Relationships of Concentrations of Endocrine Factors at Antemortem and Postmortem Periods to Carcass Weight and Backfat Thickness in Pigs

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ABSTRACT : Carcass weight and backfat thickness are two of important elements in determining the carcass trait in pigs and are studied on animal genetics, nutrition, and endocrinology. Growth factors stimulate or inhibit the proliferation and differentiation of various cells. In particular, insulin-like growth factors (IGFs), transforming growth factor (TGF)-β, and epidermal growth factor (EGF) are involved in the growth and maintenance of muscle. Also, dehydroepiandrosterone-sulfate (DHEA-S) and cortisol are known to be related to the obesity and subcutaneous fat depth in pigs. Therefore, this study was performed to relate growth factors (IGFs, TGF-β1, and EGF) and hormones (cortisol and DHEA-S) concentrations at antemortem and postmortem periods to carcass traits including carcass weight and backfat thickness. Blood and *m. Longissimus* were collected in pigs at antemortem (30 days before slaughter) and postmortem periods. After slaughtered, carcass weight and backfat thickness were measured. Growth factors and hormones in serum and *m. Longissimus* were measured by radioimmunoassay or enzyme-linked imuunosorbent assay. Before antemortem period, serum IGF-I and -II concentrations were positively correlated with the carcass weight and backfat thickness in gilts, and the concentrations of TGF-β1 and cortisol in barrows show the correlation with only carcass weight. Also, the positive correlations of muscular IGFs and TGF-β1 at postmortem 45 min with the carcass weight and backfat thickness were detected. Consequently, these results suggest that the serum and muscular endocrine factors are involved in the carcass weight and backfat thickness in pigs. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 3 : 335-341)***

Key Words : Endocrine Factor, Growth Factor, Carcass Weight, Backfat Thickness, Pigs

INTRODUCTION

Endocrine factors comprised of growth factors, cytokines, and hormones, affect several aspects of animal metabolism and physiology. Among them, growth factors are multifunctional proteins that regulate cell division, differentiation, and function by auto/paracrine and endocrine manners. Insulin-like growth factors (IGFs) interact with IGF receptor and IGF binding proteins (IGFBPs) and modulate proliferation and differentiation of several cell types (Jones and Clemmons, 1995). In myoblast, IGF stimulated myofiber hypertrophy and increased myofiber diameter (Florini et al., 1996). Especially, McGuinness and Cogburn (1990) have reported that circulating IGF-I is highly correlated with relative growth rate. Transforming growth factor (TGF)-β is the disulfide-linked homodimer protein that influences wide range of biological processes, such as stimulation or inhibition of cellular replication and differentiation depending on cell types. In muscle cells, TGF-β inhibits fusion, elevation of creatine kinase, and differentiation (Florini et al., 1988; Zappelli et al., 1996). Epidermal growth factor (EGF) is an acidic polypeptide that has strong mitogenic activity for various cell types. Peng et al. (1997) have reported that EGF is highly involved in the growth and maintenance of skeletal muscle. Among hormones affecting muscle development, dehydro-epiandrosterone-sulfate (DHEA-S) and cortisol are reportedly related to anti-obesity effect and subcutaneous fat depth (Clore, 1995; Wise et al., 1995)

In pigs, the carcass weight and fat thickness have been studied for various respects of animal breeding and feeding management since they are important factors for determining the carcass trait (Corino et al., 1999; O'Quinn et al., 2000; Szabo et al., 2001). In addition, we have recently reported the measurement and relationship of cytokine concentration in day 150 male pigs and suggested the possibility of cytokine as marker for carcass trait in pigs (Yoon et al., 2001). Based on those, this study was performed to relate endocrine factors (growth factors and hormones) concentrations in serum and muscle to carcass traits including carcass weight and backfat thickness and to investigate the possibility of endocrine factors as indicator for the selection of carcass traits in pigs

MATERIALS AND METHODS

Animals

Blood from jugular vein was collected in day 150 pigs (three-way cross breed ; Duroc×Yorkshire×Landrace; 30 barrows and 28 gilts) at antemortem (30 day before slaughter) and postmortem periods. Pigs slaughtered by electrical stimulation were weighed and the backfat thickness was measured. The *m. Longissimus* was removed from pigs

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and stored at -80°C. Sera were made by allowing blood samples to stand at room temperature for 1-2 h followed by centrifugation at 1,000×g for 30 min and aliquots were stored at -70°C until use.

Protein extraction

The *m. longissimus* was mixed with pre-chilled lysis solution [0.1 M sodium phosphate, pH 7.2, 1 mM phenylmethylsulfonylfluoride, 10 mM EDTA, 10 mM EGTA, 10 mM NaF, 1% (w/v) sodium deoxycholate, 1% (v/v) Triton X-100, 0.1% (w/v) SDS] containing 200 kallikrein inhibitor units of aprotinin/ml) (Burr et al., 1980). The mixture was chapped, incubated for 30 min on ice, and centrifuged at 10,000×g for 15 min at -4°C. The supernatant was stored at -70°C until use.

IGFs rdioimmunoassay (RIA)

The recombinant human IGF-I and -II (GroPep, Pty Ltd., Australia) was iodinated by the chloramine T method (Lee and Henricks, 1990). Iodinated IGFs were purified on a Sephadex G-50 column and aliquots were stored at -20°C until used.

Serum concentrations of IGF-I and -II were determined by RIA (Daughaday et al., 1980) with minor modifications (Yun et al., 2000). The assay employed anti-human IGF-I polyclonal antiserum (GroPep, Pty Ltd., Australia) or anti-human IGF-II polyclonal antiserum (GroPep, Pty Ltd., Australia). The intra- and inter-assay variation of IGF-I RIA were 9.0% and 13.4%, and the assay in IGF-II had an intra-assay variation of 7.5% and inter-assay variation of 14.7%.

Concentrations of srum TGF-β1 and EGF

Serum TGF-β1 was determined by TGF-β1 Emax ImmunoAssay System (Promega Co., USA). To measure the active form of TGF-β1, serum was diluted with DPBS. Then, 1 μl of HCl was added to 50 μl of diluted sample and the mixture was incubated for 15 min at room temperature.

After incubation, 1 μl of NaOH was added and the neutralized mixture was used for the assay. Flat-bottom 96 well plates were coated with TGF-β1 coat monoclonal antibody (mAb) and the captured TGF-β1 was bound by a specific second polyclonal antibody. The amount of specifically bound polyclonal antibody (pAb) was detected using a species-specific antibody conjugated to horseradish peroxidase.

Serum EGF concentration was analyzed by the quantitative sandwich enzyme immunoassay technique. A mAb (R&D Systems Inc., USA) specific for EGF was coated onto a microplate. Standards and samples were pipetted into the wells and any EGF present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked pAb specific for EGF was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution that blended hydrogen peroxide with chromogen was added to the wells and color was developed in proportion to the amount of EGF bound in the initial step. The color development was stopped and the intensity of the colors was measured at 450 nm.

Concentrations of hormones

Serum cortisol concentration was analyzed by solid-phase radioimmunoassay using a Coat-A-Count Cortisol kit (Diagnostic Products Co., USA), wherein ^{125}I-labeled cortisol competes for a fixed time with cortisol in the sample for antibody sites. Because the antibody was immobilized to the wall of a polypropylene tube, simply decanting the supernatant suffices to terminate the competition and to isolate the antibody-bound fraction of the radiolabelled cortisol. The tubes were counted for radioactivity.

Serum DHEA-S levels were measured by using RIA kit (Radim Inc., Italia) according to standard procedure based upon a competition between labeled antigens and non-labeled antigens for binding to a limited amount of specific sites on the antiserum coated tubes. After the incubation, the liquid in the tubes was removed by aspiration and the radioactivity is measured in a gamma counter.

Statistical analysis

Data were analyzed using one-way ANOVA and Pearson's correlation coefficients in SAS package (1995).

RESULTS

General performance and endocrine factor concentrations in pigs

Carcass weight, backfat thickness, growth factors and hormones concentrations of barrows and gilts were measured in antemortem and postmortem periods. Although any difference in carcass weight between barrows and gilts was not detected, backfat thickness in barrows was significantly higher than that in gilts (27.03±4.85 vs

Table 1. Means of carcass weight and backfat thickness in pigs (Mean±SD)

Sex	No.of pigs	Carcass weight(kg)	Backfat thickness (mm)
Barrow	30	82.00±8.53	27.03±4.85 [a]
Gilt	28	80.79±9.00	21.11±4.92 [b]

[a,b] Mean±SD within a row with different superscripts differ ($p<0.01$).

21.11±4.92 mm,

Table 2. Concentrations of serum growth factors and hormones at antemortem and postmortem periods

Factors		Antemortem	Postmortem
IGF-I (ng/ml)	Barrows (n=30)	311.7±155.97	268.7±179.88
	Gilts (n=28)	274.9±140.62	257.8±138.92
IGF-II (ng/ml)	Barrows	367.7± 81.37	378.0±70.46
	Gilts	367.7±91.75[a]	501.2±210.76[b]
EGF (pg/ml)	Barrows	ND	ND
	Gilts	ND	ND
TGF-β1 (ng/ml)	Barrows	174.2±62.08	163.7±56.63
	Gilts	172.5±49.41	175.1±42.68
Cortisol (ng/ml)	Barrows	51.86±24.45[c]	129.46±55.34[d]
	Gilts	40.40±29.65[c]	116.58±49.55[d]
DHEA-S (ng/ml)	Barrows	11.73±13.85	11.98±11.69
	Gilts	13.46±11.71	16.18±11.91

[a,b] Mean±SD within a row with different superscripts differ ($p<0.05$).
[c, d] Mean±SD within a row with different superscripts differ ($p<0.01$).
ND: Not detected

$p<0.01$; Table 1). Concentrations of serum growth factors and hormones in barrow and gilts were described in Table 2 and 3. Generally, although significant differences in serum IGF-II and cortiosol concentrations of gilts between antemortem and postmortem periods were detected (IGF-II: 367.7 vs 501.2 ng/ml, $p<0.05$; cortisol: 40.40 vs 116.58 ng/ml, $p<0.01$), other growth factor and hormone concentrations between antemortem and postmortem periods were not different. Also, muscle IGF-II concentration at 45 min postmortem was higher than that at 24 h postmortem (Table 3).

Correlation coefficients of carcass traits and endocrine factors

Table 4 shows the correlation coefficients between carcass traits and serum endocrine factors concentrations in pigs. In gilts, the correlations between carcass weights and serum IGFs at antemortem period were significantly positive (IGF-I: 0.7602, $p<0.001$; IGF-II: 0.6273, $p<0.01$). In addition, IGF-I and cortisol at postmortem also showed positive correlations with carcass weight (IGF-I: 0.5694, $p<0.01$; cortisol: 0.4422, $p<0.05$). The positive correlations between backfat thickness and serum IGFs at antemortem period were also found (IGF-I: 0.5253, $p<0.01$; IGF-II: 0.5490, $p<0.05$). In barrows, although TGF-β and cortisol concentrations at antemortem period showed positive and negative correlations with carcass weight, backfat thickness was not correlated with any endocrine factors. Correlation coefficients between carcass traits and muscle growth factors during postmortem period are shown in Table 5. Like the serum IGF-II, the concentration of muscular IGF-II in gilts at 45 min postmortem was highly correlated to carcass weight (45 min: 0.4495, $p<0.05$; 24 h: 0.5857, $p<0.01$). Also, TGF-β concentration in muscle at postmortem 45 min showed a positive correlation with carcass weight in gilts (0.4130, $p<0.05$). Although the correlation of endocrine factors with backfat thickness in barrow was not detected, the concentration of IGF-I in muscle was positively correlated to backfat thickness in gilts (45 min: 0.4082, $p<0.05$; 24 h: 0.3958, $p<0.05$).

Table 3. Concentrations of growth factors in muscle during postmortem period.

Factors		45 min	24 h
IGF-I (ng/ml)	Barrows (n=30)	3.45±0.98	3.90±0.97
	Gilts (n=28)	3.28±0.73	3.65±0.80
IGF-II (ng/ml)	Barrows	14.97±13.10	10.02±8.89
	Gilts	23.86±12.52[a]	16.32±8.26[b]
TGF-β1 (ng/ml)	Barrows	5.06±2.48	5.21±1.39
	Gilts	5.37±2.78	5.66±2.26
EGF (pg/ml)	Barrows	5.35±7.68	11.40±24.95
	Gilts	6.58±7.64	12.24±23.63

[a,b] Mean±SD within a row with different superscripts differ ($p<0.05$).

Table 4. Correlation coefficients between carcass traits and concentrations of endocrine factors in serum

Factors		IGF-I		IGF-II		TGF-β		Cortisol		DHEA-S	
		A[1)]	P[2)]	A	P	A	P	A	P	A	P
Carcass weight	Barrows	-0.1090	-0.1662	0.0163	0.0042	0.4673*	0.1915	-0.4165*	-0.0664	-0.0172	0.0328
	Gilts	0.7602**	0.5694**	0.6273**	-0.0632	-0.0622	-0.2612	0.2525	0.4422*	0.1306	0.2456
Backfat thickness	Barrows	0.0511	-0.1011	0.3503	0.5281	0.1970	-0.0334	-0.3197	0.0107	-0.0302	-0.0604
	Gilts	0.5253**	0.1576	0.5490*	0.2739	-0.2406	-0.1507	0.0506	0.4321*	0.1606	0.1334

* $p<0.05$, ** $p<0.01$.
1) Antemortem period
2) Postmortem period

Table 5. Correlation coefficients between carcass traits and concentrations of growth factors in muscle during postmortem periods

Factors		IGF-I		IGF-II		TGF-β		EGF	
		45[1)]	24[2)]	45	24	45	24	45	24
Carcass weight	Barrows	-0.1054	-0.2176	0.5396	0.5989	0.1779	-0.0045	0.0055	0.1699
	Gilts	0.1362	0.1674	0.4495*	0.5857**	0.4130*	0.1787	-0.2922	-0.1105
Backfat thickness	Barrows	-0.1654	-0.1749	0.3468	0.5021	0.3588	-0.0900	-0.0582	0.0420
	Gilts	0.4082*	0.3958*	0.3036	0.4176	0.0735	0.1024	-0.1266	0.0688

* $p<0.05$, ** $p<0.01$
1) Postmortem 45 min
2) Postmortem 24 h

Table 6. Correlation coefficients among concentrations of growth factors in serum

		Antemortem		Postmortem		
		IGF-II	TGF-β1	IGF-I	IGF-II	TGF-β1
Ante-mortem	IGF-I	0.3935*	-0.4466***	0.6881***	-0.1563	-0.4894***
	IGF-II	-	0.0034	0.0787	-0.0295	-0.1450
	TGF-β1	-	-	-0.4556***	-0.1607	0.6692***
Post-mortem	IGF-I	-	-	-	-0.2583	-0.3653**
	IGF-II	-	-	-	-	-0.1217

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

Correlation coefficients among growth factors and hormones

Correlation coefficients among serum IGF-I, IGF-II, and TGF-β1 concentrations in antemortem and postmortem periods were shown in Table 6. Serum IGF-I concentration at antemortem period showed a high correlation with IGF-II at antemortem period and IGF-I concentration at postmortem period (IGF-II: 0.3935, $p<0.05$; IGF-I: 0.6881, $p<0.001$). But, negative correlations of IGF-I with TGF-β1 concentration were detected at antemortem and postmortem periods (antemortem: -0.4466; postmortem: -0.4894, $p<0.001$). While serum IGF-II concentration at antemortem periods did not show any correlation with growth factor profiles at postmortem periods, TGF-β1 concentration at antemortem period was highly correlated to IGF-I and TGF-β1 expression at postmortem periods (IGF-I: -0.4556; TGF-β1: 0.6692; $p<0.001$).

Next, the correlation coefficients among concentration of growth factors in serum and muscle at antemortem and postmortem periods were analyzed (Table 7). Serum IGF-I concentration was positively correlated to muscular TGF-β1 at 45 min and 24 h postmortem, while serum TGF-β1 concentration showed a high negative correlation of muscular IGF-I concentration at 45 min and 24 h postmortem.

DISCUSSION

In the present study, concentrations of growth factors and hormones in serum and *m. longissimus* of day 150 pigs were measured to elucidate relationship of endocrine concentration with carcass weight and backfat thickness.

Although other growth factors and hormones concentrations between antemortem and postmortem periods were not different, significant differences in the serum IGF-II and cortisol concentration between antemortem and postmortem periods was detected (Table 2). In gilts, serum IGF-II concentration at antemortem period (30 day before slaughter) also showed the high positive correlation with carcass weight (0.6273, $p<0.01$, Table 5). In addition,

Table 7. Correlation coefficients among concentrations of growth factors in serum and muscle

		Postmortem							
		45 min				24 hr			
		IGF-I	IGF-II	TGF-β1	EGF	IGF-I	IGF-II	TGF-β1	EGF
Ante-mortem	IGF-I	0.1973	0.3138	0.4548***	-0.2159	0.2603*	0.4287*	0.3003*	-0.1962
	IGF-II	0.0258	0.5159**	0.1168	-0.1201	0.4800*	0.5170**	0.4600**	0.1346
	TGF-β1	-0.3248*	-0.0526	-0.2386	-0.0883	-0.5143***	-0.0426	-0.0343	-0.0095
Post-mortem	IGF-I	0.3820**	0.1294	0.4033**	-0.1288	0.3826**	0.1832	0.3094*	-0.2268
	IGF-II	0.4190*	0.2004	0.1135	-0.1787	0.1892	0.4066*	0.4480*	-0.2839
	TGF-β1	-0.3248*	-0.0426	-0.1854	-0.0883	-0.4135**	-0.0526	-0.0343	-0.0010

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

muscular IGF-II concentration at postmortem period is related to the carcass weight (45 mm: 0.4495, $p<0.05$; 24 h: 0.5857, $p<0.01$, Table 6). These results indirectly suggest that serum IGF-II is involved the hypertrophy of muscle. Generally, IGFs stimulate the catabolism in animal by stimulating the synthesis of glycogen and protein, the uptake of glucose and amino acid, and inhibiting the degradation of protein (Gluckman et al., 1991; Nissley and Rechler, 1984; Jones and Clemmons, 1995). Especially, similar to our result, the correlation of circulating IGF-II concentration with growth traits is detected in pig (Lamverson et al., 1996), human (Reece et al., 1994), and cow (Lee et al., 1995). Serum IGF-I concentration has the positive correlation with growth rate in pig (Lamberson et al., 1995; Owens et al., 1999; Lee and Chung, 2000) and human (Yang and Yu, 2000), and mouse (Blaire et al., 1987). The present study also suggests that the concentrations of serum IGF-I at antemortem and postmortem periods were significantly correlated to carcass weight in gilt (0.7602, $p<0.01$, Table 5). Owens et al., (1999) suggests that circulating IGF-I, originating from either liver, muscle, or fat (Coleman et al., 1994; Brameld et al., 1996), may either act via endocrine mechanisms to stimulate lean tissue growth or may reflect somatic tissue production of IGF-I, which is acting as a paracrine growth factor. But the present result suggests that circulating IGF-I is at least not originate from *m. longissimus*, indicating that any relationship of muscular IGF-I concentration at 45 min and 24 h postmortem is not detected.

In barrows, serum concentration of TGF-β1 at antemortem period is positively related to the carcass weight (0.4673, $p<0.05$, table 4). TGF-β is involved in the stimulation of the amino acid uptake and cell proliferation and the suppression of proteolysis (Florini and Ewton, 1988). In addition, TGF-β1 modulate the myogenic differentiation including fusion of myoblast, elevation of creatine kinase activity, and expression of other muscle-specific mRNAs and the corresponding proteins in a concentration-dependent fashion (Florini et al., 1991). The present study and previously reports present the association of TGF-β1 with the proliferation and differentiation of muscle cell.

As shown in Table 4, the correlation of cortisol with carcass weight is dependent on sex. The level of cortisol secreted from adrenal cortex increases to stimulate the metabolism of glucose, lipid, and cholesterol in blood as a countermeasure about stress (Parker et al., 1985). Therefore this result show the difference of the response against stress between gilt and barrow.

In generally, the adipose tissue expansion is a consequence of adipocyte hypertrophy and hyperplasia, and IGF plays an important role in this processes including preadipocyte growth and differentiation (Boney et al., 1994; Boney et al., 2000; Marques et al., 2000). Similar to these previously reports, the result of the present study shows that serum IGF-I and -II at antemortem period have the positive correlation with backfat thickness in gilt (IGF-I: 0.5253, $p<0.01$; IGF-II: 0.5490, $p<0.05$, Table 5). In particular, Bunoma and Klindt (1993) reported that plasma IGF-II is higher genetically selected for increased backfat depth in similarly aged pigs and that IGF-II is play a important role in adipose deposition in pigs. Also, plasma IGF-II concentration is higher from animals with more fat mass (Lee et al., 1993), indicating that IGF-II may regulate fat deposition. Unlike our results of IGF-I, several studies (Lamberson et al., 1995; Owens et al., 1999; Lee and Chung, 2000) reported that the correlation of IGF-I with backfat thickness is negative or not detected. But the present result also shows the positive relationship of muscular IGF-I at postmortem 45 min and

24 h with backfat depth (45 min: 4082; 24 h: 0.3958, $p<0.05$, table 5). Therefore we suggest that adipose growth might be promoted by IGF-I as well as IGF-II originating from liver or muscle (Lee et al., 1993) and that IGF-I and -II positively involve in the backfat deposition in barrows and gilts.

Parker et al. (1985) reported that cortisol have a negative correlation with DHEA-S, involving an anti-obesity effect and the subcutaneous fat depth (Clore, 1995; Wise et al., 1995). Like this report, in barrows, cortisol concentration at postmortem period is positively correlated to backfat thickness and carcass weight, while the correlations of serum DHEA-S concentration with backfat thickness and carcass weight are not detected. This result shows that the pathway of cortisol and DHEA-S on backfat deposition is either differed.

In summary, we have examined possible of growth factors (IGFs, TGF-β1, and EGF) and steroid hormones (corisol, DHEA-S) with carcass weight and backfat thickness in pigs. At antetmortem period, the positive correlations of serum concentrations of IGF-I and IGF-II with the carcass weight and backfat thickness in gilts are detected. Contrary to the gilt, the carcass weight and backfat thickness is related to the concentration of TGF-β1 and cortisol. Therefore we indirectly suggest that serum and muscular growth factors at antemortem period maybe involved in the carcass weight and backfat thickness in pigs.

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