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Decreased expression of the IGF-II gene during porcine adipose cell differentiation☆

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ABSTRACT

IGF-I and IGF-II are known to regulate cell development and recent data suggest a possible role of IGF-II on adipose tissue development. This study was undertaken to examine the IGF system gene expression in porcine differentiating adipocytes. Both adipocytes and stromal–vascular (s/v) cells were isolated from subcutaneous adipose tissue collected from 7-day-old piglets. s/v cells were cultured in chemically defined medium. Compared with isolated adipocytes, IGF-II and IGFBP-5 mRNA levels were very high in freshly isolated s/v cells, whereas IGF-I mRNA levels were lower in s/v cells than in adipocytes. Between day 0 and day 6 of culture, IGF-II and IGFBP-5 gene expression decreased whereas expression levels of late markers of adipocyte differentiation were up-regulated. Cell differentiation was also associated with an increase in the expression of IGF-I, insulin and IGF receptor genes. The current findings suggest that IGF-I and IGF-II have different effects on porcine adipose cell development.

1. Introduction

Insulin-like growth factor-II (IGF-II) is a single chain polypeptide that belongs to the family of insulin-related peptides, which includes also insulin and IGF-I (Jones and Clemmons, 1995; O'Dell and Day, 1998). IGFs interact with two types of cell surface receptors, type I and type II, that differ in their amino acid sequence, secondary structure and ligand-binding specificity; and they also interact with six high-affinity IGF binding proteins (IGFBP-1 to IGFBP-6; Jones and Clemmons, 1995; Firth and Baxter, 2002). These factors are potent growth regulators that exert biological effects in an *endo-*, *para-* and/or autocrine manner (Jones and Clemmons, 1995). Whereas IGF-II is known to play a key role in placental and fetal growth (Constância et al., 2002; Fowden, 2003), its action during postnatal life is much less documented (O'Dell and Day, 1998; Chao and D'Amore, 2008). An increasing number of studies argue for a possible role of IGF-II on adipose tissue development in mammals other than rodents.

Indirect evidence for effects of IGF-II on adipose tissue growth and development is provided by studies of igf2 gene polymorphism, with igf2 genotype influencing fat deposition in the pig (Nezer et al., 1999). In other studies, adipose tissue and isolated adipocytes have been shown to express IGF-II and other members of the IGF system in the pig (Hausman et al., 2002, 2006; Gardan et al., 2006, 2008). Furthermore, we have found a higher IGF-II mRNA level in isolated intramuscular adipocytes than in subcutaneous or perirenal adipocytes in growing pigs (Gardan et al., 2006). Because intramuscular fat is the latest adipose tissue to develop in the pig (Lee and Kauffman, 1974; Hauser et al., 1997), we hypothesized that IGF-II gene expression may vary with the stage of development of the adipose cells. However, the expression pattern of IGF-II in relation with other components of the IGF system in differentiating adipose cells is still unknown. Therefore, the present study was undertaken to examine the expression of some genes of the IGF system in freshly isolated cells and during differentiation of stromal–vascular (s/v) cells in primary culture.

2. Materials and methods

2.1. Animals and sample collection

Studies were conducted in compliance with the French guidelines for care and use of animals in research. Male crossbred piglets (Pietrain × (Large White × Landrace)) were killed after an overnight fast by exsanguination after electrical stunning at 7 days of age (2.6 ± 0.3 kg body weight, $n = 6$). Subcutaneous dorsal adipose tissue was aseptically removed immediately after death. Samples of 6 g were used for cell preparation.

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Table 1
Primers used for analysis of gene expression by real-time RT-PCR

Gene[a]	Accession number	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)
IGF-I	M31175	GCTGGACCTGAGACCCTCTGT	TACCCTGTGGGCTTGTTGAAAT
IGF-II	X56094	AGGGCATCCAAACCACAAAC	GGGTTCAATTTTTGGTATGTAACTTG
IGFBP-2	AF120326	GCACCTGTACTCCTTGCACATC	CGCTGCCCATTCAGAGACAT
IGFBP-3	AF085482	CATCCCCAACTGCGACAAG	ATCCACGCACCAGCAGAAG
IGFBP-4	BX921877	ATCGAGGCCATCCAGGAAA	CCCCGATGACCTTCATCTTG
IGFBP-5	NM_214099.	CGTGGACAAGTACGGGATGA	CGAAGCTGTGGCACTGGAA
IGF-I R	U58370	CAACCTCCGGCCTTTTACTTT	CAGGAATGTCATCTGCTCCTTCT
IGF-II R	CB469268	TGCCCGGTGAAGAGCAA	TTGTCCCCACACACGATAATGT
Insulin R	AF102858	CAGCGATGTATTTCCATGTTCTGT	GCGTTTCCCTCGTACACCAT
ME	X93016	TGGTGACTGATGGAGAACGTATTC	CAGGATGACAGGCAGACATTCTT
PPARγ	AF103946	ATTCCCGAGAGCTGATCCAA	TGGAACCCCGAGGCTTTAT

[a] IGF-I and IGF-II, insulin-like growth factor-I and -II; IGFBP, insulin-like growth factor binding protein; R, receptor; ME, malic enzyme; PPAR-γ, peroxisome proliferator-activated receptor gamma.

2.2. Cell isolation

Cells were isolated by collagenase digestion as described by De Clercq et al. (1997). Briefly, subcutaneous adipose tissue was minced and digested in a HEPES phosphate buffer (2 mL/g of tissue) containing 2% BSA and 2 mg/mL collagenase II and XI (800 U/mg; Sigma, St-Quentin Fallavier, France) in a shaking water bath for 45 min at 37 °C. Flask contents were filtered through nylon meshes (200 μm). A fivefold excess of buffer containing no collagenase was added, before cells were centrifuged at 400 × *g* for 10 min to separate floating adipocytes from the pellet of s/v cells. Floating adipocytes were collected in DMEM at 37 °C, and then immediately processed for mRNA extraction. The pellet containing adipocyte progenitors is successively filtrated through a 100- and 25-μm nylon mesh. An aliquot (20 μL) of cell suspension was stained with Trypan blue stain, and counted on a hemocytometer. A fraction of s/v cells was then processed for mRNA extraction, whereas the other fraction was cultured.

2.3. Cell culture

s/v cells were seeded into 24-well plates (2 cm^2/well) at a density of 7.5×10^4 cells/cm^2 for morphological measurements, or in 6-well plates at a similar density for gene expression analyses. Cells were maintained in a humidified 5% CO_2 atmosphere for 24 h at 37 °C. Plating medium consisted of Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, Cergy Pontoise, France) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL fungizone, 0.25 μg/mL nystatine, 4 mM L-glutamine, 2.7 nM insulin, 100 nM cortisol. Cells were then cultured in a serum-free medium (DMEM/Ham's Nutrient Mixture F-12 (1:1) (Invitrogen), 100 U/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL fungizone, 0.25 μg/mL nystatine, 50 mM β-mercaptoethanol, 0.1 nM ascorbic acid, 2.5 mM L-glutamine, 10 μg/mL transferrin, 100 nM cortisol, 0.2 nM triiodothyronine). Media were supplemented with either insulin, and/or IGF-I or IGF-II (GroPep, Adelaïde, Australia, 10 nM). The media were then changed every other day. Cell differentiation was assessed by oil red-O and Hemalun Mayer staining (Gerfault et al., 1999). Cell diameters were also determined using a coulter counter (Coulter Multisizer, Beckmann) as previously described (Etherton, 1980). Cells with a diameter below 5 μm were not considered.

2.4. Malic enzyme assay

Serum-free media were replaced with a 0.25 M sucrose solution and cells were then collected. After sonication, homogenates were stored at −70 °C until use. Activ-

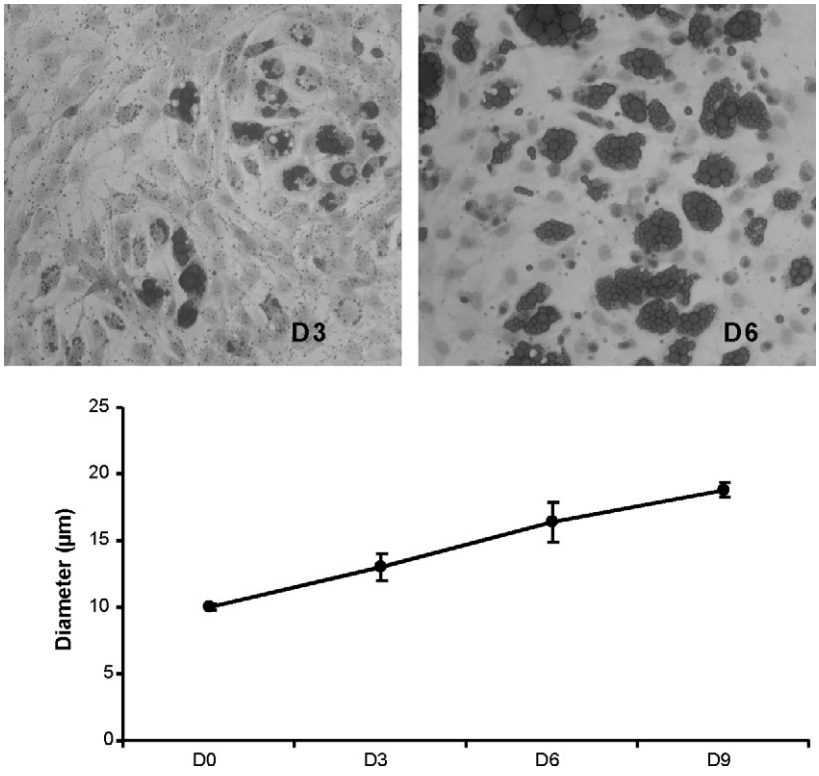

Fig. 1. Adipose cells during differentiation in primary culture. Cells were first cultured in a plating medium and were then cultured from day 1 to day 9 in a defined medium supplemented with insulin (10 nM). (A) Photomicrographs of cultured stromal–vascular (s/v) cells at day 3 and day 6 of culture (original magnification 200×). (B) Mean diameters (μm) were measured at day 0, day 3, day 6 and day 9 as described in Section 2. Data represent means ± S.E.M. of experiments performed in duplicate ($n = 4$).