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Expression profiles of key transcription factors involved in lipid metabolism in Beijing-You chickens

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ABSTRACT

Intramuscular fat (IMF) is a crucial factor for the meat quality of chickens. With the aim of studying the molecular mechanisms underlying IMF deposition in chickens, the expression profiles of five candidate transcription factors involved in lipid metabolism in several tissues were examined in Beijing-You (BJY) chickens at five ages (0, 4, 8, 14 and 20 wk). Results showed that accumulation of IMF in breast (IMFbr), thigh (IMFth) and abdominal fat weight increased significantly ($P < 0.01$) after 8 wk. Accumulation of both IMFbr and IMFth from 8 to 14 wk exceeded that from 14 to 20 wk; IMFth was 4–7 times of IMFbr. As for the expression profiles of key transcription factors: 1) expression of *C/EBPα* and *PPARγ* in abdominal fat was significantly higher than that in breast and thigh muscles at all ages. The expression of *C/EBPα* was positively correlated with *PPARγ* in both breast and thigh muscles, which indicated that both *C/EBPα* and *PPARγ* promoted fat deposition and might act through a unified pathway; 2) the expression of *SREBP-1* in 0, 4, and 8 wk in thigh muscle was significantly higher than that in breast; 3) expression of *C/EBPβ* at 4 and 8 wk was significantly higher than that at 14 and 20 wk; and it was positively correlated with IMFth and IMFbr from 0 to 8 wk; 4) expression of *PPARα* in breast and thigh muscles was significantly higher than that in abdominal fat. Taken together, all five transcription factors studied play roles in lipid metabolism in chickens with *C/EBPα* and *PPARγ* being important effectors.

1. Introduction

It is commonly known that intramuscular fat (IMF) contributes to flavor and juiciness. It is an important factor for the palatability of chicken meat. The content of IMF is the result of lipogenesis and lipolysis with complex molecular mechanisms. Chickens differ from mammals in having minimal dissectible adipose tissue associated with the connective elements of skeletal muscle and lipid accumulation is more influenced by uptake of blood lipids and subsequent lipogenesis rather than *de novo* fatty acid synthesis (Griffin et al., 1987, 1992). Liver serves as the main site of fatty acid synthesis and exports lipids as lipoproteins along with those derived from the gut (Griffin et al., 1987, 1992; Leclercq et al., 1984; Leveille et al., 1975). Together, these serve as substrates for use by other tissues, including deposition in muscle as well as in adipose tissue. The regulation of lipid deposition in adipose tissue is incompletely understood but can be expected to involve adipogenesis (differentiation and maturation), lipid transport, lipogenesis and lipolysis; the latter three would be of additional importance in muscle.

There is evidence, from a variety of species, for certain transcription factors playing roles in controlling adipocyte differentiation, lipogenesis and lipolysis (Haraguchi et al., 2003; Hausman et al., 2009; Hummasti et al., 2008). Adipogenesis is a well-regulated process controlled by a highly coordinated activation of various transcription factors. Temporal expression, in a highly coordinated cascade, of transcripts for *CCAAT/enhancer-binding protein factors* (*C/EBPα*, *C/EBPβ*), *sterol regulatory element-binding protein 1* (*SREBP1*), and *peroxisome proliferator-activated receptors* (*PPARα* and *PPARγ*) is especially important (Elam et al., 2001; Koo et al., 2001; Rosen et al., 2000). *SREBP1* plays an important role in the early stages of adipogenesis (Kim and Spiegelman, 1996). In addition to *C/EBPβ* being expressed in the early stages of adipogenesis (Darlington et al., 1998; Timchenko et al., 1996), *C/EBPβ* as a transcriptional relay might be under the direct control of some effects of insulin and/or *SREBP1* in mature fat cells (Le Lay et al., 2002; Timchenko et al., 1996). Subsequently, *C/EBPβ* induces the expression of *C/EBPα* and *PPARγ* at later stages of cell differentiation (Rosen et al.,

Abbreviations: AbFW, abdominal fat weight; ADD-1, adipocyte determination and differentiation-dependent factor 1; ANOVA, analysis of variance; BJY, Beijing-You; *C/EBPα*, CCAAT/enhancer-binding protein factors α; *C/EBPβ*, CCAAT/enhancer-binding protein factors β; GLM, generalized linear model; IMF, intramuscular fat; IMFbr, IMF in breast muscle; IMFth, IMF in thigh muscle; MCE, mitotic clonal expansion; *PPARα*, peroxisome proliferator-activated receptor α; *PPARγ*, peroxisome proliferator-activated receptor γ; *SREBP1*, sterol regulatory element-binding protein 1.

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Table 1
Primers for the targeted and reference transcripts.

Gene name	Primer sequence[a]	Product length (bp)	GenBank Accession
SREBP1	F CTGAAGGGTGACGAGGAGGG R GCTGCTGCCACAGGTTGGT	301	AY029224.1
C/EBPβ	F ATTACGAGGCGGACTGTTTGG R CGGGTGAGGCTGATGTAGGTG	304	NM_205253.1
C/EBPα	F GTGGACAAGAACAGCAACGAGTACCGC R TGCCTGAAGATGCCCCGCAGAGT	200	NM_001031459.1
PPARα	F TCAGAATAAGGAAGCCGAAGT R GATTGGAGAAGCCAGGGAT	110	AF163809.1
PPARγ	F CACAAGCGGAGAAGGAG R TTTGGTCAGAGGGAAGG	134	AF163811.1
β-actin	F GAGAAATTGTGCGTGACATCA R CCTGAACCTCTCATTGCCA	152	L08165

[a] Each primer is shown 5′ to 3′.

2002; Timchenko et al., 1996) and these participate in a positive feedback loop, promoting and maintaining the differentiated state (Yeh et al., 1995a). The two transcription factors, *C/EBPα* and *PPARγ*, play essential roles in activating terminal differentiation of adipocytes, lipid synthesis and other specific programs (Hausman et al., 2009; Olofsson et al., 2008; Rosen et al., 2002). *PPARα* is another important contributor to lipid metabolism by increasing fatty acid β-oxidative and lipid oxidation, hence reducing lipid accumulation (Muoio et al., 2002; Tsuchida et al., 2005; Ye et al., 2001).

The dynamics of these transcription factors (*SREBP1*, *C/EBPβ*, *C/EBPα*, *PPARγ* and *PPARα*) in important tissues across maturation in chickens are still unclear, as are the possible effects of the transcription factors on fat deposition. Beijing-You (BJY) chickens are a local variety, with a high fat content and an excellent flavor. The objective of this study was to describe developmental changes and explore possible relationships between expression of transcription factors and fat deposition in economically important tissues during growth of chickens.

2. Materials and methods

2.1. Animals and sample collection

All experimental procedures were performed according to the Guidelines for Experimental Animals established by the Ministry of Science and Technology (Beijing, China). Fifty BJY male hatchlings came from conservation stock (Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China) and were randomly assigned to five groups, each of 10 birds. All birds were raised under recommended conditions with *ad libitum* feed and water.

At each age tested (day of hatch, 4, 8, 14 and 20 wk), the 10 birds of a group were fasted for 12 h, weighed then killed by stunning and exsanguination. Samples (50–100 mg) of each right breast muscle, thigh muscle, and abdominal fat were rapidly removed and snap-frozen in liquid nitrogen for storage at −80 °C. The remainder of each sampled tissue was stripped of obvious connective tissues, weighed, and then stored at −20 °C.

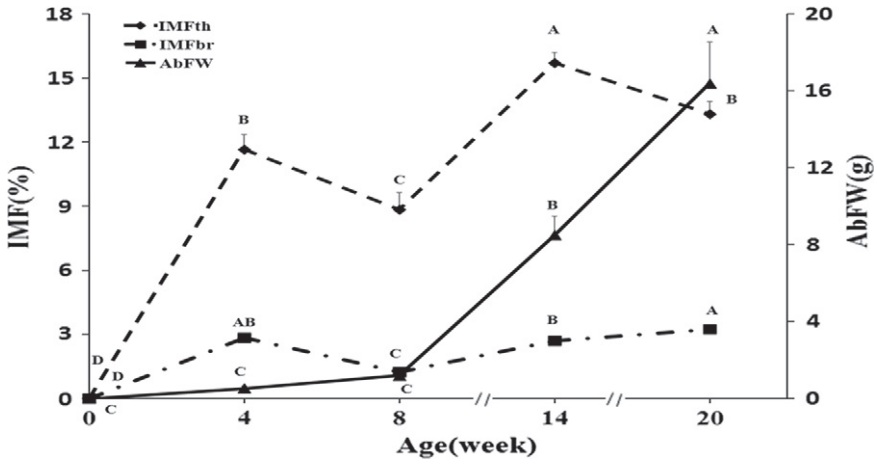
2.2. Intramuscular fat (IMF)

IMF in the right breast muscle (IMFbr) and IMF in thigh muscle (IMFth) were measured by Soxhlet extraction, exactly as described by Zerehdaran et al. (2004) and Cui et al. (2012).

2.3. RNA extraction and reverse transcription (RT)

Total RNA was isolated at 4 °C using the Trizol reagent (Invitrogen, US), then any residual genomic DNA and protein were removed with Dnase I (TaKaRa, Japan) and RNA clean kit (TIANGEN, Beijing). The purified RNA was dissolved (200–400 ng/ml, $OD_{260}/OD_{280} = 1.8–2.0$), and stored at −70 °C.

Total RNA was used for RT (in 20 μl final volume) following the manufacturer's instruction (Promega, USA) and cDNA was stored at −70 °C for subsequent real time-PCR.



Note: different letters indicate significant differences between ages within each trait ($P<0.05$, n=10). IMF could not be measured and there was no abdominal fat at 0 wk (all shown as 0)

Fig. 1. IMF in breast and thigh and AbFW across ages of BJY. Note: different letters indicate significant differences between ages within each trait ($P < 0.05$, n = 10). IMF could not be measured and there was no abdominal fat at 0 wk (all shown as 0).