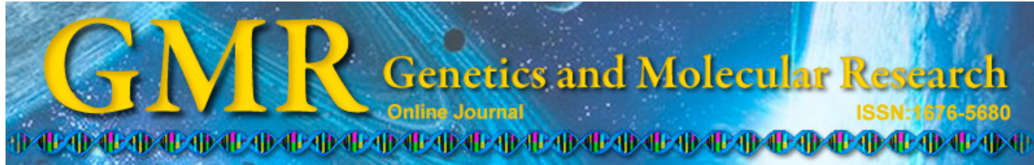

Overexpression of the *A-FABP* gene facilitates intermuscular fat deposition in transgenic mice

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ABSTRACT. Adipocyte fatty acid-binding protein (A-FABP), the most abundant FABP in adipocytes, controls fatty acid uptake, transport, and metabolism in fat cells. We constructed a transgenic mice model that overexpressed the cattle *A-FABP* gene to investigate the relationship between A-FABP expression and intermuscular fat deposition. There was no significant difference in body weight and serum biochemical indexes between transgenic and wild-type mice. Further, there were no significant differences in intermuscular triglyceride content and A-FABP expression levels over three generations of transgenic mice. However, abdominal adipose rate, A-FABP protein content, and intermuscular triglyceride levels of transgenic mice were significantly higher than those of wild-type mice. In addition, triglycerides were remarkably higher in the skeletal muscle but lower in the myocardium of transgenic mice.

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Thus, overexpression of cattle *A-FABP* gene promoted fat deposition in the skeletal muscle of transgenic mice.

Key words: Transgenic mice; Cattle A-FABP; Overexpression; Fat deposition

INTRODUCTION

Adipocyte fatty acid-binding protein (A-FABP), a member of the FABP family, is also known as FABP4 (Su et al., 2004). It is an important factor that controls intermuscular fat content (Gerbens et al., 1998), which in turn controls meat tenderness and flavor (DeVol et al., 1988; Gerbens et al., 1998). In addition, Michal et al. (2006) indicated that A-FABP was closely related to subcutaneous fat content and marbling. Thus, many researchers investigated A-FABP to determine ways to improve meat quality. For example, the A-FABP gene was found to be strongly associated with the function and development of intramuscular fat accretion (Chen et al., 2013) and was selected as candidate gene for regulating intermuscular fat metabolism in pigs (Gerbens et al., 2001). In cattle, genetic polymorphisms of *FABP4* gene were found to be related to meat quality grades (Gao et al., 2011; Shin et al., 2012) and carcass weight and marbling (Lee et al., 2010). Avilés et al. (2013) proposed that the A-FABP gene was significantly related to fat accumulation in cattle. A-FABP was also found to be significantly related to meat tenderness in sheep (Xu et al., 2011). In chicken, the A-FABP gene was used as a marker to identify intramuscular fat accretion (Ye et al., 2010).

Many researchers have extensively investigated the A-FABP gene that is involved with fat deposition. A-FABP, the most abundant intracellular lipid transport proteins in mature adipocytes, plays a role in fatty acid transport protein and triglyceride (TG) metabolism (Van der Horst et al., 1993; Chmurzyńska, 2006). Chmurzyńska (2006) showed that A-FABP had a high affinity binding site with long-chain fatty acids, which was required to transport fatty acids across membranes and organelle, resulting in the regulation of fatty acid oxidation and TG metabolism. Smith et al. (2007) suggested that hormone sensitive lipase (HSL), which is known to regulate adipocyte steatolysis, in adipocytes was activated by A-FABP. Thus, they indicated that A-FABP could bind with HSL in adipose cells and regulate their lipolysis. Thompson et al. (2009) reported that A-FABP could interact with Janus kinase 2, leading to increased intracellular lipid metabolism. Cabre et al. (2007) indicated that the fatty acid, peroxisome proliferator-activated receptor-γ, could regulate A-FABP gene expression level, thereby affecting TG metabolism. Taken together, these findings suggest that *A-FABP* is significantly associated with fat metabolism.

In the present study, we generated a cattle A-FABP transgenic mice model. This study aimed to determine whether the overexpression of cattle A-FABP gene could promote fat deposition in transgenic mice. The findings of this study could improve our knowledge about A-FABP and transgenic cattle.

MATERIAL AND METHODS

Transgenic and wild-type mice preparation

A-FABP transgenic mice were generated by injecting a specific foreign plasmid

(pEGFP-C1-A-FABP) into fertilized eggs by using the microinjection method. Non-transgenic mice were used as wild-type (WT) mice. Transgenic and WT mice were housed in a barrier facility with 12-h light/12-h dark cycles and fed in a standard and uniform condition.

Routine detection

Body weight and abdominal adipose tissue accumulation rate determination

Nine transgenic and WT mice each were selected randomly at the 60th day of age and used for subsequent analyses. Subsequently, body weight, abdominal fat weight, and the rate of abdominal adipose tissue accumulation were determined.

Serum biochemical index determination

Blood was collected in 2-mL tubes during the enucleation of eyeballs, centrifuged at 3500 *g* for 15 min, and serum was collected and processed for determining the biochemical indexes of alanine aminotransferase (ALT), aspartate transaminase (AST), creatinine (CRE), cholesterol (CHO), glucose (GLU), and TG by using an automatic biochemical analyzer (GLAMOUR 3000, MD), followed with manufacturer instruction of Reagent for ALT Test Kit, AST Test Kit, CRE Test Kit, CHO Test Kit, GLU Test Kit, and TG Test Kit (InTec).

A-FABP protein content determination

Tissues (30 mg), including myocardium and skeletal muscle, were collected from transgenic and WT mice and homogenized using pestles in phosphate buffered saline. The tissues were frozen and thawed under -80°C, and centrifuged at 5000 *g* for 5 min, liquid supernatant was seclected and enzyme-linked immunosorbent assay (ELISA) was used to determine the total A-FABP protein content according to the manufacturer instruction of ELISA Kit for FABP4 (SEB693Mu, Uscn).

TG content determination

Tissues (30 mg), including myocardium and skeletal muscle, were collected from transgenic and WT mice and homogenized using pestles in isoamylol. The homogenate was incubated for 48 h at 4°C, centrifuged at 5000 *g* for 5 min, supernatant was separated, and the absorbance was measured according to the manufacturer protocol for the TG kit operation. TG content was calculated as follows: TG content = A sample / A standard x concentration of standard solution.

Real-time polymerase chain reaction (RT-PCR) analysis of gene expression patterns

RT-PCR was performed using standard curves generated on the basis of total RNA from heart and skeletal muscle tissues. TRIzol reagent was purchased from Invitrogen. Tissues were homogenized in TRIzol, left for 5 min, centrifuged at 12,000 *g* for 10 min at 4°C, separated in chloroform, precipitated with isopropanol, and eluted with ethanol. RNA was then dissolved in diethylpyrocarbonate-treated water. Total

RNA was reverse-transcribed into cDNA for subsequent PCR amplification. The following A-FABP primers were used: forward primer, 5'-GTGGGCGTGGGCTTTGCT-3'; reverse primer, 5'-CCATTTTGTCGTCCATGAGTTTTCT-3'. After amplification, a 377-bp fragment was obtained. β-actin was used as the house-keeping gene (forward primer, 5'-TGAGCTGCGTTTTACACCCTT-3'; reverse primer, 5'-GCGACCATCCTCCTCTTAGG-3'); a 384-bp fragment was obtained. PCR temperature program was as follows: 1) 95°C for 2 min, 2) 95°C for 10 s, 3) 60°C for 40 s, 4) 72°C for 30 s, 5) step 2 again for 40 cycles, and 6) 72°C for 5 min. PCRs were performed in triplicate by using the Gene Expression Macro Software (Bio-Rad) and the comparative Ct ($2^{-\Delta\Delta Ct}$) value method.

Statistical analysis

Data are reported as means ± SD. Statistical differences were analyzed using analysis of variance and the *t*-test to determine entire treatment effects (SPSS 11.0). A two-tailed P value of 0.01 was used to reveal statistical significance.

RESULTS

Routine detection analysis

There was no significant difference in body weight between transgenic and WT mice (Figure 1A). However, abdominal adipose content of transgenic mice was significantly higher than that of WT mice (Figure 1B). According to serum biochemical index test, serum contents of ALT and AST were higher and those of CRE, CHO, and GLU were slightly lower for A-FABP transgenic mice than for WT mice. The TG content in the serum of transgenic mice was significantly lower than that of WT mice (Figure 2A-F).

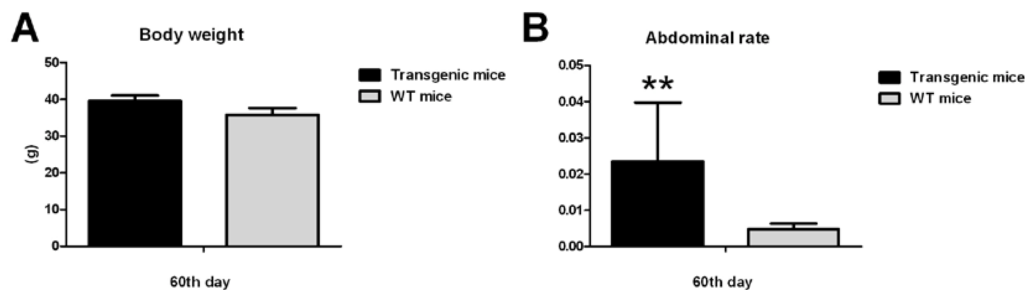

Figure 1. Routine detection between transgenic and wild-type (WT) mice. **A.** Body weight was not significantly different between transgenic and WT mice (N = 9). **B.** Abdominal adipose tissue accumulation rate of transgenic mice was significantly higher than that of WT mice (N = 9; **P < 0.01).

A-FABP levels and fat deposition analysis between parental transgenic mice and WT mice

A-FABP protein content in the skeletal muscle and myocardium of parental transgenic mice was significantly higher than that of WT mice (Figure 3A); A-FABP protein content in the

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myocardium was higher than that in the skeletal muscle of both parental transgenic mice and WT mice (Figure 3A). In addition, TG content in the skeletal muscle and myocardium of parental transgenic mice was significantly higher than that in WT mice (Figure 3B); further, TG content in the skeletal muscle was higher than that in the myocardium of both parental transgenic mice and WT mice. Thus, upregulation of A-FABP gene expression facilitated fat deposition.

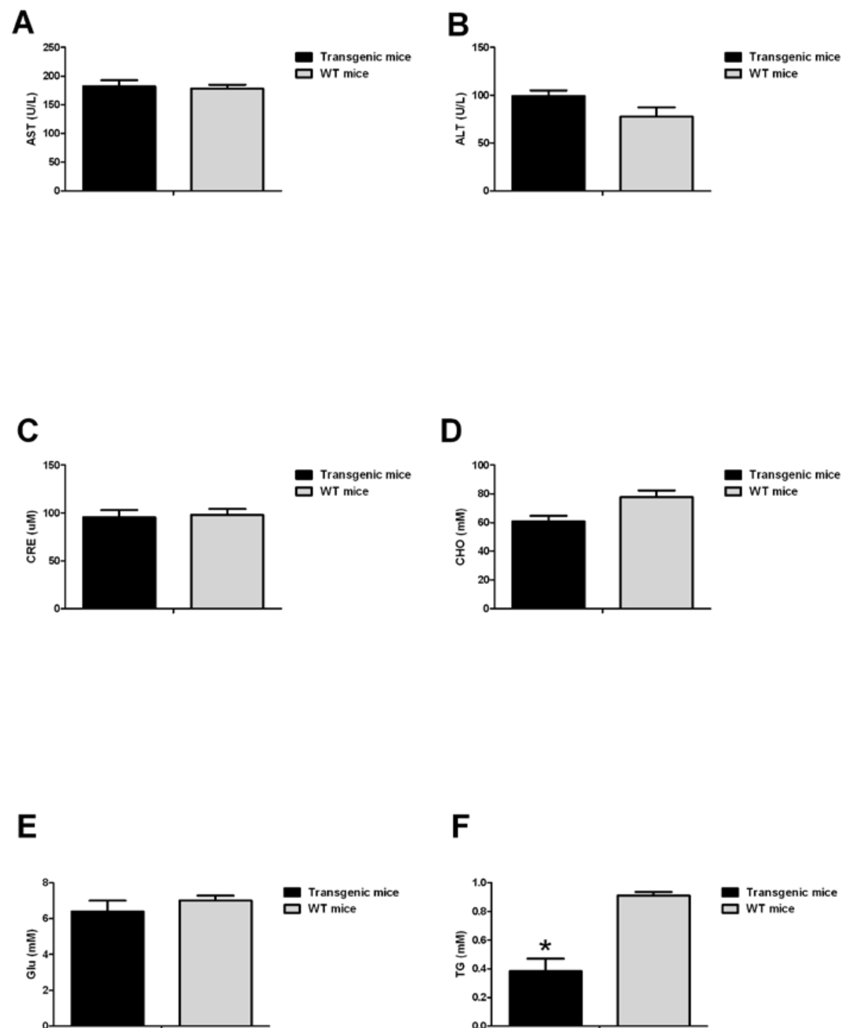

Figure 2. Serum biochemical index determination of transgenic and WT mice. **A.** Aspartic acid aminotransferase (AST) content in the serum of transgenic mice was not significantly different from that of WT mice. **B.** Alanine aminotransferase (ALT) content in the serum of transgenic mice was not significantly different from that in WT mice. **C.** Creatinine (CRE) content in the serum of transgenic mice was not significantly different from that in WT mice. **D.** Cholesterol (CHO) content in the serum of transgenic mice was not significantly different from that in WT mice. **E.** Glutamine (GLU) content in the serum of transgenic mice was not significantly different from that in WT mice. **F.** Triglyceride (TG) content in the serum of transgenic mice was significantly different from that in WT mice ($0.01 < *P < 0.05$).

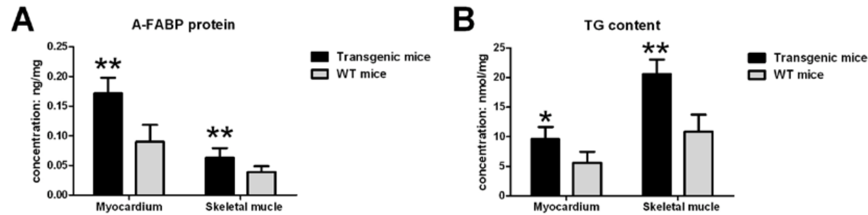

Figure 3. A-FABP and TG levels in the myocardium and skeletal muscle of parental transgenic mice and WT mice. **A.** A-FABP levels in the myocardium and skeletal muscle of A-FABP transgenic mice and WT mice (N = 9). A-FABP content of transgenic mice in the myocardium and skeletal muscle has increased more than 50% (**P < 0.01). **B.** TG levels in the myocardium and skeletal muscle of A-FABP transgenic mice and WT mice (N = 9; 0.01 < *P < 0.05; **P < 0.01).

A-FABP levels and TG content over three generations of transgenic and WT mice

A-FABP expression level was analyzed using RT-PCR. No significant difference in A-FABP expression levels were found in the skeletal muscle and myocardium among the three generations of A-FABP transgenic mice. A-FABP gene expression was absent in WT mice (Figure 4A). Further, there was no significant difference in A-FABP protein content over the three generations in transgenic mice determined using the ELISA method, but A-FABP protein content was significantly higher in the transgenic mice than in the WT mice (**P < 0.01; Figure 4B). Similarly, TG content was not significantly different over the three generations of transgenic mice, but was significantly higher than that in WT mice (0.01 < *P < 0.05, **P < 0.01; Figure 4C). A-FABP gene expression levels remained almost constant across the three generations of transgenic mice. These findings suggest that A-FABP transgenic mice are hereditarily stable and promote fat deposition.

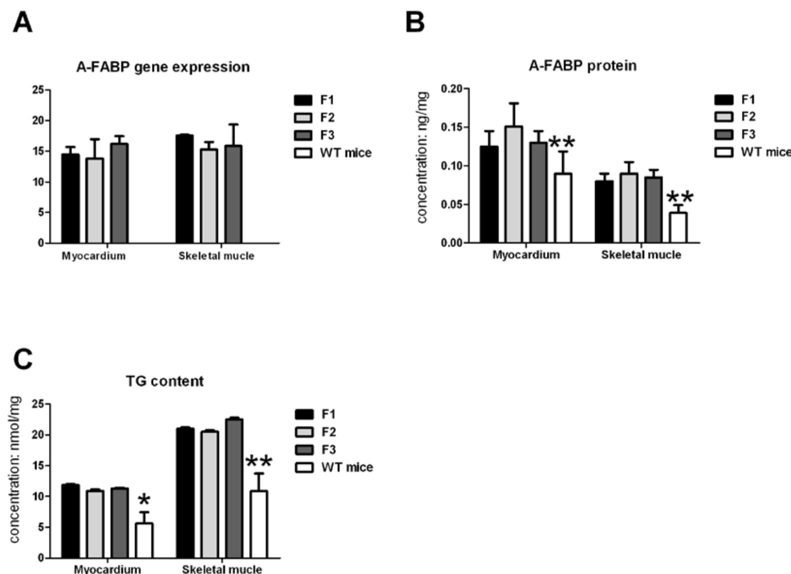

Figure 4. A-FABP and TG levels over three generations of A-FABP transgenic and WT mice. **A.** A-FABP gene expression over three generations of transgenic and WT mice (N = 9). **B.** A-FABP protein content over three generations of transgenic and WT mice (N = 9). **C.** TG content over three generations of transgenic and WT mice (N = 9).

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DISCUSSION

A-FABP has been shown to be associated with fat deposition (Houben et al., 1998); our results confirmed this finding. Overexpression of cattle A-FABP gene in transgenic mice resulted in remarkable increase in TG content, suggesting that the A-FABP gene facilitated TG deposition. In addition, abdominal adipose tissue accumulation rate of transgenic mice was significantly higher than that of WT mice, confirming that overexpression of A-FABP could facilitate fat deposition. A-FABP transgenic mice is the model for transgenic cattle research. In our study, fat deposition in the skeletal muscle of transgenic mice was significantly higher than that of WT mice, whereas that in the myocardium was only slightly higher in transgenic mice than in WT mice. Thus, we could forecast that fat deposition was remarkably increased in the skeletal muscleof the transgenic cattle.

To our knowledge, this is the first study reporting the generation of cattle A-FABP transgenic mice model; in previous studies, the A-FABP gene was knocked down to regulate fat deposition. For example, Wei et al. (2013) showed that A-FABP gene expression was associated with lipid metabolism-related gene expression, including ADIPOQ, LEP, and LEPR, and knocking down of A-FABP gene remarkably decreased the expressions of ADIPOQ and LEP genes, resulting in the upregulation of fat deposition. Further, Yang et al. (2011) knocked down A-FABP gene and found that fat mass and body weight of mice increased. However, our study showed that overexpression of A-FABP gene could promote intermuscular fat deposition.

Interestingly, the TG content in the skeletal muscle and myocardium of transgenic mice was significantly higher than that of WT mice, whereas that in the serum of transgenic mice was remarkably lower than that in WT mice; this could be related to body lipid homeostasis. In addition, there were no significant differences in serum biochemical indexes between transgenic and WT mice, which was consistent with the findings of transgenic cattle. However, further studies are warranted to determine the mechanism underlying the action of A-FABP gene.

Further, A-FABP transgenic mice showed over-expression of A-FABP and increased intermuscular TG levels over three generations, suggesting that A-FABP transgenic mice are hereditarily stable. In addition, our study showed that over-expression of cattle A-FABP gene could promote intermuscular fat deposition in the skeletal muscles, which could form the basis for future A-FABP research and cattle meat quality research.

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