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Original article

Insulin-like growth factor II (IGF-II) mRNA expression during skeletal muscle development of double-muscled and normal bovine foetuses

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Abstract — We investigated the IGF-II gene expression in developing *Semitendinosus* muscle in foetal normal and double-muscled cattle. Samples from normal and double-muscled foetuses ranging from 90 to 210 d post-conception were collected and total RNA extracted. Northern blot analysis was performed using the human IGF-II cDNA probe. Five IGF-II transcripts, 5.1, 4.4, 3.7, 2.6 and 1.7 kb, were detected in muscle samples. Throughout gestation, all transcripts, except for the 5.1 kb one, decreased similarly in both genetic types. In double-muscled foetuses, the amount of the 5.1 kb transcript was higher than those of the other transcripts and its expression remained stable throughout the gestational stages analysed. These results indicated that the regulation of IGF-II gene transcription was distinct in both genetic types. The IGF-II foetal plasma concentrations increased throughout gestation. In bovine foetuses, the first muscle cell differentiation was concomitant with a high autocrine IGF-II mRNA expression and low plasma IGF-II levels in both genetic types. The second step of muscle cell differentiation was associated with high IGF-II plasma concentrations and the autocrine expression of IGF-II was reduced. © Inra/Elsevier, Paris

muscle / bovine / mRNA / insulin-like growth factor-II / double-muscled

Résumé — Expression du gène de l'IGF-II au cours du développement musculaire chez les fœtus Charolais normaux ou présentant une hypertrophie musculaire (Charolais « culards »). L'expression du gène de l'IGF-II au cours du développement du muscle demi-tendineux de fœtus Charolais normaux ou dits « culards » a été étudiée par analyse en northern blot et hybridation avec une sonde d'ADNc d'IGF-II humain. Les échantillons ont été prélevés au cours du développement fœtal

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entre 90 et 210 j post-conception. Cinq ARN messagers (ARNm) spécifiques de l'IGF-II, (5,1, 4,4, 3,7, 2,6 et 1,7 kb) ont été détectés dans tous les échantillons de muscle analysés. Mis à part le transcrit de 5,1 kb, les ARNm de l'IGF-II présentent une diminution au cours du développement des fœtus des deux types génétiques. Chez les animaux culards, le transcrit de 5,1 kb est plus abondant que les autres transcrits et son niveau reste stable tout au long de la gestation. Ces résultats attestent d'une régulation différentielle de la transcription du gène IGF-II entre les deux types génétiques. Chez les fœtus, les taux d'IGF-II plasmatiques augmentent tout au long de la gestation. Ainsi la première vague de différenciation des cellules musculaires apparaît en présence d'une expression autocrine d'ARNm IGF-II forte et de taux plasmatiques d'IGF-II bas, pour les deux types génétiques étudiés. La seconde vague est accompagnée par des taux d'IGF-II circulants plus élevés et d'une expression autocrine d'ARNm IGF-II réduite. © Inra/Elsevier, Paris

muscle / bovin / ARNm / *insulin-like growth factor-II* / culards

1. INTRODUCTION

Expression of the insulin-like growth factor-II (IGF-II) gene is greater during foetal development than at any other period in the life of mammals [29, 30, 40]. Moreover, the disruption of this gene greatly diminishes prenatal growth in mice [6]. Together, these observations support the hypothesis that IGF-II is the major regulator of foetal growth. More specifically, IGF-II is expressed in developing muscle tissues of several species (rat [2]; human [13]; bovine [24]) and in several myoblast cell lines [8, 46]. Recently, we demonstrated that IGF-II gene expression in muscle cell bundles, isolated from bovine foetuses, has a greater intensity during early foetal development than compared to stages prior to and after birth [24]. These results suggest that IGF-II participates in myogenesis as an autocrine/paracrine-acting growth factor, stimulating both proliferation and differentiation of muscle cells.

Double-muscled cattle provides an excellent model for studying muscle development. At birth, these animals have nearly 40 % more muscle fibers than normal muscled cattle [7]. Moreover, hyperplasia was demonstrated by the higher potency of myoblast proliferation in double-muscled foetuses than in normal foetuses [37]. Previous work showed that serum from double-muscled foetuses stimulated proliferation of L6 myoblasts to a greater extent than serum from normal foetuses [10]. In addition, in foetal double-muscled cattle, Picard et al. [33] showed the existence of a delay in differentiation compared to normal muscled cattle. However, the regulation of increased muscle and delay in differentiation associated with double-muscled cattle remains unknown.

In order to determine the potential role of IGF-II in hyperplasia of double-muscled cattle during foetal development, we studied the IGF-II gene expression time-course changes in *Semitendinosus* muscle in both genetic types during gestation. The northern blot analysis of IGF-II expression gene revealed several transcripts which exhibited a differential regulation between double-muscled and normal foetuses.

2. MATERIALS AND METHODS

2.1. Animals

This study was carried out as part of a research programme approved by the 'Institut national de la recherche agronomique' (Inra, France) Ethical Committee. Animals were bred and slaughtered and samples collected at the Inra Research Center (Theix, France) according to ethical guidelines concerning animal care.

2.2. Tissue and plasma samples

Embryos from the INRA95 strain [28] were transplanted into Charolais/Salers crossbreed cattles of an average age of 2 years. *Semitendinosus* muscle samples were taken from normal and double-muscled Charolais foetuses ranging in age from 90 to 210 d post-conception (pc). Tissue samples were removed immediately after exsanguination of the dam. Tissue samples collected for total RNA preparation were frozen in liquid nitrogen and stored at –80 °C. Foetal blood samples were collected in sterile heparinised syringes, centrifuged at 4 °C and the plasma stored at –20 °C until the determination of IGF-II concentration.

2.3. Total RNA preparation

Total RNA were extracted according to the method of Puissant and Houdebine [36] as follows: the tissues were homogenised in 4 mol·L^{-1} guanidinium thiocyanate solution, containing 25 mmol·L^{-1} sodium citrate, pH 7, 0.5 % sarkosyl, 100 mmol·L^{-1} mercaptoethanol, at 4 °C (1:10, w:v). The homogenate was acidified with 0.1 vol. of 2 mol·L^{-1} sodium acetate, pH 5.2, 1 vol. of Tris-EDTA saturated phenol (100 mmol·L^{-1} Tris-HCl pH 7.5, 10 mmol·L^{-1} EDTA), and 0.2 vol. isoamylic alcohol-chloroform (1:49) were added successively to the homogenate. After a 15 min incubation at 4 °C, and centrifugation (5 000 *g*, 10 °C, 20 min), the upper phase was saved and one equal volume of isopropanol was added to precipitate the RNA overnight, at –20 °C. The RNA pellet was recovered by centrifugation (5 000 *g*, 20 min, 4 °C), rinsed with 70 % ethanol, and dissolved in sterile water. RNA were precipitated by adding 5.5 vol. of 4 mol·L^{-1} LiCl and kept at 4 °C for 2 h. RNA were recovered by centrifugation (5 000 *g*, 20 min, 4 °C) and the pellet was dissolved in 2 vol. of 10 mmol·L^{-1} Tris-HCl pH 7.5, 1 mmol·L^{-1} EDTA and 0.5 % SDS. After one additional extraction by phenol/isoamylic alcohol-chloroform (vol/vol), the aqueous phase was precipitated in the presence of 300 mmol·L^{-1} sodium acetate and 2.5 vol. ethanol. The RNA were kept in this precipitated form at –20 °C, until quantification by optical density measurements and analysis by northern blot.

2.4. Probes

A 663 bp fragment containing the entire coding region of the human IGF-II (hIGF-II) gene with 15 bp of the untranslated leader to exon 6 and 99 bp of the 3' untranslated sequences was used [21]. cDNA was used to synthesise radioactive probes for northern blot analysis. This cDNA fragment was labelled with α ^{32}P-dCTP (3 000 Ci·mmol^{-1}; ICN, Orsay, France) to a specific activity of 10^8 cpm·µg^{-1} DNA using a random priming kit (Boehringer Mannheim, Meylan, France). A mouse 18S rRNA [38] and a mouse β-actin cDNA probe [1] were also used and labelled as previously described.

2.5. Northern blot analysis

The samples of RNA (20 µg) were denatured in 2.2 mol·L^{-1} formaldehyde, 50 % formamide (5 min, 65 °C) and size separated by electrophoresis in agarose (1.5 %) gel, in the presence of 2.2 mol·L^{-1} formaldehyde in 10 mmol·L^{-1} sodium phosphate buffer. RNA were transferred to a Zeta probe membrane (Biorad, Ivry-sur-Seine, France) by capillary blotting overnight under high ionic strength (10 × SSC = 0.15 mol·L^{-1} sodium chloride, 0.15 mol·L^{-1} sodium citrate). Autoradiograms were obtained by exposure to Amersham Hyperfilm with two amplifying screens, at –80 °C. Laser density scans were converted to relative numerical values (Image Master, Pharmacia, Les Ulis France).

2.6. Determination of plasma IGF-II concentrations

The extraction of plasma samples from normal and double-muscled foetuses and the competitive protein binding assay for IGF-II were similar to those reported previously [3]. Briefly, prior to determining IGF-II concentrations, plasma samples were chromatographied on an Ultrogel AcA54 column (IBF, Villeneuve-Garenne, France) in 1 mol·L^{-1} CH_3COOH, 0.15 mol·L^{-1} NaCl, 0.1 % BSA to ensure effective removal of IGF binding proteins. The yield after calibration of each column ranged between 75 and 80 %. Total IGF-II was measured by assays using IGF binding proteins extracted from human cerebrospinal fluid which have selective affinity for IGF-II (> 10^{-10} M^{-1}). The IGF-II standard concentrations ranged from 0.03 to

1 ng/tube. IGF-II exhibited 10-fold more reactivity than IGF-I with the cerebrospinal binding proteins. No cross reaction was found with insulin (25 $\mu g \cdot mL^{-1}$). Each unknown sample was assayed at three different concentrations and each concentration was assayed in duplicate. The intra-assay coefficient of variation was 4.8 %.

2.7. Statistical analysis

The statistical significance of the differences between foetuses of different ages and genotypes was determined by variance and covariance tests with the assistance of the SAS software [41].

3. RESULTS

3.1. Comparison of the developmental expression of the IGF-II gene between normal and double-muscled foetuses

Northern blot analysis, using a human IGF-II cDNA probe, identified multiple IGF-II mRNA with sizes of 5.1, 4.4, 3.7, 2.6 and 1.7 kb in each muscle sample studied (*figure 1A*). These multiple transcripts were similar to those previously reported for rat [5, 25, 43] and bovine foetal tissues [24]. The ethidium bromide profile (*figure 1B*) confirmed the integrity of the RNA loaded in each lane. In order to normalise the muscle IGF-II gene expression data, northern blots were hybridised with a 18S probe (*figure 1C*). The patterns of developmental expression of each transcript for normal and double-muscled foetuses are shown in *figure 2*. The results are expressed as the ratio between the signals obtained with the IGF-II and 18S probes. For normal foetuses, the relative amount of each IGF-II transcript progressively decreased throughout gestation (90 d pc versus 210 d pc, $P < 0.001$); the decrease was already significant at 130 d pc ($P < 0.001$). For double-muscled foetuses, the same pattern was observed, i.e. a decrease of IGF-II gene expression throughout the gestation, except for the 5.1 kb transcript. The general trend of IGF-II gene expression reported here, was the same as that reported in the literature for rat, sheep and pig [5, 22, 32]. At each stage of gestation, there was no significant difference in the relative amounts of the different transcripts between genetic types. At 210 d pc, in both genetic types, all the transcripts, except for the 5.1 kb one in double-muscled foetuses, reached the same relative amount.

The 5.1 kb transcript exhibited a specific pattern. From 90 to 170 d pc and for both bovine genetic types, the 5.1 kb transcript amounts were higher than those of the other transcripts (for example, at 170 d pc in normal and double-muscled foetuses, 5.1 versus 4.4 kb transcript, $P < 0.1$ and 0.001, respectively). In double-muscled foetuses, as early as 130 d pc and up to 210 d pc, the 5.1 kb transcript remained stable throughout the gestation stages analysed. At 170 and 210 d pc, its relative amount was significantly higher ($P < 0.001$) in double-muscled animals than in normal animals. These results indicate that the developmental regulation of multiple IGF-II transcripts was distinct between both genotypes.

3.2. Comparison of the developmental expression of the β-actin gene between the two genotypes

Since the mouse β-actin cDNA cross hybridises to α-actin mRNA in muscles [1, 48], this probe was used as a control of the differentiation grade of *Semitendinosus* muscle during foetal development. Northern blot hybridisation of muscle RNA preparations with an actin cDNA identified a 2.2 kb mRNA in normal and double-muscled foetuses (*figure 3A*). In both normal and double-muscled foetuses, the actin mRNA's relative level progressively increased during the foetal period studied ($P < 0.001$). At the first stages studied (130 and 170 d pc), the relative level of actin mRNA was similar in muscles of normal and double-muscled foetuses. However, at the last stage studied (210 d pc), the actin mRNA level was significantly higher in muscles of normal animals compared to double-muscled animals

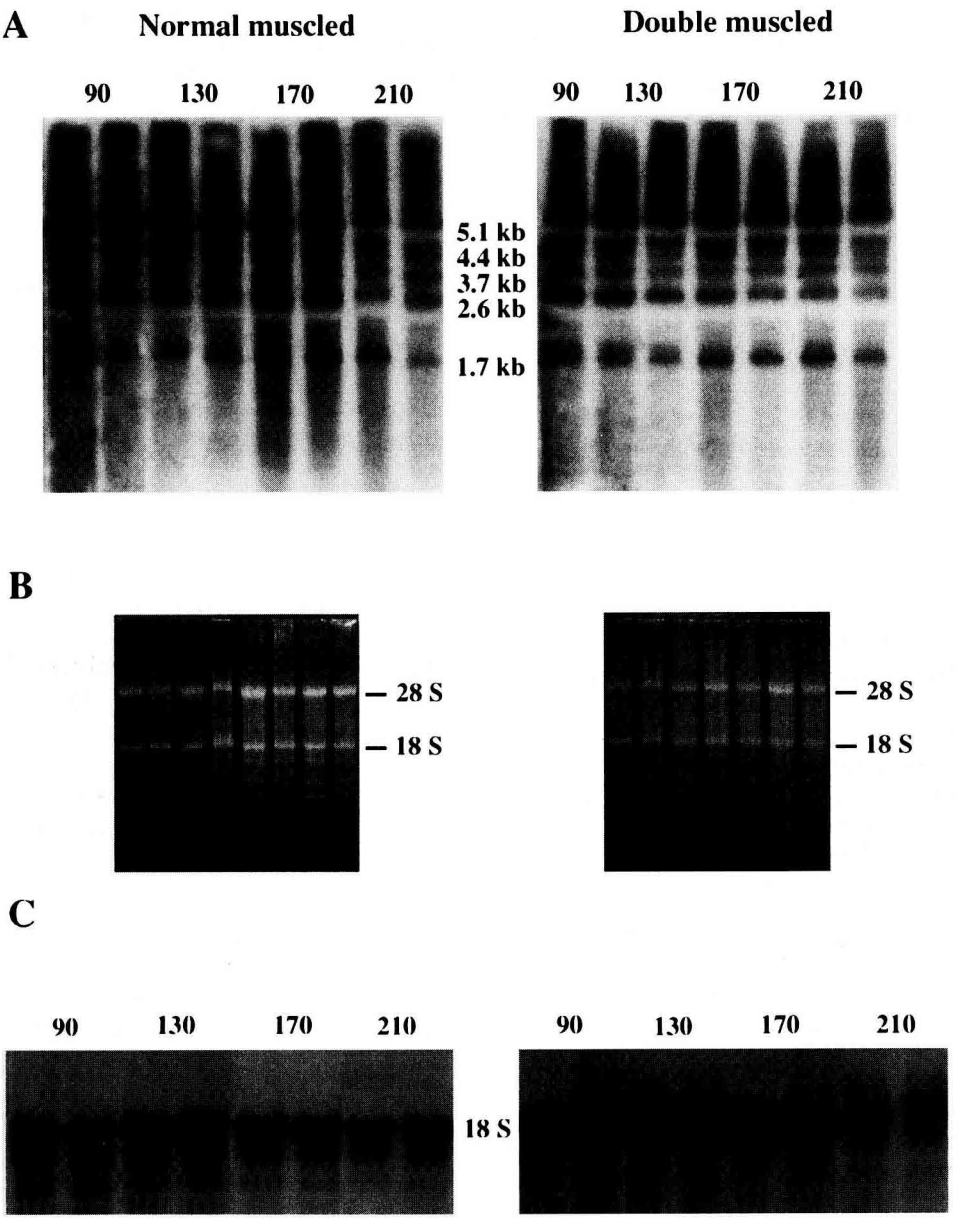

Figure 1. Northern blot analysis of IGF-II gene expression in *Semitentidinosus* muscle from normal and double-muscled foetuses (**A**). Total RNA (20 µg) were denaturated in formaldehyde, subjected to electrophoresis and transferred to Zeta-probe membranes. The membranes were hybridised with the human IGF-II cDNA probe. Autoradiographs lasted 7 d. The intermediate panel (**B**) depicts ethidium bromide staining of the 28S and 18S ribosomal RNA for each sample. In order to standardise the IGF-II gene expression, the same membranes were hybridised with a 18S RNA probe (**C**). In this case, autoradiographs lasted for 30 min.

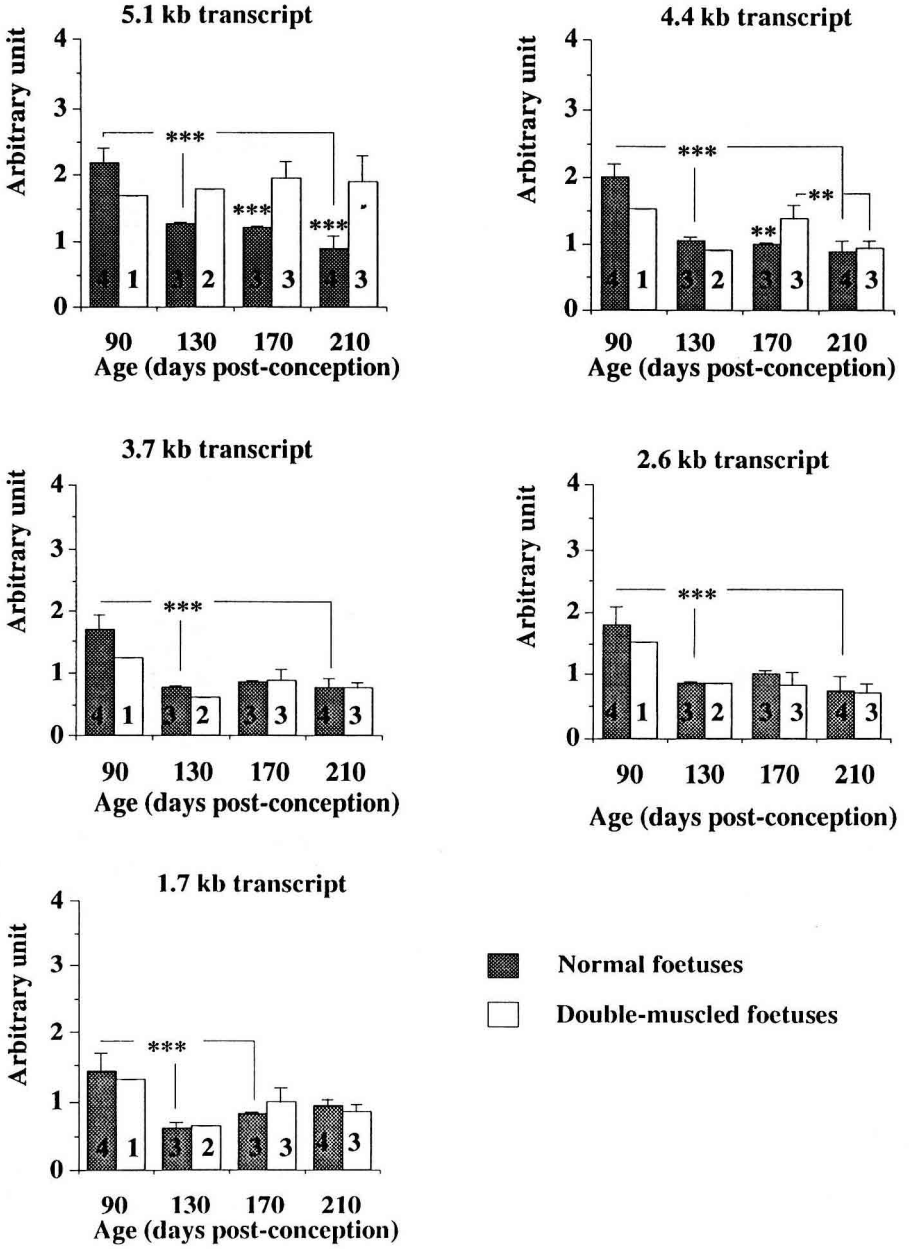

Figure 2. Analysis of the developmental pattern of multiple IGF-II transcripts in *Semitendinosus* muscle from normal and double-muscled foetuses. The autoradiographic films were scanned with the Image Master System (Pharmacia). The results are presented as the mean ± SEM of individual determinations and expressed in arbitrary densitometric units taking into account individual values of 18S hybridisation. The number of analysed foetuses is indicated inside the bars. Small asterisks indicate when the means are significantly different between gestational stages (**, $0.01 < P < 0.1$; ***, $0.001 < P < 0.01$).

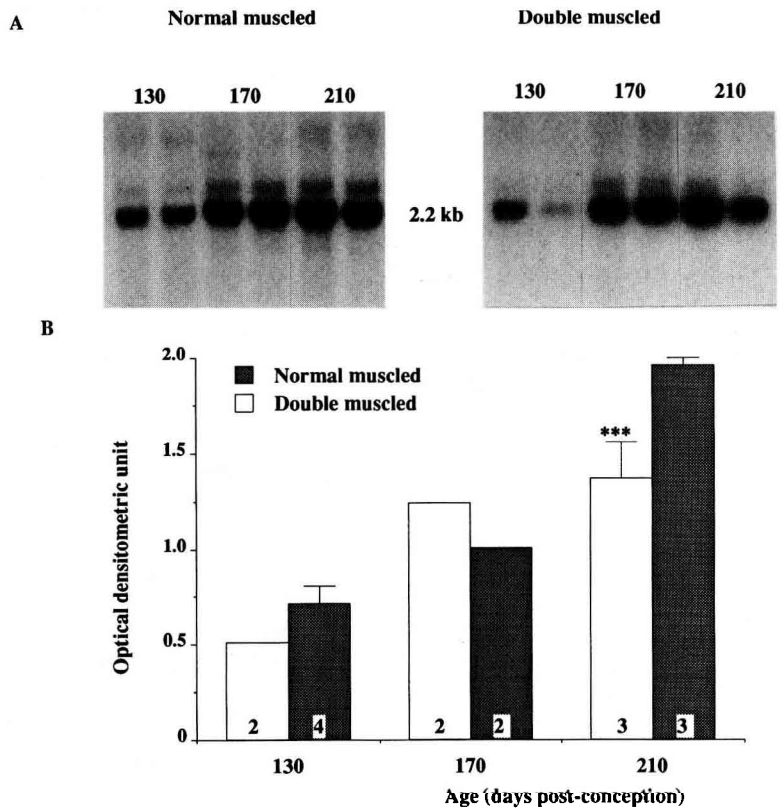

Figure 3. Northern blot analysis of actin gene expression of *Semitendinosus* muscle RNA from normal and double-muscled foetuses (**A**). The same membranes were hybridised with a mouse β-actin cDNA probe. Actin gene expression in muscle samples was quantified by laser densitometric scanning (Image Master System, Pharmacia; **B**). The results are presented as the mean ± SEM of individual determinations (the number of analysed foetuses is indicated near the bars) and expressed in arbitrary densitometric units, taking into account individual values of 18S hybridisation. Small asterisks indicate when the means are significantly different between gestational stages (*, $0.01 < P < 0.1$; ***, $0.001 < P < 0.01$).

($P < 0.001$). This difference in the amount of actin mRNA indicated a delayed differentiation of *Semitendinosus* muscle from double-muscled foetuses (*figure 3B*).

3.3. Foetal IGF-II plasma concentrations

Figure 4 shows the IGF-II levels determined in plasma from normal and double-muscled bovine foetuses. High concentrations of IGF-II existed throughout the gestation. At the earliest times sampled (90 d pc), 400 ng·mL^{-1} IGF-II were present and levels increased to peak values of approximately 1 200 ng·mL^{-1} at 250 d pc. Foetal IGF-II concentration results were analysed by linear curve analysis to test the effect of the genotypes. No significant difference between normal and double-muscled foetuses was found as depicted by the slope of the curves (6.18 versus 3.54, respectively). However, the individual variations of IGF-II amounts in normal foetuses were very low.

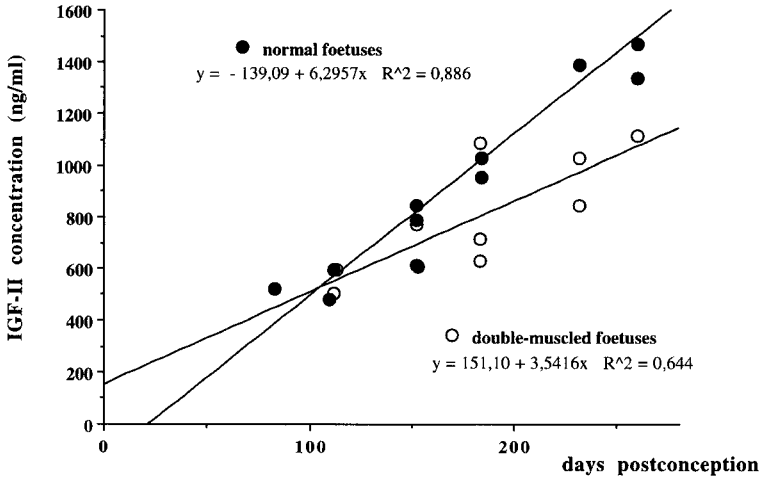

Figure 4. Plasma IGF-II levels throughout gestation were measured in normal and double-muscled foetuses. After separation of IGF-II from their endogenous binding proteins by acidic gel filtration, plasma IGF-II levels were assayed using an IGF-binding protein isolated from human cerebrospinal fluid as described in 'Materials and methods'. Individual determinations were assayed with three different concentrations (each concentration in duplicate). IGF-II profiles are described mathematically by fitting polynomial equations to test the effects of foetal genotypes

4. DISCUSSION

In *Semitendinosus* muscle from normal and double-muscled bovine foetuses, the IGF-II gene is expressed as a family of transcripts, as is the case in other mammalian species [16, 19, 25, 32]. However, during foetal life, the pattern and the relative amounts of IGF-II mRNA expression differ in liver and muscle. For instance, the 3.7 kb mRNA is only detected in foetal muscle but not in foetal liver [4]. These results indicate that bovine IGF-II gene expression exhibits developmental stage and tissue-specific regulation. The IGF-II gene generates multiple mature transcripts with different 5' untranslated regions but identical coding and 3' untranslated regions. The pattern of bovine IGF-II gene expression mimics the complex pattern of IGF-II in humans during development [4]. Strong homologies have been shown to exist between the coding regions of bovine and human genes [18]. Possible analogies between the human and bovine gene structures were also analysed using human IGF-II probes containing various untranslated exons. Thus, specific functions, defined for the different human transcripts, could be attributed to each bovine transcript. Human 4.8 kb mRNA is actively engaged in protein synthesis while human 6.0 kb mRNA exhibits a structured 5' untranslated region that functions as a cis-acting translational attenuator [31]. The bovine 5.1 kb transcript appears to be homologous to the human 6.0 kb transcript, and the bovine 4.0 kb to the human 4.8 kb transcript, respectively [4]. Thus, it may be engaged in protein synthesis of IGF-II. The 3.7 and 2.6 kb transcripts might be translated as prepro-IGF-II peptides (for review see [42]). The bovine 1.7 kb transcript is homologous to the human 1.8 kb transcript, which corresponds to the 3' end of exon 9, but is uncapped and untranslated. It is generated by endonucleolysis of the mature IGF-II transcripts [27, 31].

This study demonstrates the apparent stability of the 5.1 kb transcript expression

throughout the developmental period studied. This stability was more marked in double-muscled foetuses and resulted in a significantly higher amount of the 5.1 kb transcript in double-muscled than in normal foetuses at 210 d pc. Thus, the developmental regulation of the 5.1 kb transcript is distinct in the two genetic types. When high levels of the human 6.0 kb mRNA are found (for example in Wilms' tumours), low levels of IGF-II are detected [15]. Thus, in foetal muscle, the high level of the 5.1 kb transcript observed throughout the gestation should be associated with a low local concentration of IGF-II. Nevertheless, an alternative cap-independent way is known in the picornavirus and has recently been shown by Prats et al. [35] and Vagner et al. [47] during the translation of FGF mRNA in eukaryotic cells. In this case, greater amounts of IGF-II would be present in muscles of double-muscled animals than in those of normal animals. To our knowledge, IGF-II levels in foetal muscle have not been determined. The expression of IGF-II transcripts (except for the 5.1 kb one) decreased greatly throughout gestation (from 90 to 210 d pc) without a significant difference between either genetic types. Our results were not consistent with previously published observations [11]. Using slot-blot analysis, maximum muscle IGF-II gene expression was found to be significantly higher and delayed in double-muscled foetuses compared to normal foetuses (180 versus 150 d pc). The discrepancy between both studies could be due either to a difference between analytical methods (slot blot versus northern blot) or to a difference between standardisation methods (actin probe versus 18S probe). On the contrary, the same pattern of IGF-II gene expression has been observed by in situ hybridisation studies [24]. The IGF-II mRNA were primarily (between 60 and 162 d pc) localised in the developing muscle fiber bundles. After 162 d pc, the IGF-II transcripts started to shift away from muscle fibers and began to localise within the connective tissue. Our data showed that muscle IGF-II mRNA expression was greater during early bovine foetal development as has been observed in sheep [32] and rat [2].

Plasma IGF-II concentrations increased with advancing gestational age. The change in the serum concentrations of IGF-II during foetal development found in this study was similar to that in previous studies and coincides with foetal growth rates [17]. In most species, IGF-II circulates at higher levels than IGF-I in the foetus and is generally thought to be more active than IGF-I in regulating foetal growth [9]. During gestation, a high autocrine expression of the IGF-II gene was associated with a low plasma IGF-II concentration; then, the fall in IGF-II mRNA was associated with increasing levels of IGF-II in the foetal circulation. In vitro, the expression of IGF-II by muscle cells is under tight negative feedback control by the IGFs themselves [26]. Thus, the large increase in IGF-II in foetal plasma observed during gestation could be involved in the decrease of autocrine IGF-II production in bovine *Semitendinosus* muscle cells.

In this study, the high amount of autocrine IGF-II mRNA and low plasma IGF-II concentrations observed may play a role in regulating the development of the first muscle cell population, which was present from 39 d pc and gave rise to type I fibers. Autocrine expression of IGF-II has been shown to contribute to myotube formation in the developing embryo [13, 14] and during muscle regeneration [23]. Antisense oligonucleotides to IGF-II can disrupt embryonic development at very early stages. The second cell generation became differentiated at about 120 d of foetal life and then developed into type II fibers. The differentiation of the second generation is concomitant with low autocrine IGF-II amounts and high circulating IGF-II [39]. There are some conflicting observations between in vivo and in vitro studies. In vitro, several myoblast cell lines express IGF-II and the extent of autocrine expression of IGF-II is correlated with the rate of spontaneous differentiation [9]. In fact, the autocrine expression of IGF-II

stimulates myogenesis in the absence of exogenous IGF-II [44, 45]. A better understanding of the role of IGF-II in bovine foetal development will necessitate further study of changes in somatomedin receptor and binding protein characteristics [20]. Using a β-actin cDNA probe, we studied the pattern of muscular actin gene expression in normal and double-muscled foetuses throughout gestation. At 210 d pc, the actin gene expression was significantly lower in double-muscled than in normal foetuses. This result suggests a delay in the differentiation grade of muscle from double-muscled foetuses. Indeed, Picard et al. [33] have shown by immunocytochemical detection that the pattern of expression of myosin isoforms is different in the two bovine genotypes. They observed more mature myosins in normal than in double-muscled muscle at all gestation stages. At 200 d pc, there was no longer any difference between the two animal types. In addition, they have shown that in vitro primary myoblasts from double-muscled animals differentiated later and were more numerous than those of normal animals when seeded at identical densities [34].

In conclusion, these results suggest a differential regulation of the IGF-II gene transcription between double-muscled and normal foetuses. Since the actin gene expression was lower in double-muscled foetuses, a delay of differentiation between both genetic types was found. Recently, Grobet et al. [12] have identified a deletion in a gene, encoding a myostatin – member of the TGFβ superfamily (GDF8) – which is responsible for the double-muscled phenotype. The deletion leads to the truncation of the bioactive carboxyl terminal peptide. In our study, the disruption of the GDF8 gene did not affect the regulation of muscular IGF-II mRNA expression.

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REFERENCES

[1] Alonso S., Minty A., Bourlet Y., Buckingham M., Comparison of three actin-coding sequences in the mouse, evolutionary relationships between the actin genes of warm-blooded vertebrates, Mol. Evol. 23 (1986) 11–22.

[2] Beck F., Samani N.J., Penschow J.D., Thorley B., Tregear G.W., Coghlan J.P., Histochemical localization of IGF-I and -II mRNA in the developing rat embryo, Development 101 (1987) 175–184.

[3] Binoux M., Lassare C., Gourmelen M., Specific assay for insulin-like growth factor (IGF) II using the IGF binding protein extracted from human cerebrospinal fluid, J. Clin. Endocrinol. Metab. 63 (1986) 1151–1155.

[4] Boulle N., Schneid H., Listrat A., Holthuizen P., Binoux M., Groyer A., Developmental regulation of bovine insulin-like growth factor-II (IGF-II) gene expression: homology between bovine transcripts and human IGF-II exons, J. Mol. Endocrinol. 11 (1993) 117–128.

[5] Brown A.L., Graham D.E., Nissely S.P., Hill D.J., Strain A.J., Rechler M., Developmental regulation of insulin-like Growth factor II mRNA in different rat tissues, J. Biol. Chem. 261 (1986) 13144–13150.

[6] De Chiara T.M., Robertson E.J., Efstratiadis A., Parental imprinting of the mouse insulin-like growth factor II gene, Cell 64 (1991) 849–859.

[7] Dumont B.L., Carcass composition and muscle structure of hypertrophied animals, in: King J.W.B., Ménissier F. (Eds.), Muscular Hypertrophy of Genetic Origin and its Use to Improve Beef Production, Curr. Top. Vet. Anim. Sci. 16, 1982, pp. 111–126.

[8] Florini J.R., Magri K.A., Ewton D.Z., James P.L., Grindstaff K., Rotwein P.S., 'Spontaneous' differentiation of skeletal myoblasts is dependent upon autocrine secretion of insulin-like growth factor, J. Biol. Chem. 24 (1991) 15917–15923.

[9] Florini J.R., Ewton D.Z., Cooligan S.A., Growth hormone and the insulin-like growth factor system in myogenesis, Endocr. Rev. 17 (1996) 481–517.

[10] Gerrard D.E., Judge M.D., Induction of myoblast proliferation in L6 myoblast cultures by foetal serum of double-muscled and normal cattle, J. Anim. Sci. 71 (1993) 1464–1470.

[11] Gerrard D.E., Grant A.L., Insulin-like growth factor-II expression in developing skeletal muscle of double-muscled and normal cattle, Domest. Anim. Endocrinol. 11 (1994) 339–347.

[12] Grobet L., Martin L.J.R., Poncelet D., Pirottin D., Brouwers B., Riquet J., Schoeberlein A., Dunner S., Ménissier F., Massabanda J., Fries R., Hanset R., Georges M., A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle, Nat. Genet. 17 (1997) 71–74.

[13] Han V.K.M., D'Ercole A.J., Lund P.K., Cellular localization of somatomedin (insulin-like growth factor) messenger RNA in the human fœtus, Science 236 (1987) 193–196.

[14] Hannon K., Smith C.K., Bales K., Santerre R., Temporal and quantitative analysis of myogenic regulatory and growth factor gene expression in the developing mouse embryo, Dev. Biol. 151 (1992) 137–144.

[15] Haselbacher G.K., Irminger J.C., Zapf J., Ziegler W.H., Humble R.E., Insulin-like growth factor II in human adrenal pheochromocytomas and Wilm's tumors: expression at the mRNA and protein level, Proc. Natl. Acad. Sci. USA 84 (1987) 1104–1106.

[16] Hedley P.E., Dalin A.M., Engström W., Developmental regulation of insulin-like growth factor II gene expression in the pig, Cell Biol. Int. Rep. 13 (1989) 857–862.

[17] Holland M.D., Hossner K.L., Williams S.E., Wallace C.R., Niswender G.D., Odde K.G., Serum concentrations of insulin-like growth factors and placental lactogen during gestation in cattle, I Fetal profiles, Domest. Anim. Endocrinol. 14 (1997) 231–239.

[18] Honneger A., Humbel R.E., Insulin-like growth factors I and II in fetal and adult bovine serum, J. Biol. Chem. 261 (1986) 569–575.

[19] Irminger J.C., Rosen K.M., Humbel R.E., Villa-Komaroff L., Tissue-specific expression of insulin-like -growth factor II mRNAs with distinct 5' untranslated regions, Biochemistry 84 (1987) 6330–6334.

[20] Jones J.I., Clemmons D.R., Insulin-like growth factors and their binding proteins: biological actions, Endocr. Rev. 16 (1995) 3–34.

[21] LeBouc Y., Noguiez P., Sondermeijer P., Dreyer D., Girard F., Binoux M., A new 5'-non-coding region for human placental insulin-like growth factor II mRNA expression, FEBS Lett. 222 (1987) 181–185.

[22] Lee C.Y., Chung C.S., Simmen F.A., Ontogeny of the porcine insulin-like growth factor system, Mol. Cell. Endocrinol. 93 (1993) 71–80.

[23] Levinovitz A., Jennische E., Oldfords A., Edwall D., Norstedt G., Activation of insulin-like growth factor II expression during skeletal muscle regeneration in the rat: correlation with myotube formation, Mol. Endocrinol. 6 (1992) 1227–1234.

[24] Listrat A., Gerrard D.E., Boulle N., Groyer A., Robelin J., In situ localization of muscle insulin-like growth factor-II mRNA in developing bovine foetuses, J. Endocrinol. 140 (1994) 179–187.

[25] Lund P.K., Moats-Statts B.M., Hynes M.A., Simmons J.G., Jansen M., D'Ercole A.J., Van Wyk J.J., Somatomedin-C/insulin-like growth factor-I and insulin-like growth factor-II mRNA in rat foetal tissues, J. Biol. Chem. 261 (1986) 14539–14544.

[26] Magri K.A., Benedict M.R. Ewton D.Z., Florini J.R., Negative feedback regulation of insulin-like growth factor-II gene expression in differentiating myoblaste in vitro, Endocrinology 135 (1993) 53–62.

[27] Meisma D., Scheper W., Holthuisen P.E., Van den Brande J.L., Sussenbach J.S., Site specific cleavage of IGF-II mRNAs requires sequence elements from two distinct regions of the IGF-II gene, Nucl. Acid Res. 20 (1992) 5003–5009.

[28] Ménissier F., Advantages of using double-muscled sires in crossbreeding and the selection of a specialised double-muscled sire line in France, in: King J.W.B., Ménissier F. (Eds.), Muscle Hypertrophy of Genetic Origin and its Use to Improve Beef Production, Curr. Top. Vet. Anim. Sci. 16, 1982, pp. 480–536.

[29] Milner R.D.G., Hill D.J., Fetal growth control: the role of insulin and related peptides, Clin. Endocrinol. 21 (1984) 415–433.

[30] Moses A.C., Nissley S.P., Short P.A., Rechler M.M., White R.M., Knight A.B., Higa O.Z., Increased levels of multiplication-stimulating activity, an insulin-like growth factor, in foetal rat serum, Proc. Natl. Acad. Sci. USA 77 (1980) 3649–3653.

[31] Nielsen F.C., Christiansen J., Posttranscriptional regulation of insulin-like growth factor II mRNA, Scand. J. Clin. Lab. Invest. 55 (1995) 37–46.

[32] O'Mahoney J.V., Brandon M.R., Adams T.E., Developmental and tissue-specific regulation of ovine insulin-like growth factor II (IGF-II) mRNA expression, Mol. Cell. Endocrinol. 78 (1991) 87–96.

[33] Picard B., Gagnière H., Robelin J., Geay Y., Comparison of the foetal development of muscle in normal and double-muscled cattle, J. Muscle Res. Cell Motil. 16 (1995) 629–639.

[34] Picard B., Gagnière H., Listrat A., Robelin J., Geay Y., Différenciation musculaire au cours du développement foetal et néonatal chez les bovins normaux et culards, Prod. Anim. 9 (1996) 218–219.

[35] Prats A.-C., Vagner S., Prats H., Amalric F., Cis-acting elements involved in the alternative translation initiation process of human basic fibroblast growth factor mRNA, Mol. Cell Biol. 12 (1992) 4796–4805.

[36] Puissant C., Houdebine L., An improvement of the single-step method of RNA isolation by acid guanidinium-thiocyanate-phenol-chloroform extraction, Biotechniques 8 (1990) 148–149.

[37] Quinn L.S., Ong L.D., Roeder R.A., Paracrine control of myoblast proliferation and differentiation by fibroblasts, Dev. Biol. 140 (1990) 8–9.

[38] Raynal F., Michot B., Bachellerie J.P., Complete nucleotide sequence of mouse 18S rRNA gene comparison with other available homologs, FEBS Lett. 167 (1984) 263–269.

[39] Robelin J., Picard B., Listrat A., Jurie C., Barboiron C., Pons F., Geay Y., Myosin transitions in *semitendinosus* muscle during fetal development of the cattle: Immunocytochemical and electrophoretic analyses, Reprod. Nutr. Dev. 33 (1993) 25–41.

[40] Sara V.R., Hall K., The biosynthesis and regulation of foetal somatomedin, in: Ellendorff F. et al. (Eds.), Fetal Endocrinology, Academic Press, New York, 1984, pp. 213–229.

[41] SAS, SAS/STAT guide for personal computer, Version 6 Edition, 1988.

[42] Schofield P.N., Molecular biology of the insulin-like growth factors: gene structure and expression, Acta Paediatr. Scand. (suppl.) 372 (1991) 83–90.

[43] Soares M.B., Turken A., Ishii D., Mills L., Episkopou V., Cotter S., Zeitlin S., Efstratiadis A., Rat insulin-like growth factor-II gene. A single gene with two promoters expressing a multi-transcript family, J. Mol. Biol. 192 (1986) 737–752.

[44] Stewart C.E.H., Rotwein P., Insulin-like growth factor is an autocrine survival factor for differentiating myoblasts, J. Biol. Chem. 271 (1996) 11330–11338.

[45] Stewart C.E.H., James P.L., Fant M.E., Rotwein P., Overexpression of insulin-like growth factor induces accelarated myoblast differentiation, J. Cell. Physiol. 169 (1996) 23–32.

[46] Tollefsen S.E., Sadow J.L., Rotwein P., Coordinate expression of insulin-like growth factor II and its receptor during muscle differentiation, Proc. Natl. Acad. Sci. USA 86 (1989) 1543–1547.

[47] Vagner S., Gensac M.-C., Maret A., Bayard F., Amalric F., Prats H., Prats A.-C., Alternative translation of human fibroblast growth factor 2 mRNA occurs by internal entry of ribosomes, Mol. Cell. Biol. 15 (1995) 35–44.

[48] Vandekerckhove J., Weber K., The complete amino acid sequence of actins from bovin aorta, bovine heart, bovine fast skeletal muscle and rabbit slow skeletal muscle, Differentiation 14 (1979) 123–133.