (Saibaisheng, Beijing, China). Quality and quantity of RNA was estimated using the ratio of absorbance at 260 to 280 nm. Next, 2 μg of RNA were DNase treated (Promega, Madison, WI, USA) and reverse-transcribed using random primers (Invitrogen, Carlsbad, Guangzhou, China) and Moloney murine leukemia virus reverse transcriptase (Promega) according the manufacturer’s protocol. A total of 1 μl of cDNA was used in a 20 μl PCR. The real-time PCR was performed with an ABI 7500 Sequence Detection System (Applied Biosystems, CA, USA) using a two-step PCR procedure with the following protocol. Temperature was held at 95°C for 1 min. Forty PCR cycles of 95°C (15 s), 58°C (15 s) and 72°C (40 s) were completed, followed by melt curve analysis to confirm a single PCR product repeated twice each reaction. Fragments (75 to 150 bp) of the target genes (including FATP-1, mFABP, FAT/CD36, caveolin-1 and β-actin) were cloned and inserted into pMD18-T simple vector as standard plasmids. Table 1 presents the forward and reverse primers for the tested genes and for the internal reference gene (β-actin; Yun et al., 2005). Q-PCR (SYBR Green Kit, TOYOBO, Osaka, Japan) technology was employed to detect the mRNA expression level. The mRNA expression levels of the target genes were normalized by that of β-actin according to the 2^−ΔΔCt method.

**Cell culture**

Chinese hamster ovary (CHO) cells were grown in 1640 (Hyclone, Logan, UT, USA) medium supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY, USA),

---

**Table 1** Primer sequences used in Q-PCR and gene cloning*<ref>

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Forward primers</th>
<th>Reward primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken FATP-1</td>
<td>5’TCAAGCTTTCGAGTCTGAA3’</td>
<td>5’CAAGCTTGGTTGAAAGAGC3’</td>
</tr>
<tr>
<td>Chicken mFABP</td>
<td>5’GCAGGGATCCTTCGGAGT3’</td>
<td>5’GGAGGGTTCCTCTCTCT3’</td>
</tr>
<tr>
<td>Chicken FAT/CD36</td>
<td>5’CTGGGAAGGTTACCTGGAT3’</td>
<td>5’GGCGGACCCAGTAGAGTG3’</td>
</tr>
<tr>
<td>Chicken caveolin-1</td>
<td>5’CAAGCACTCTACGACGAC3’</td>
<td>5’GGCGGTAAGCTCTCTCTCT3’</td>
</tr>
<tr>
<td>CHO β-actin</td>
<td>5’TTCCACCGCTTCTCTCT3’</td>
<td>5’GGGTATTCGAGTCCACAG3’</td>
</tr>
<tr>
<td>Chicken FAT/CD36 ORF</td>
<td>5’CGGCGGATCCTTCGAGTCTCTG3’</td>
<td>5’CCGCGGATCCTCTCTCTCTCT3’</td>
</tr>
</tbody>
</table>

FATP-1 = fatty acid transport protein-1; mFABP = membrane fatty acid-binding protein; FAT = fatty acid translocase; CHO = Chinese hamster ovary; ORF = open reading frame.

*Underlined sequence indicated site of BamHI and XhoI.

---

https://doi.org/10.1017/S1751731112001619
Table 2. FA composition in muscle samples

<table>
<thead>
<tr>
<th>Breed</th>
<th>Avian (s.e.)</th>
<th>Silky (s.e.)</th>
<th>IP (s.e.)</th>
<th>BF (s.e.)</th>
<th>Significance difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>0.05 (0.02)</td>
<td>0.25 (0.07)</td>
<td>0.00 (0.00)</td>
<td>0.31 (0.10)</td>
<td>ns</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.00 (0.00)</td>
<td>0.02 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.02 (0.00)</td>
<td>ns</td>
</tr>
<tr>
<td>C16:0</td>
<td>28.77 (3.74)</td>
<td>32.57 (2.93)</td>
<td>34.53 (3.93)</td>
<td>26.99 (2.73)</td>
<td>**</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.52 (0.06)</td>
<td>1.13 (0.10)</td>
<td>0.25 (0.04)</td>
<td>1.39 (0.12)</td>
<td>ns</td>
</tr>
<tr>
<td>C18:0</td>
<td>17.78 (2.91)</td>
<td>13.71 (2.71)</td>
<td>15.66 (2.69)</td>
<td>15.83 (2.93)</td>
<td>ns</td>
</tr>
<tr>
<td>C18:1</td>
<td>19.96 (4.53)</td>
<td>22.94 (4.37)</td>
<td>20.54 (4.40)</td>
<td>22.36 (4.50)</td>
<td>ns</td>
</tr>
<tr>
<td>C18:2</td>
<td>15.29 (2.83)</td>
<td>17.35 (1.91)</td>
<td>11.78 (2.98)</td>
<td>20.87 (1.41)</td>
<td>ns</td>
</tr>
<tr>
<td>C20:1</td>
<td>0.01 (0.00)</td>
<td>0.03 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.04 (0.00)</td>
<td>ns</td>
</tr>
<tr>
<td>C20:2</td>
<td>0.02 (0.00)</td>
<td>0.03 (0.02)</td>
<td>0.00 (0.02)</td>
<td>0.07 (0.03)</td>
<td>ns</td>
</tr>
<tr>
<td>C20:3</td>
<td>0.11 (0.01)</td>
<td>0.19 (0.02)</td>
<td>0.17 (0.02)</td>
<td>0.13 (0.01)</td>
<td>ns</td>
</tr>
<tr>
<td>C20:4</td>
<td>15.67 (4.68)</td>
<td>10.69 (2.77)</td>
<td>10.49 (3.08)</td>
<td>10.30 (3.08)</td>
<td>ns</td>
</tr>
<tr>
<td>C20:5</td>
<td>0.11 (0.01)</td>
<td>0.19 (0.02)</td>
<td>0.20 (0.02)</td>
<td>0.00 (0.00)</td>
<td>ns</td>
</tr>
<tr>
<td>C22:4</td>
<td>0.98 (0.10)</td>
<td>0.45 (0.10)</td>
<td>0.62 (0.14)</td>
<td>0.80 (0.06)</td>
<td>**</td>
</tr>
<tr>
<td>C22:6</td>
<td>0.57 (0.10)</td>
<td>0.45 (0.12)</td>
<td>0.52 (0.08)</td>
<td>0.61 (0.12)</td>
<td>ns</td>
</tr>
</tbody>
</table>

FA = fatty acid; IP = m. intercostalispectoralis; BF = m. biceps femoris; GC–MS = gas chromatography–mass spectrometry.

Data are presented as mean (s.e.) for breed and anatomical location. s.e. is standard error of difference between means. ns = no significant difference (P > 0.05); *P < 0.05, **P < 0.01.

2 mM glutamine, 10 μg/ml streptomycin and 10 units/ml penicillin in a 5% CO₂/95% air atmosphere at 37°C.

Stable transfection of chicken FAT/CD36 in CHO cells

To determine the FA transport preference of chicken FAT/CD36 at the cellular level, a stable chicken FAT/CD36 overexpressing CHO cell line was created. The sequence of the chicken FAT/CD36 open reading frame (ORF) was cloned by Ex Taq (Takara Bio Inc., Shiga, Japan) and then inserted into pcDNA3.1 (+) via BamHI and XhoI restriction enzyme sites. After sequencing, the recombinant plasmid was extracted using the Endo-free Plasmid Kit (MN, Germany) and transfected into CHO cells with the Polyfect Kit (Qiagen, Hilden, Germany). After a 10-day selection with 1250 mg/l G418, the monoclonal strain was obtained by using the chicken FAT/CD36-CHO cell model. For this purpose, various FAs (palmitic acid (PA), stearic acid (SA), oleic acid (OA), LA and AA, Sigma, St. Louis, MO, USA) were combined and added to the cellular experiments, as follows. After washing twice with 1640, the chicken FAT/CD36-CHO cells and the pcDNA3.1 (+)-transfected CHO cell controls were treated with 0.5 ml serum-free medium (GIBCO) containing mixed FA (at a final concentration of 0.5 mM PA, SA, OA, LA and AA, respectively) and 2.5 mM bovine serum albumin (Gao and Serrero, 1999; Eyre et al., 2007; Lobo et al., 2009; Sigma, USA) for 15, 30 and 60 min, respectively, with six replicates for each treatment. The supernatant was collected to detect FA composition by GC–MS. FA methyl esters were identified by comparing retention times with external standard, methylated penta-decanoic acid (C15:0). The chromatographic peak area of various FAs was counted and converted into absolute content of them. The decrease of FA in supernatant was considered as the molar weight of FA uptake. Then, the uptake of total FAs in each treatment was normalized by the quantity of total cell protein as ‘mol/g cell protein’ (Bruce et al., 2011). The uptake of each FA was compared with its proportion in total FAs.

Statistical analysis

The data were described as X ± s.e. and analyzed statistically with SPSS 17.0 software by univariate Student–Newman–Keuls comparison. The significance level was considered at P < 0.05. The Spearman method was used to assess the significance of correlation between FA composition and membrane transporters, with significance levels at P < 0.01 and P < 0.05.

Results

Correlation between FA composition and respective membrane transporter expression

The FA composition of muscle samples is presented in Table 2. Together, PA (C16:0), SA (C18:0), OA (C18:1), LA (C18:2) and AA (C20:4) represented more than 96% of the
Selective transport of LCFAs by FAT/CD36

Table 3 mRNA abundance of transporters in muscle samples

<table>
<thead>
<tr>
<th>Breed</th>
<th>Anatomical location</th>
<th>Significance difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Avian (mean (s.e.))</td>
<td>IP (s.e.)</td>
</tr>
<tr>
<td>FATP-1/β-actin (×10⁻⁵)</td>
<td>9.80 (1.81)</td>
<td>14.80 (3.14)</td>
</tr>
<tr>
<td>mFABP/β-actin (×10⁻⁵)</td>
<td>1.86 (0.43)</td>
<td>2.49 (0.25)</td>
</tr>
<tr>
<td>FAT/CD36/β-actin (×10⁻⁵)</td>
<td>15.60 (2.76)</td>
<td>6.10 (0.90)</td>
</tr>
<tr>
<td>Caveolin-1/β-actin (×10⁻⁵)</td>
<td>4.79 (0.57)</td>
<td>4.65 (0.64)</td>
</tr>
</tbody>
</table>

IP = m. ipsilateral pectoralis; BF = m. biceps femoris; FAT = fatty acid-binding protein; mFABP = membrane fatty acid-binding protein; FAT = fatty acid translocase.

The total RNA of BF and IP samples was extracted with TRIZOL Reagent for quantitative PCR. The mRNA expression levels of the target genes were normalized by that of β-actin according to the 2⁻ΔΔct method (n = 10).

Data are presented as mean (s.e.) for breed and anatomical location. s.e. is standard error of difference between means.

ns = no significant difference (P > 0.05); *P < 0.05, **P < 0.01.

Table 4 The correlations of FA composition and membrane transporters were analyzed with Spearman correlation coefficient

<table>
<thead>
<tr>
<th>FA</th>
<th>FATP1</th>
<th>mFABP</th>
<th>FAT/CD36</th>
<th>Caveolin-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>0.165</td>
<td>0.035</td>
<td>0.568</td>
<td>0.090</td>
</tr>
<tr>
<td>C18:0</td>
<td>0.180</td>
<td>0.124</td>
<td>0.150</td>
<td>0.165</td>
</tr>
<tr>
<td>C18:1</td>
<td>−0.083</td>
<td>−0.267</td>
<td>0.126</td>
<td>0.181</td>
</tr>
<tr>
<td>C18:2</td>
<td>−0.344</td>
<td>−0.276</td>
<td>0.567</td>
<td>0.124</td>
</tr>
<tr>
<td>C20:4</td>
<td>0.106</td>
<td>0.503</td>
<td>−0.233</td>
<td>−0.262</td>
</tr>
</tbody>
</table>

FA = fatty acid; FAT = FA-binding protein; mFABP = membrane FA-binding protein; FAT = FA translocase.

Correlation coefficient with P < 0.05 are significant.

ns = no significant difference (P > 0.05); *P < 0.05, **P < 0.01.

total long-chain FAs (LCFA) in chicken. Compared with Avian farm broiler breeder chickens, local Silky fowl breeder had significantly higher (P < 0.05) proportions of LA (C18:2) and PA (C16:0) but much lower (P < 0.05) proportions of SA (C18:0) and AA (C20:4). With regard to the anatomical locations, myristic acid (C14:0), myristoleic acid (C14:1) and eicosenoic acid (C20:1 and C20:2) were specifically detected in m. biceps femoris, whereas eicosapentaenoic acid (C20:5) was found only in pectoralis. Of the five major FAs of chicken, LA (C18:2) represented a greater (P < 0.05) proportion of the total LCFA in the m. biceps femoris than in the m. ipsilateral pectoralis, whereas PA (C16:0) and AA (C20:4) followed the opposite trend (P < 0.01).

FATP-1, mFABP, FAT/CD36 and caveolin-1 mRNA were all observed to be abundantly expressed in skeletal muscles from both anatomical locations of Avian farm broiler breeder chickens and local Silky fowl breeder. Among these four FA transporters, only FAT/CD36 showed significant differences (P = 0.007) in expression (Table 3), whereas the other three genes exhibited no significant difference (P > 0.05) with regard to either breed or anatomical location.

The correlation analysis revealed that there was a positive significant correlation (P < 0.01) between FAT/CD36 and LA (C18:2), whereas a negative correlation (P < 0.01) between FAT/CD36 and PA (C16:0) (Table 4).

Construction and validation of the chicken FAT/CD36-CHO cell model

As shown in Figure 1, 1460 bp chicken FAT/CD36 ORF was cloned and the FAT/CD36-pcDNA3.1 (+) was further constructed. As shown in Figure 2, the monoclonal CHO strain transfected with FAT/CD36-pcDNA3.1 (+) was confirmed by western blot. The results showed that FAT/CD36 protein was highly expressed in FAT/CD36-pcDNA3.1 (+)-transfected CHO cells, whereas not detected in pcDNA3.1 (+) negative control cells.

FA uptake of the chicken FAT/CD36-CHO cell model

As shown in Figure 3, the uptake of FA in the control group increased slowly as the duration of treatment increased. In contrast, the chicken FAT/CD36-CHO cell model demonstrated enhanced FA uptake throughout the experiment, especially at the 60th min, when the FA uptake was dramatically increased (P < 0.01). The amount of total FA uptake in the FAT/CD36-CHO cell model was nearly 10 times greater than that of the control.

As shown in Figure 4, the proportion of palmitic acid (C16:0) was higher than SA (C18:0), OA (C18:1), LA (C18:2) and AA (C20:4) in the total cellular uptake of FAs. Compared with pcDNA3.1-CHO control, FAT/CD36-CHO took up a higher proportion of LA (C18:2) and lower proportion of PA (C16:0) both in 30 min (P < 0.05) and 60 min (P < 0.01).
In addition, the uptake of AA (C20:4) in FAT/CD36-CHO was also significantly greater ($P < 0.01$) than that in pcDNA 3.1-CHO control in 60 min. With regard to the uptake of SA (C18:0) and OA (C18:1), no significant difference was observed ($P > 0.05$).

**Discussion**

Normally, the FA composition was determined by several factors including species, age and nutrition. To analyze the relationship between transcriptome and some commercial traits, the broiler breeder of chicken was generally adopted to maintain the consistency of specific genetic background (Lyon et al., 2003; Trindade et al., 2004). In this paper, we also used the broiler breeder of Avian farm and Silky fowls. With regard to the age at the first egg and the growth curve, the physiological age of the broiler breeder of Avian farm and Silky fowls does not have much differences. Therefore, we selected the birds at 100 days of age to compare their FA composition and gene expression level, which was similar to other publications (Li et al., 2003). FA composition results demonstrated that PA, OA, LA, AA and SA were consistently observed to be the major components of muscles, consistent with the regularity and stability of FA composition in chicken. However, different FAs varied in terms of the relative proportion between anatomical locations and breeds. In this experiment, OA and LA were observed in a higher proportion in Silky fowl breeder than in Avian farm broiler breeder chickens; however, Silky fowl breeder had less SA than Avian farm breeder chickens, which was consistent with the consensus that local breeds are superior to fast-growing chickens in terms of meat quality (Shu et al., 2001; Wang et al., 2004). Compared with the *m. ipsilateral pectoralis*, the *m. biceps femoris* had significantly less PA and more LA proportion. The FA composition in muscles had been reported to be associated with myofiber type. Here, the main type of muscle fiber in *m. biceps femoris* was different from that in *m. ipsilateral pectoralis* (Belichenko et al., 2004). The presence of fast-twitch glycolytic fibers, rich in chicken *m. ipsilateral pectoralis*, was positively correlated with the FA composition of muscles, consistent with the regularity and stability of FA composition in chicken.

Caveolin-1, FATP-1 and mFABP mRNA expression levels between breeds and anatomical locations were not different. In particular, the level of caveolin-1 was nearly the same in both breeds and anatomical locations. It was not difficult to understand these observations given that caveolin-1 is a kind of scaffold protein with the highest expression level among these transport proteins. Caveolin-1 also had no correlation with any FA. The transport preference of mFABP for AA was shown by the finding that mFABP mRNA was highly expressed in the brain and correlated with the concentration of AA, which has an important physiological function in brain.
development (Murphy et al., 2005; Motohashi et al., 2009). This opinion was also supported by our results that mFABP had a positive correlation with AA. We also found that FAT/CD36 demonstrated differences between breeds and anatomical locations. Its mRNA expression level was higher in the m. biceps femoris than in the m. ipsilateral pectoralis, which was in accordance with recent reports (Marotta et al., 2004; Feng et al., 2007). Interestingly, FAT/CD36 was found to positively correlate with the LA content but negatively correlate with PA content, which is significant for its effect on meat quality. However, if we separately contrasted the relationship between FAT/CD36 mRNA expression and LA content in different species, the result may well be different. The differences may be caused by the following two reasons. First, many publications had reported the translocation of FAT/CD36 both to the plasma membrane for transport of FAs and to the mitochondria for increasing FA oxidation (Koonen et al., 2005; Bezaire et al., 2006). For example, exercise induces the translocation of FAT/CD36 to mitochondria, thus increasing muscle FA oxidation (Campbell et al., 2004). Furthermore, FAT/CD36 co-immunoprecipitated with carnitine palmitoyltransferase 1 (CPT1) in cells, and its overexpression increased mitochondrial FA oxidation efficiency, showing an additive effect when co-overexpressed with CPT1 (Campbell et al., 2004). Second, mitochondria show the highest oxidation rates with C12:0-C16:0 and with C18:2 (Alexson and Cannon, 1984). Thus, in summary, FAT/CD36 expression in a certain range could increase transport of LA into cells. However, it may also promote β-oxidation when FAT/CD36 expression levels are significantly higher.

By using the overexpressing CHO model, it was demonstrated that FAT/CD36 did enhance cellular FA uptake. Among the five major FAs, PA represented a greater proportion than SA, OA, LA and AA both in the FA composition of chicken and in the cellular uptake experiment, suggesting that PA itself traverses the cell barrier more easily than the other four FAs. Interestingly, in the uptake experiment, PA represented a lower proportion of total FA uptake in FAT/CD36-CHO than in the control, whereas LA represented a greater proportion in FAT/CD36-CHO, with a significant difference at both 30 and 60 min. On the basis of equimolar FA within physiological range, FAT/CD36-CHO revealed a preference for LA over PA relative to the control group. We speculated that the preference of FAT/CD36 might be due to anatomical differences. First, the recognition of FA by FAT/CD36 might have structure differences. FAT/CD36 was found abundantly at the apical region of the taste cell (Fukuwatari et al., 2003), and as a transmembrane glycoprotein it was even considered to be a fat sensor (Dransfield, 2008). These assumptions were strongly supported by the experiments in FAT/CD36-null mice. The FAT/CD36-null mice were unable to discriminate the LA-enriched solutions from the control, whereas the wild-type mice could (Laugerette et al., 2005). Second, FAT/CD36 and LA might interact with each other.
As we know, FAT/CD36 also plays an important role in mitochondrial oxidation, which involves CPT I as a key enzyme (Holloway et al., 2006; Holloway et al., 2008). When LA was added to the diet, the mRNA expression of CPT I increased significantly in red muscles, and its half-life was prolonged by 50% (Morash et al., 2009). As a ligand of peroxisome proliferator-activated receptor-γ (Belury et al., 2002), LA influenced the level of activation, resulting in increased FAT/CD36 (Teboul et al., 2001). The preceding line of reasoning demonstrates the close interaction between FAT/CD36 and LA. In addition, LA is an essential FA and a primary member of the ω-6 PUFA family, which can be transformed to γ-linolenic acid and AA by elongation/desaturation. The increased levels in chicken are of great value in the improvement of meat quality.

Furthermore, chicken FAT/CD36-CHO significantly enhanced the proportion of AA during the uptake of FA, but no positive correlation was detected in the FA composition in this study. The difference might be due to the difference of an in vivo vs. a primary member of the ω-6 PUFA family, which can be transformed to γ-linolenic acid and AA by elongation/desaturation. The increased levels in chicken are of great value in the improvement of meat quality.

References


