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Postnatal Development of *IGF-I* and *IGF-II* Genes in Goat Skeletal Muscle and Liver Tissues

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Abstract: Insulin like Growth Factors (IGF-I and IGF-II) play an important role in regulation of skeletal muscle growth and act as mediator of growth hormone during muscle development and differentiation. In this study, the expression profiling of goat *IGF-I* and *IGF-II* genes was investigated in liver and skeletal muscle at six postnatal development stages (3, 15, 30, 60, 90 and 120 days) and two types of muscles (longissimus dorsi muscle, LD; triceps brachii muscle, TB) using relative real-time quantitative PCR. The results showed that *IGF-I* gene was expressed at the highest level in the liver with relatively high expression levels in spleen, skeletal muscle and heart. *IGF-II* gene was predominantly expressed in spleen, followed by liver and longissimus dorsi muscle, whereas expression in heart was relatively weak. *IGF-I* and *IGF-II* genes had different expression profiles during the postnatal period of the goat liver and skeletal muscle development. Moreover, *IGF-I* was expressed at higher levels in triceps brachii muscles compared with longissimus dorsi muscles. The present study provided the comprehensive understanding of IGF-I and IGF-II expression during liver and muscle development and laid the basis for further studies on their roles in goat liver and skeletal muscle development.

Key words: Goat, Nanjiang Mongolian Grazelle, IGF-I, IGF-II, skeletal muscle, liver

INTRODUCTION

Insulin like growth factors I and II (IGF-I and IGF-II) are produced primarily by the liver as an endocrine hormone as well as in target tissues in a paracrine/autocrine fashion (Florini *et al*., 1996). They are primary mediators of the effects of Growth Hormone (GH) to promote the prenatal and postnatal myofiber development and growth (Oksbjerg *et al*., 2004). Muscle cell cultures produce IGFs and IGF Binding Proteins (IGFBPs) in various degrees depending on the origin (species, muscle type) and the state of muscle cells development (Clemmons, 1998), suggesting an autocrine/paracrine mode of action of IGF related factors. Transgenic over expression of IGF-I induces myofiber hypertrophy and muscle growth were increased approximately 30%. In addition, there was a substantial increase in weights of spleen, pancreas, brain and kidney (Liu *et al*., 1993; Coleman *et al*., 1995). All of these observations are consistent with the view that IGFs plays an essential role during skeletal muscle and another tissues normal growth and development.

During postnatal skeletal muscle growth *in vivo* or in fully differentiated muscle cells, IGFs stimulate the rate of protein synthesis and inhibits the rate of protein degradation thereby enhancing myofibre hypertrophy (Le Grand and Rudnicki, 2007; Rhoads *et al*., 2009). It suggested that IGFs is sufficient to regulate myofibre hypertrophy and transformation postnatally which is associated with muscle mass and meat quality respectively in economically livestock. However, little is known of the regulation of mRNA levels of *IGF-I* and *IGF-II* gene in goat liver and skeletal muscle development.

In this study, goat *IGF-I* and *IGF-II* genes tissue expression patterns were analyzed. To further understand their roles in liver and skeletal muscle development, expression differences were detected in liver and muscle samples from different developmental stages in goats. The findings of transcriptional characterization of goat *IGF-I* and *IGF-II* genes will undoubtedly help in further understanding their roles in goat liver and muscle development.

MATERIALS AND METHODS

Animals and collection of tissues: The Nanjiang Mongolian Grazelle used in the expression profiling analysis were raised at the Station of Nanjiang Mongolian Grazelle breeding center (Nanjiang, Sichuan, China). All experimental procedures were approved by Sichuan

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Table 1: Primer sequences used in this study

GenBank Accession No.	Genes name	Primer sequence (5'–3')	Size (bp)	Temp. (°C)
D11378	*IGF-I*	CTGCGGGGCTGAGTTGGTGG	201	63.5
		GAGCGGGCTGACTTGGTGGG	-	-
DQ645739	*IGF-II*	GACCATCCAGCCGCATAAA	138	62.6
		CGGTCGTAGAGGCAGACACA	-	-
AJ431207	*GAPDH*	TTGTGATGGGCGTGAACC	127	63.0
		CCCTCCACGATGCCAAA	-	-

Province Committee on Laboratory Animal Care. The longissimus dorsi muscles and liver were collected from goats at six different postnatal periods (3, 15, 30, 60, 90 and 120 days after birth, six samples at each stage) for expression levels analysis of the *IGF-I* and *IGF-II* genes at different developmental stages. To examine the target genes expression in different muscle fiber types, two different fiber types of muscles were collected from six 120 day goats (fast-twitch muscle fiber type, longissimus dorsi muscle; slow-twitch muscle fiber type, triceps brachii). Three female goats adult (120 days) heart, liver, spleen, lung, kidney and longissimus dorsi muscle were also collected, respectively for spatial expression analysis. The all tissues were collected within 30 min after slaughter and immediately frozen in liquid nitrogen then stored at -80°C until RNA extraction.

RNA isolation and cDNA synthesis: The RNA was extracted using Trizol reagent (Invitrogen) according to the manufacturer's protocol, treated with RNase-free DNase I (Takara, Japan) to remove contaminating genomic DNA incubate at 80°C with 2.5 μL of 0.5 M EDTA for 2 min to inactivate DNase I and stored at -80°C. The first strand cDNAs was synthesized from 2 μg total RNA using M-MLV reverse transcriptase (Promega, Madison, WI, USA) as described in the manufacturer's protocol. The corresponding cDNA was stored at -20°C.

Quantitative real-time RT-PCR analysis: Real-time RT-PCR was used to quantify the expression level of goat IGF I and IGF II in different tissues and at six different postnatal periods in liver and longissimus dorsi muscle using ABI 7300 real-time PCR thermal cycle instrument (ABI, USA), according to the supplied protocol. Gene specific primers (Table 1) were designed based on the sequences (GenBank: D11378 and DQ645739) using Primer 5.0 software. Each real-time PCR (in 25 μL) reaction contained 12.5 μL SYBR® Green Real time PCR Master Mixture (contains ROX Dye. Toyobo, Jap), 0.25 μM primers and 1 μL normalized template cDNA. The cycling conditions consisted of an initial, single cycle for 3 min at 95°C followed by 40 cycles of cycling consisting of 20 sec at 94°C, 20 sec at 62°C,15 sec at 72°C and final extension for 5 min. The specificity of PCR products were confirmed by melting curve analysis. All PCR amplifications were performed in triplicate for each RNA sample. Gene expression levels were quantified relatively to the expression of GAPDH using Gene expression macro software (ABI, USA) by employing an optimized comparative Ct ($2^{-\Delta\Delta Ct}$) value method.

Statistical analysis: For each gene, one-way ANOVA was employed using SPSS version 17.0 to compare the difference of gene expression in different tissues and in different developmental stages and Duncan's new multiple rang test was used to analyze statistical significance. The $p<0.05$ was deemed to be significant and $p<0.01$ highly significant level. Values were presented as mean±SD.

RESULTS AND DISCUSSION

Tissue expression pattern of goat *IGF-1* and *IGF-2* genes: The tissues expression analysis of goat *IGF-I* and *IGF-II* genes was performed by qRT-PCR. Goat IGF-I mRNA was expressed at the highest level in the liver with relatively high expression levels in spleen whereas expressions in longissimus dorsi muscle and heart were relatively low. Goat IGF-II mRNA was predominantly expressed in spleen. In contrast, low levels of expression of IGF-II were evident in liver and much lower levels in heart and longissimus dorsi muscle (Fig. 1). The results are generally in agreement with the expression of *IGF-I* and *IGF-II* genes in sheep and pig.

Differential expression of IGF-I and IGF-II during goat liver development: To analyze the postnatal developmental patterns of goat *IGF-I* and *IGF-II* genes in liver, the researchers performed the qRT-PCR analyses of six liver developmental stages. As shown in Fig. 2, the expression level of IGF-I was relatively high at 3rd day then significantly decreased at 15th day ($p<0.05$). Goat IGF-I was up-regulated from the 15-90 days, reaching its highest expression at 90th day and then significantly decreased at 120th day ($p<0.05$). No significant expression differences between 3, 30 and 120 days were observed.

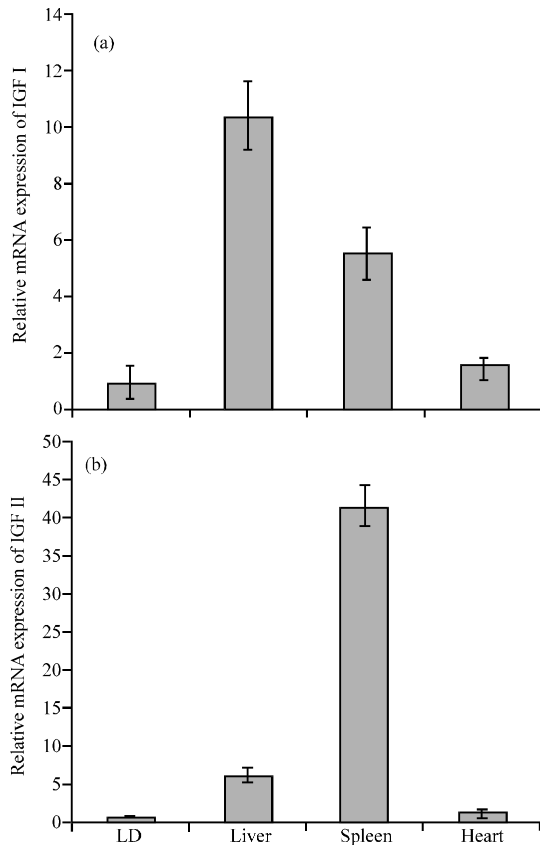

Fig. 1: Spatial expression profile of *IGF-I* and *IGF-II* genes in different tissues of 120 days goats by real-time PCR. The relative expression levels of this gene mRNA was analyzed using the comparative Ct method, employing GAPDH as the reference gene in each sample. Error bars represent SD (n = 3). Significant levels were analyzed by t-test. Different letters above the bars indicate a significant difference ($p<0.05$) between different tissues

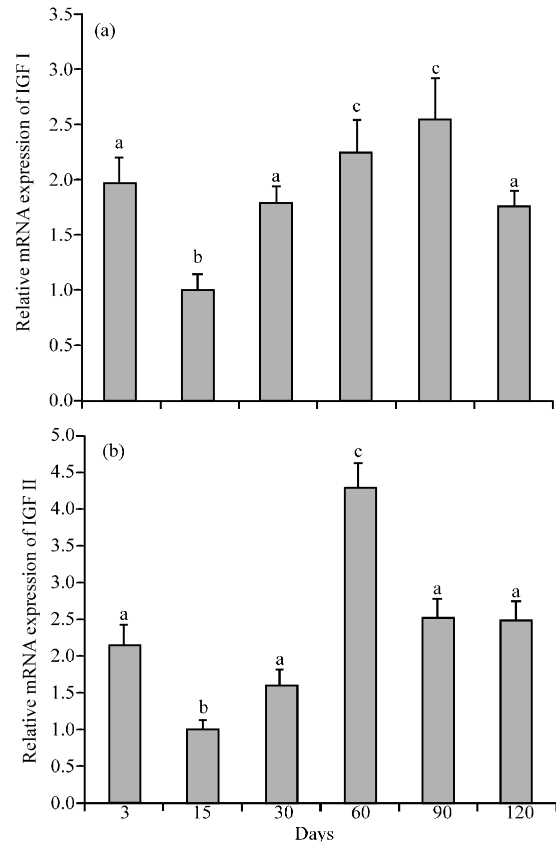

Fig. 2: Goat *IGF-I* and *IGF-II* genes mRNA expression level in different liver development stages in the Nanjiang Mongolian Grazelle. Significant levels were analyzed by t-test. Different letters above the bars indicate a significant difference ($p<0.05$) between six different liver development stages

The expression trend of IGF-II from the 3-30 days is generally coincident with IGF-I. However, IGF-II expression peaked at 60th day and then significantly decreased at the 90th day ($p<0.05$). No significant expression differences between 90 and 120 days were observed.

Differential expression of IGF-I and IGF-II during goat skeletal muscle development: To analyze expression patterns of IGF-I and IGF-II during goat skeletal muscle development, the researchers performed the qRT-PCR analyses of six skeletal muscle developmental stages. As shown in Fig. 3, goat IGF-I was expressed at 3rd day with a relatively high level then decreased at 15th day and then significantly ($p<0.05$) increased at the 60th day. No significant expression differences in longissimus dorsi muscles between 60, 90 and 120 days were observed. Goat IGF-II was expressed at 3rd day with a relatively high level then decreased at 15th day and was up-regulated from the 15-60 days ($p<0.05$), reaching its highest expression at the 60 days and was subsequently down-regulated from the 60-120 days ($p<0.05$). Goat IGF-I and IGF-II provided similar expression patterns from the 3-60 days but not from the 60-120 days in postnatal muscles.

Differential expression of IGF-I and IGF-II in two different muscles contained different muscle fiber type: To detect the expression pattern of IGF-I and IGF-II in muscles contained different muscle fibers, the researchers also performed qRT-PCR analyses of samples from three individual 120 days female goats. In this study, triceps

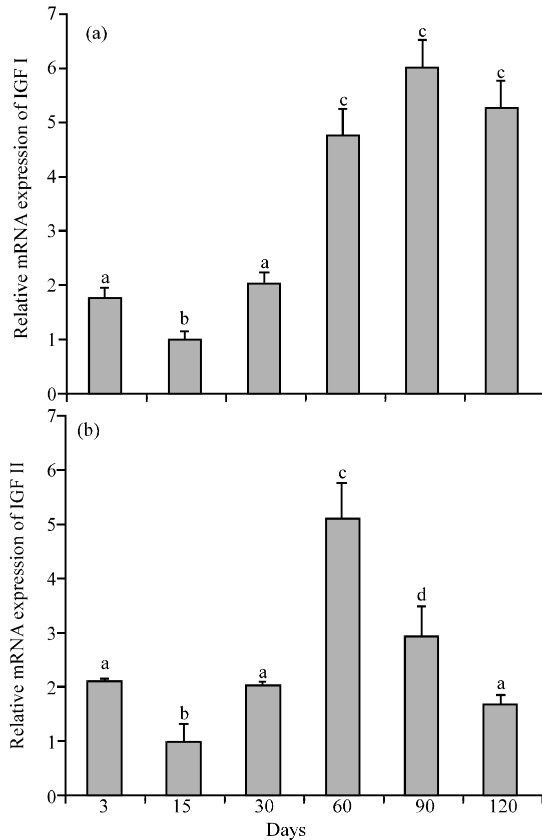

Fig. 3: The *IGF-I* and *IGF-II* gene expression in the skeletal muscle from six different stages of the Nanjiang Mongolian Grazelle by qRT-PCR. The expression level was normalized to GADPH and measured with 2^(-ΔΔCt) value. Significant levels were analyzed by t-test. Different letters above the bars indicate a significant difference ($p<0.05$) between six different muscle development stages. day 3: muscle from 3rd day after birth; day 15: muscle from 15th day after birth; day 30: muscle from 30 day after birth; day 60: muscle from 60 days after birth; day 120: muscle from 120 days after birth

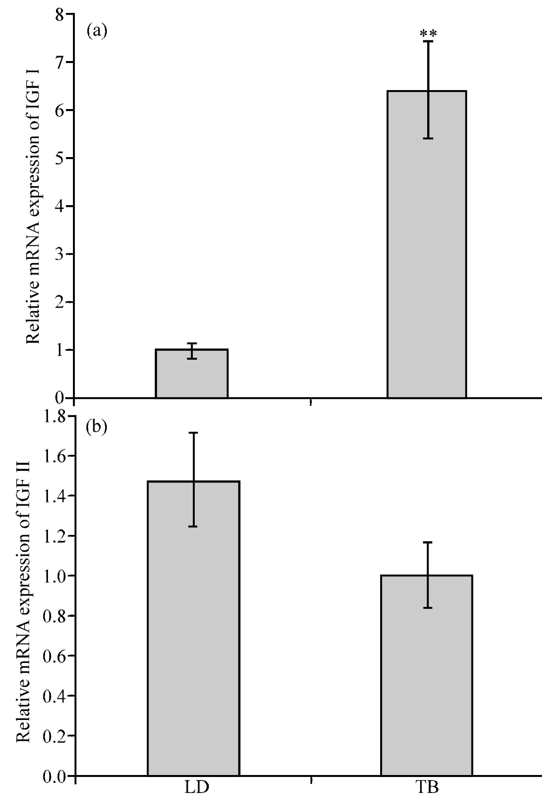

Fig. 4: a) The *IGF-I* and *IGF-II* genes expression in two different muscles contained different muscle fiber type in the Nanjiang Mongolian Grazelle by qRT-PCR; b) represented the IGF-I and IGF-II. The expression level was normalized to GADPH. Results are averaged from three independent replicates. Error bars represent SD (n = 3). Differences in IGF-I and IGF-II mRNA levels are presented relatively to Longissimus Dorsi (LD). Two different muscles: Longissimus Dorsi (LD) and Triceps Brachii muscle (TB) were analyzed. Significant levels were analyzed by t-test. **$p< 0.01$; *$p<0.05$

brachii muscles which are composed mostly of type I, slow-twitch oxidative fibers and longissimus dorsi muscles contained predominantly type IIb, fast-twitch glycolytic fibers. As shown in Fig. 4, the triceps brachii muscles displayed a greater abundance of IGF-I mRNA than longissimus dorsi and muscles in Nanjiang Mongolian Grazelle ($p<0.01$). IGF-II showed no significant expression differences between two different muscles. These analyses showed that IGF-I is preferentially expressed in slow-twitch oxidative fibers in goat skeletal muscle. IGF-I and IGF-II is an autocrine survival factor during myogenesis (Stewart and Rotwein, 1996). Before birth, the IGFs are known to have an important paracrine role in the growth and development of fetal tissues (Fowden, 1995; Fowden and Silver, 1995). Pigs with a regulatory mutation in the *IGF-II* gene exhibit significantly greater skeletal muscle mass than their wild type counterparts and its expression levels have been shown to differ between obese and lean genotypes in postnatal pigs (Van Laere *et al*., 2003). The majority of IGF-I and IGF-II mRNA was localized to developing muscle fibers in pigs (Gerrard *et al*., 1998). Previous studies suggest that fetal IGF-I and IGF-II are independently regulated in the sheep fetal circulation and

have a different expression pattern (Fowden and Silver, 1995; Cheung *et al*., 1996; Oliver *et al*., 1996). However, the expression pattern of *IGF-I* and *IGF-II* genes during postnatal goat skeletal muscle and liver development remain poorly understood. Therefore, the researchers established a real-time RT-PCR method for *IGF-I* and *IGF-II* gene expression analysis.

To understand the roles of *IGF-I* and *IGF-II* genes in goat muscle development, the researchers examined their expression differences among six postnatal development stages. The results showed that goat IGF-I was expressed at 3rd day with a relatively high level then up-regulated from the 15-60 days ($p<0.01$), reaching its highest expression at the 60 days. In pig, IGF-II mRNA peaked in 59 days fetal muscle and decreased after birth. In contrast, IGF-I expression increased to maximal levels around birth and is down regulated in postnatal skeletal muscle development (Gerrard *et al*., 1998). IGF-I has the ability to stimulate both satellite cell proliferation and differentiation (Engert *et al*., 1996; Pesall *et al*., 2001). In contrast, IGF-II is thought to be involved solely with satellite cell differentiation. So, the researchers infer that IGF-I and IGF-II may play important role on early component of myofibril formation or signal transduction during goat muscle development.

The expression of *IGF-I* and *IGF-II* genes depend on the origin of the cells and their state of development stage. IGF-I and IGF-II mRNA was up-regulated in rat and ovine muscle satellite cells from proliferation to differentiation. In contrast, *IGF-II* gene expression is decreased in turkey satellite cells from proliferation to differentiation (Ernst *et al*., 1996). In addition, IGF-I expression is not detected in human (Crown *et al*., 2000) and avian (Kocamis *et al*., 2001) satellite cells. The expression patterns of IGF expression *in vitro* muscle cells suggest an autocrine regulation of myogenesis by the IGFs.

When we analyzed the IGF-I expression in two different muscles found IGF-I expressed at higher levels in triceps brachii muscles contained mostly of slow-twitch oxidative fibers compared with longissimus dorsi muscles contained predominantly fast-twitch glycolytic fibers in Nanjiang Mongolian Grazelle. However, IGF-II showed no significant expression differences between two different muscles. In pigs, an increased number of insulin-like Growth Factor-I Receptor (IGF-IR) has been found in membranes isolated from slow twitch muscles compared to fast-twitch muscles (Louveau *et al*., 1996). However in rabbit, the expression of IGF-I and IGF-II were similar in line with the higher proliferation rate of satellite cells derived from slow twitch muscles compared to fast-twitch muscles (Barjot *et al*., 1996). And this difference is probably related to the actions and levels of other growth factors (Martelly *et al*., 2000). Passive stretch not only induced an increase in IGF-I mRNA expression within the individual muscle fibres but also an increase in the percentage of slow-twitch oxidative fibers (Kocamis *et al*., 2001).

CONCLUSION

The study shows that the expression profile of goat IGF-I indicated that *IGF-I* gene could contribute to the skeletal muscle development is higher in slow-twitch than in fast-twitch muscles.

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