Feeding daidzein to late pregnant sows influences the estrogen receptor beta and type 1 insulin-like growth factor receptor mRNA expression in newborn piglets

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Abstract

The present study was undertaken to determine the tissue-specific expression of estrogen receptor beta (ERβ), and the effects of a daidzein supplement to the diet of pregnant sows on the expression of ERβ, and type 1 insulin-like growth factor receptor (IGF-1R) genes in newborn piglets by using semi-quantitative RT-PCR. Eight sows received a dietary supplement of daidzein at a dosage of 8 mg per kg feed from day 85 of gestation, and six sows were used as controls. After parturition, 2 male neonatal piglets were selected from each litter for sampling. ERβ mRNA was detected in intestine, lung, thymus, kidney, pituitary and hypothalamus tissues, but not in heart, adrenal, skeletal muscle, liver or placental tissues. Daidzein treatment significantly increased the birth weight of male piglets and markedly reduced the level of ERβ mRNA in the hypothalamus, but not in the pituitary. An up-regulation of IGF-1R gene transcription was observed in skeletal muscles of newborn piglets. In addition, the IGF-1R mRNA was found to be most abundant in pituitary and hypothalamus, followed by skeletal muscle, thymus, and liver tissues in decreasing order. Our results demonstrate that (1) ERβ is expressed in a tissue-specific manner in newborn piglets, (2) daidzein down-regulates ERβ gene expression in the hypothalamus, possibly indicating central effects of daidzein, and (3) daidzein influences fetal growth associated with higher IGF-IR gene expression in skeletal muscle.

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Introduction

Previous studies suggest that plant-derived phytoestrogens, especially isoflavones, are potentially therapeutic compounds for a range of estrogen-dependent diseases, such as breast cancer, menopausal symptoms, cardiovascular disease and osteoporosis (Anderson 1999, Adlercreutz *et al.* 2000). Isoflavones are structurally similar to mammalian endogenous estrogens (Setchell & Cassidy 1999), and thus may act as estrogen agonists or antagonists (Setchell *et al.* 1998). Recent studies suggest that isoflavones act mainly through binding to estrogen receptor beta (ERβ) (Kuiper *et al.* 1998*b*, Casanova *et al.* 1999, Makela *et al.* 1999). ERβ is the second subtype estrogen receptor expressed not only in reproductive organs, but also in hypothalamus, pituitary gland, lung, and other tissues in rats and humans (Kuiper *et al.* 1996, Enmark & Gustafsson 1999). ERβ and ERα have high homology in the DNA binding domain (>95% amino-acid identity), but the homology in the ligand binding domain is relatively low, about 55% (Gustafsson 1999). These structural differences may contribute to the different characteristics in ligand binding affinities, as well as in biological functions (Kuiper *et al.* 1997). It was suggested that ERβ plays important roles in brain function (Gustafsson 1999) mediating estrogen in brain development during embryogenesis (Toran-Allerand 1999). However, whether ERβ is expressed in porcine brain or other tissues is still unclear.

Daidzein, an aglycone, is an isoflavone present in large quantities in soybeans and other legumes. Recently, Liu *et al.* (1999) demonstrated that feeding pregnant sows with a daidzein-supplemented diet improved pre- and postnatal growth in newborn male piglets. However, the mechanisms mediating the effects of daidzein on animal growth are still unclear. Daidzein is a lipophilic substance with a molecular mass of 254 Da, thus it is able to pass through the placental barrier (Adlercreutz *et al.* 1999). It was demonstrated that children in Asia are born with similar plasma levels of phytoestrogens as those of their mothers, indicating a free transfer of these compounds to the fetus. It is well known that insulin-like growth factor-I (IGF-I) and -II (IGF-II) play important roles in fetal growth, and

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they both exhibit growth promoting effects via the type 1 IGF receptor (IGF-1R) (Anthony *et al.* 1995, Gluckman 1997). Evidence exists that estrogen regulates expression of the IGF system *in vitro* and *in vivo*. In rhesus monkey uteri and human breast tissues, the IGF-I and IGF-1R mRNA levels have been shown to be up-regulated by estradiol (Adesanya *et al.* 1996, Clarke *et al.* 1997). It is therefore hypothesized that one of the mechanisms of the daidzein prenatal growth promoting effects might be by regulating the IGF-1R and ERβ at the level of gene transcription. In order to test our hypothesis, we used RT-PCR to investigate the distribution of ERβ in pig non-reproductive organs, and examined the effects of daidzein on the mRNA levels of the ERβ and IGF-1R gene in different tissues of newborn piglets.

Materials and Methods

Animals and feeding

All animals were cared for according to guidelines set by the Animal Protection Committee from the Ministry for Agriculture and Nature Protection, Schwerin, Germany. Sixteen multiparity sows (German Landrace, Pig Breeding Association North/Eastern, Germany) were mated to one German Landrace boar. Sow pregnancy was confirmed at day 28 of gestation by ultrasound. Sows had live weights of 180 ± 7 kg, backfat depths of 19 ± 2 mm (Piglog 105, SFK Technology A/S, Herlev, Denmark) and parities of 3·0 ± 0·3. Sows were divided into experimental (n=8) and control (n=8) groups with balanced weight, backfat depth and parity. However, during the experiment two control sows were excluded due to skeletal system illness and premature farrowing respectively. The sows were housed individually at the pig experimental station of the Research Institute, under controlled environmental conditions (19 °C, 60–80% relative humidity). All sows were fed twice daily with the same commercial pregnancy diet (Denkavit, Trede & Pein GmbH & Co. KG, Itzehohe, Germany) containing 11·8 MJ metabolizable energy per kg dry matter and 14·0% crude protein. All animals had free access to water. Throughout pregnancy, sows were fed manually corresponding to the following schedule: a daily feed ration of 2·6 kg at the beginning of the pregnancy was increased gradually to 5 kg at the end of the pregnancy. This schedule guaranteed an enhanced nutritional supply to the sows during pregnancy. From day 85 of gestation until parturition the ration of the experimental sows was supplemented with 8 mg daidzein per kg feed. Live weights, backfat depths, and sow parities were not significantly different between the two groups at day 85 of gestation. To induce farrowing, on day 114 of pregnancy all sows were injected intramuscularly with 1 ml of a synthetic prostaglandin analog (cloprostenol, 75 mg/ml: AniMedica West, Chemische Produkte GmbH, Senden, Germany).

Sample collection

After sow parturition, body weight and sex of newborn piglets were recorded. Two male piglets with body weights close to the mean were selected from each litter. Within 6 h after birth, daidzein piglets (n=16) and control piglets (n=12), were anesthetized with 1 ml of a mixture (v:v=1:1) of ursotamin and combelen and were then killed. Brains were removed immediately from the skull and the pituitary glands were collected. Boundaries used for dissecting the hypothalamus were as follows: rostral edge of the optic chiasm, immediate rostral to the mamillary body, width of the optic chiasm. The thymus, liver, *longissimus dorsi* muscle, heart (ventricle), small intestine (ileum), lung, adrenal gland and kidney (cortex) were also collected. Additionally, after parturition fresh placental tissue (endometrium) was collected. All samples were immediately frozen in liquid nitrogen and stored at − 70 °C until RNA isolation.

RNA extraction, cDNA synthesis, and polymerase chain reaction

Total RNAs were isolated from tissue samples using the RNeasy mini kit (QIAGEN, Atsworth, CA, USA) according to the manufacturer's instructions. RNA yields and purities were assessed by absorbance at 260 and 280 nm in a RNA/DNA Calculator (Amersham Pharmacia Biotech Europe, Freiburg, Germany). Ratios of absorption (260/280 nm) of all preparations were between 1·8 and 2·0. Aliquots of RNA samples were subjected to electrophoresis to verify their integrity.

The cDNA was synthesized using 1·0 μg total RNA from each sample. RNA samples were denatured at 65 °C for 15 min and placed on ice for 5 min before reverse transcription (RT). The final reaction volume, 25 μl, contained 1 × reaction buffer, 5 mM $MgCl_2$, 1 mM dNTPs, 3·2 μg random primer $p(dN)_6$, 50 units RNase inhibitor, 0·01 mg/ml gelatin and 20 units AMV reverse transcriptase (1st strand cDNA synthesis kit, Boehringer Mannheim Corp., Indianapolis, IN, USA). The reaction was performed at 25 °C for 10 min, 42 °C for 60 min, 99 °C for 5 min for enzyme heat inactivation, and 4 °C for 5 min. RT products were either stored at − 20 °C or used directly for PCR. To eliminate residual genomic DNA from the RNA sample, prior to the RT reaction, 1 unit DNaseI (Roche Diagnostics, Mannheim, Germany) was added and incubated at 37 °C for 30 min followed by heat-inactivation of the enzyme at 75 °C for 5 min (Huang *et al.* 1996). Genomic DNA amplification contamination was checked periodically by control experiments in which reverse transcriptase (positive control) or RNA (negative control) were omitted during the RT step.

PCR was performed in a 50 μl reaction volume containing 2·0 μl tissue-specific cDNA (equivalent to 80 ng of starting RNA), 1·5 mM $MgCl_2$, 2 units Taq DNA

polymerase (Boehringer Mannheim GmbH, Mannheim, Germany), 0·2 mM dNTPs and 0·4 μM of each primer. For amplification of the target genes, the following primer pairs were used: ERβ (GeneBank accession no. AF164957) forward 5′-TCTCCTGTCTCCTACAACT GCA and reverse 5′-GGCATCCCTCTTTGAACTT GGA, for amplification of a 396-bp fragment of pig ERβ cDNA; IGF-1R (GeneBank accession no. AB003362) forward 5′-CCCAAAGTCTGTGAGGAAGAGA and reverse 5′-TTAGTCCCTGTCACTTCCTC-CA, for amplification of a 422-bp fragment of pig IGF-1R cDNA. Additional reactions were run using primers for β-actin (GeneBank accession no. U07786) forward 5′-GGAGA TCGT GCGGGACATCAAG and reverse 5′-GGCGTA GAGGTCCTTCCTGATG, to serve as a control. These primers amplify a 269-bp fragment of pig β-actin cDNA. To obtain optimal conditions for amplification, in the exponential phase of PCR the cycle numbers were tested first for each target gene. Plotting PCR signal intensity (as expressed by net intensity) against the number of amplification cycles revealed a strong linear relationship between cycles 32 and 40 for ERβ (correlation coefficient r^2=0·996), and between cycles 24 and 32 (r^2=0·983) for IGF-1R.

QuantumRNA 18S primer and competimer (Ambion, Inc. Austin, TX, USA) were used as internal controls of amplification. This primer pair (catalog no. 1716) amplifies a 488-bp fragment. The ratio of 18S primer to competimer was 1:9 for ERβ and IGF-1R genes. Amplifications were performed in a Biometra Personal Cycler (Biomedizinische Analytik GmbH, Göttingen, Germany). For ERβ the following cycle parameters were used: 120 s at 94 °C, 36 cycles at 94 °C for 40 s, 60 °C for 50 s, 72 °C for 40 s. For IGF-1R, we used 28 cycles at 94 °C for 40 s, 57 °C for 45 s, 72 °C for 45 s. Each reaction was followed by 5 min at 72 °C and continuous hold at 4 °C. After amplification, 10 μl of each PCR product were analyzed by agarose gel electrophoresis (2%).

Quantitation of PCR products (image analysis)

Gels were stained with ethidium bromide and photographed with a 3-CCD color camera using an image analysis system (Quantimet 570, Leica Cambridge Ltd, Cambridge, UK). Net intensities of individual bands (same area) were measured using Kodak Digital Science 1D software (Eastman Kodak Company, Rochester, NY, USA). Ratios of net intensity of target genes to that of the internal control bands (QuantumRNA 18S) were calculated before statistical analysis. To minimize the between-assay error, samples from two groups were always processed in parallel.

Statistical analysis

All values are reported as means ± S.E.M. Data were analyzed by ANOVA (STATISTICA program V5·0, StatSoft Inc., Tulsa, OK, USA) using a mixed model. For piglet performance data, treatment and piglet gender were employed as fixed factors, sow as a random factor and litter size as a covariant factor. Tissue mRNA levels were processed with the same model including tissue as a fixed factor, without including gender. Means were compared by Tukey HSD (honest significant difference) unequal number multiple comparison test. Student's *t*-test was used in the analysis of unpaired data.

Table 1 Pregnancy characteristics in the daidzein group (sows suppplemented with 8 mg daidzein per kg feed) compared with the control group. Values are means ± S.E.M.

	Daidzein	Control	Significance
Parameter			
No. of sows	8	6	
No. of piglets/litter	13·3 ± 1·0	14·7 ± 1·2	ns
Birth weight (kg)			
Male	1·31 ± 0·04	1·17 ± 0·04	P<0·05
Female	1·25 ± 0·03	1·17 ± 0·04	ns
Survival (%)	98·35 ± 1·75	90·43 ± 1·87	P<0·05

ns, not significant.

Results

Body weights of newborn piglets and percentage of survival

The birth weight of male piglets in the daidzein-treated group was significantly higher (P<0·05) than that of the control group. However, female piglets were not found to have different birth weights. Sows fed with daidzein had a higher (P<0·05) percentage of survival than that of control sows (Table 1).

Tissue distribution of ERβ mRNA in newborn piglets

Using RT-PCR, ERβ mRNA was detected in intestine, lung, thymus, kidney, pituitary and hypothalamus, but not in heart, adrenal gland, skeletal muscle, liver or placental tissues. The highest expression of ERβ mRNA was found in the hypothalamus. This was followed closely by the pituitary and the kidney which expressed a moderate level of ERβ mRNA, while expression in intestine, lung and thymus tissues was quite low (Fig. 1).

Effects of daidzein on ERβ mRNA levels in hypothalamus and pituitary tissues

Relative mRNA levels of ERβ in the hypothalamus and the pituitary were measured by semi-quantitative RT-PCR. Compared with the control group, ERβ mRNA levels in the hypothalamus were markedly reduced in the daidzein group (P<0·05) (Fig. 2A,C).

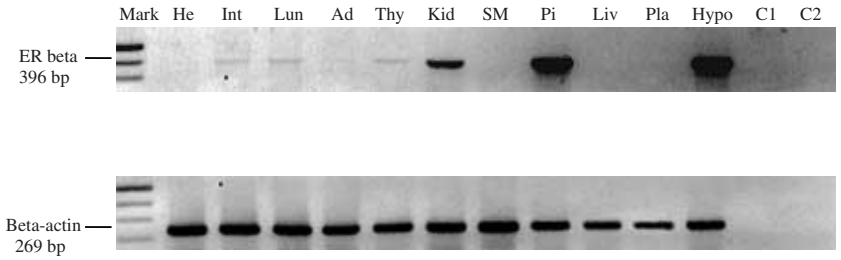

Figure 1 Tissue distribution of ERβ gene expression in newborn piglets. Qualitative RT-PCR was performed on 2 μg total RNA from newborn piglets using primers specific for pig ERβ transcripts (GeneBank accession no. AF164957), cDNA (3 μl) for PCR amplification, cycle number 38; other parameters are the same as those of quantitative RT-PCR presented in the Materials and Methods section. Gel electrophoresis (2%) shows RT-PCR products of the ERβ gene in intestine (Int), lung (Lun), thymus (Thy), kidney (Kid), pituitary (Pi) and hypothalamus (Hypo). However, no RT-PCR products are present in heart (He), adrenal gland (Ad), skeletal muscle (SM), liver (Liv), or placental (Pla) tissues. RT-PCR for β-actin was carried out as a positive control. C1 and C2 are negative controls where reverse transcriptase and RNA respectively were omitted during the RT step.

However, in pituitary tissue no differences were found between the two groups (Fig. 2B,C). In the daidzein group, expression of ERβ mRNA was lower ($P<0{\cdot}05$) in the hypothalamus compared with the pituitary, whereas in the control group there was no noticeable difference between these two tissues.

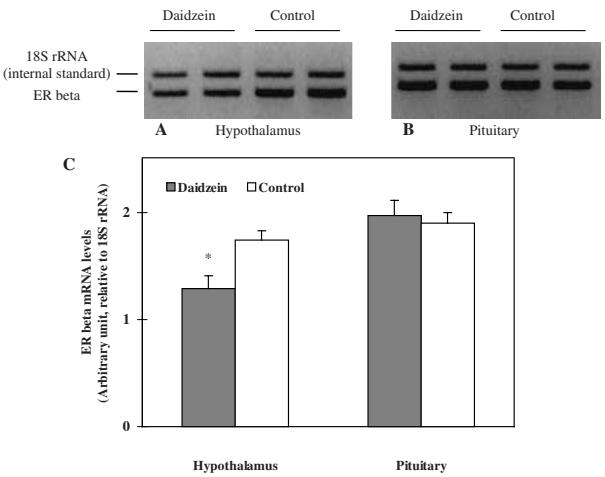

Figure 2 Effects of daidzein feeding in late pregnant sows on ERβ gene expression of newborn piglets. Gel electrophoresis (2%) shows RT-PCR products of ERβ from (A) hypothalamus and (B) pituitary tissues in two groups compared with 18S ribosomal RNA (internal standard). (C) Relative levels of ERβ mRNA – e.g. RT-PCR products of ERβ in hypothalamus and pituitary tissues in the daidzein ($n=16$) and control ($n=12$) groups were normalized with the internal standard; every sample was measured in duplicate. Expression of ERβ was significantly ($P<0{\cdot}05$) reduced in the hypothalamus in the daidzein group compared with that of the control group, but was unchanged in the pituitary. Levels are given as means ± S.E.M. *$P<0{\cdot}05$ vs levels from corresponding control (ANOVA followed by Tukey HSD unequal number multiple comparison test).

IGF-1R mRNA levels in newborn piglet tissues

The expression of IGF-1R in different tissues of newborn piglets in the daidzein and control groups is presented in Fig. 3. Compared with the control group, relative muscle IGF-1R mRNA levels in the daidzein-fed group were significantly higher ($P<0{\cdot}05$). IGF-1R mRNA levels in thymus and liver tissues increased in the daidzein group ($P=0{\cdot}08$ and $P=0{\cdot}09$ respectively), while no significant changes were detected in the hypothalamus or the pituitary. In addition, relative mRNA levels of IGF-1R gene were highest in the hypothalamus and pituitary, moderate in skeletal muscle and thymus, and were lowest in the liver.

Discussion

Since the discovery of the ERβ subtype by Kuiper *et al.* (1996), many studies have shown that this receptor is expressed in multiple rat, mouse, and human tissues, in addition to the reproductive system tissues (Kuiper *et al.* 1998*a*, Casanova *et al.* 1999). These discoveries have improved our knowledge of the mechanism of action of estrogen and its analogs. However, information about ERβ distribution in pigs is limited. Here, we have shown that there is ERβ mRNA expression in hypothalamus and pituitary tissues of newborn piglets. High amounts of ERβ transcripts were previously found in the pituitary of the pre-pubertal rat (Mitchner *et al.* 1998, Wilson *et al.* 1998), human (Shupnik *et al.* 1998), and rhesus monkey (Pau *et al.* 1998), whereas levels in mouse pituitary appeared to be low or nearly undetectable (Couse *et al.* 1997). It has been reported that the expression of ERβ is high in rat and human fetal hearts (Saunders 1998). In the present experiment we failed to detect ERβ mRNA in the heart of newborn piglets. Levels of ERβ expression in fetal human

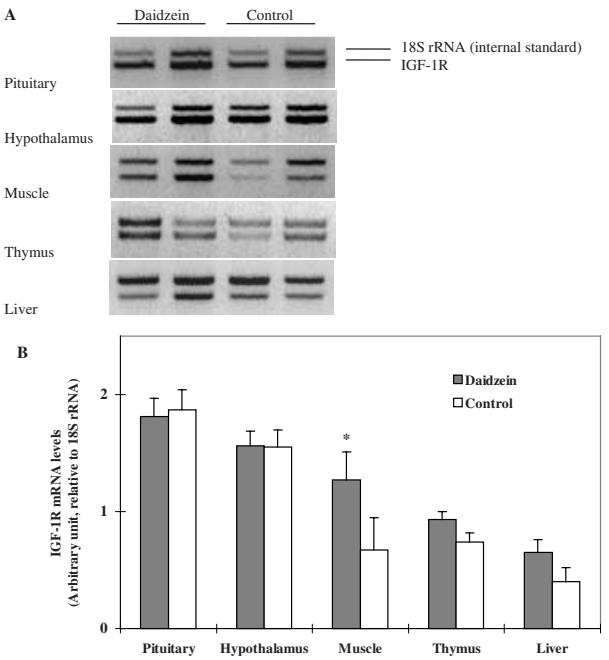

Figure 3 Expression of IGF-1R in different tissues of newborn piglets in the daidzein and control groups. (A) Gel electrophoresis (2%) shows RT-PCR products of IGF-1R from different tissues in two groups compared with 18S ribosomal RNA (internal standard). (B) Semi-quantitative RT-PCR of IGF-1R mRNA (duplicate representative tests) – e.g. RT-PCR products of IGF-1R of each tissue in the daidzein ($n=16$) and control ($n=12$) groups were normalized with internal standard. Compared with those of the control group, relative IGF-1R mRNA levels in muscle were significantly enhanced ($P<0{\cdot}05$), and IGF-1R mRNA levels of thymus and liver were inclined to increase in the daidzein group ($P<0{\cdot}1$). No significant changes were detected in hypothalamus or pituitary tissues. However, expression of IGF-1R gene was found to be highest in the hypothalamus and the pituitary, modest in muscle and thymus, and lowest in the liver. Levels are reported as means ± S.E.M. *$P<0{\cdot}05$ vs levels from corresponding control (ANOVA followed by Tukey HSD unequal number multiple comparison test).

and rat kidneys are low or undetectable (Brandenberger *et al.* 1997), but we detected moderate expression in newborn piglets. ERβ signals detected in the thymus and the intestinal tract of newborn piglets were very weak; however very high expression in humans and rats has been reported (Enmark & Gustafsson 1999). High expression has been reported in the adrenal gland of human fetuses (Brandenberger *et al.* 1997). In our experiment, ERβ expression in the adrenal gland was not detected in newborn piglets which is in accord with results reported from prepubertal rats (Saunders 1998). No ERβ transcripts have been found in rat or human livers (Enmark & Gustafsson 1999). Using RT-PCR, mRNAs of ERβ in skeletal muscle, liver and placental tissue of newborn piglets were also undetectable in our experiment. Expression of the ERβ gene thus appears to be drastically different among tissues and species.

Since the hypothalamus and pituitary expressed the most abundant ERβ mRNA, we chose these two types of tissue to try and elucidate further the effects of daidzein. The results of ERβ expression in the pituitary are similar to those reported by Mitchner *et al.* (1998) who demonstrated that estradiol did not alter the expression of ERα or ERβ in rat pituitaries. However, observations by Schreihofer *et al.* (2000) showed that ERβ mRNA levels fell 40% on the morning of proestrus and were suppressed by estradiol or dihydrotestosterone in ovariectomized female rat pituitaries. Schreihofer *et al.* (2000) also reported that progesterone or progesterone plus estradiol suppressed ERβ gene expression in rodent pituitary cell lines, yet the relative levels of ERβ mRNA in testes were increased after neonatal estrogen exposure (Tena-Sempere *et al.* 2000). Very recently, it was found that daidzein is capable of significantly down-regulating the androgen receptor and the ERα mRNA expression in rat uteri (Diel *et al.* 2000). Data accumulated so far suggest that the effects of estrogen or its agonists on ER gene expression are tissue-specific and vary in different species. Our results demonstrate that daidzein regulates ERβ expression in a tissue-specific manner at the transcription level. The expression of ERβ in the hypothalamus was significantly down-regulated by daidzein, suggesting a direct effect of daidzein acting as an estrogen agonist on the hypothalamus–pituitary neuroendocrine functions. Dietary genistein exerted estrogenic effects upon the hypothalamic–pituitary axis in rats, increased plasma prolactin (Santell *et al.* 1997), and enhanced growth hormone (GH) release in rat anterior pituitary cells (Ogiwara *et al.* 1997). The serum levels of luteinizing hormone and prolactin in the pig were increased by supplemented daidzein feeding (Liu *et al.*1999).

Both *in vitro* and *in vivo* experiments have shown that exogenous estrogens enhance cell (Lee *et al.* 1999) and tissue (Klotz *et al.* 2000) proliferation and growth. The growth promoting effect of exogenous estrogen in domestic animal production has been known for a long time (Lamming 1957). The exact mechanism of this phenomenon is still unclear, but it is partially correlated with estrogen modulating growth-axis functions. Research indicates that exogenous estrogen augments serum concentrations of GH in cattle (Breier *et al.* 1988) and sheep (Phelps *et al.* 1988), and of IGF-I in cattle (Coxam *et al.* 1990). Zeranol, a synthetic estrogen used for stimulating growth in domestic animals, was found to increase serum concentrations of IGF-I in lambs (Hufstedler *et al.* 1996), and GH gene expression in pituitaries of wethers (Thomas *et al.* 2000). Our results, together with previous findings (Liu *et al.* 1999) suggest that daidzein improves male fetal growth, while no significant influence was observed in female newborn piglets. Wang *et al.* (1995) found that daidzein injected subcutaneously into rats increased male rat growth and was attended by higher growth hormone levels; the results in the female rats were the opposite.

Daidzein has demostrated both agonistic and antagonistic effects. At low plasma estrogen levels, daidzein acted agonistically, while at high plasma levels it acted antagonistically (Setchell & Cassidy 1999). Recently, it was demonstrated that estrogen is important for male animal growth (Vanderschueren *et al.* 1997, Sharpe 1998, Toran-Allerand *et al.* 1999). Results from our study and others (Wang *et al.* 1995, Liu *et al.* 1999) show that daidzein may act agonistically in males by promoting fetal growth, while potentially acting antagonistically in female piglets.

It is clear that both IGF-I and IGF-II play major roles in controlling the growth of skeletal muscles (Florini *et al.* 1996). *In vitro* (Ewton *et al.* 1987) and *in vivo* (Yu & Czech 1984) studies demonstrated that IGF-1R mediates several anabolic actions of IGF-I and IGF-II, including stimulation of amino acid uptake, proliferation and differentiation in skeletal muscle. Results indicate a possible association between higher birth weight and daidzein-enhanced IGF-1R gene expression in skeletal muscle. However, we did not detect ERβ in newborn piglet muscle. ERα but not ERβ has been proven to mediate effects of estrogen in the skeleton of male mice during growth and maturation (Vidal *et al.* 2000). Therefore, it is reasonable to assume that daidzein-influenced muscle IGF-1R gene expression might be effected via ERα.

In rat hypothalamus, Pons *et al.* (1991) found that IGF-1R was highest during the fetal phase and steadily decreased thereafter to low levels in adult rats. Our results support this observation. Results suggest an important role for IGF-I in the growth and differentiation of the brain in the fetus (D'Ercole *et al.* 1996), and are consistent with phenomena observed in postnatal brain growth retardation. In breast and uterus tissues, IGF-I and IGF-1R levels were increased by estradiol or combinations of estradiol and progesterone (Clarke *et al.* 1997). In rat hypothalamic cell cultures, Pons & Torres-Aleman (1993) found that the addition of 17β-estradiol elicited a significant increase in type-1 IGF receptor protein levels in neurons. In the present study, IGF-1R mRNA levels in the hypothalamus and pituitary were unchanged after daidzein feeding. However, the IGF-1R mRNA levels in skeletal muscle were up-regulated, and the IGF-1R mRNA levels were increased in liver and thymus tissues. These results suggest that estrogen and/or other agonists regulate IGF-1R levels also in a tissue-specific manner and indicate that the IGF system may be a critical regulator of estrogen-mediated growth (Klotz *et al.* 2000).

To our knowledge, this is the first study to investigate the distribution of ERβ in pigs, and is also the first study to provide *in vivo* evidence that daidzein inhibits ERβ gene expression in the hypothalamus of pigs. Knowledge about the expression levels of ERβ in different tissues may be valuable for re-evaluating mechanisms of action for estrogen agonists and antagonists in a tissue-specific manner. Our results suggest that one way daidzein may influence fetal growth is via up-regulation of IGF-1R expression in skeletal muscle. The down-regulation of ERβ gene expression in the hypothalamus indicates the possible central effects of daidzein on the neuroendocrine system.

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