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DIFFERENT SERUM INSULIN-LIKE GROWTH FACTOR-I (IGF-1) BINDING CHARACTERISTICS ACCOMPANY REDUCED GROWTH ASSOCIATED WITH LOW THYROID HORMONE AND/OR LOW TOTAL IGF-1 STATUS IN YOUNG PIGS

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Serum IGF-I binding patterns were determined for 3-month-old pigs exhibiting a wide range of total IGF-I and thyroid hormone (TH) concentrations. Both variables positively correlated with their growth rate. No differences in labelled IGF-I binding patterns after molecular sieve chromatography or in ligand blots were found in pig sera with different TH levels and high IGF-I concengrations (>13.1 nmol/). When serum IGF-I concentrations were low, ligand blots in the position expected for IGF binding protein-2 (IGFBP-2) were more intense and relative binding to 40 kDa serum proteins greater. Since at high TH concentrations binding to the 150 kDA fraction was unchanged, the total bound/free ratio was significantly higher than in sera from the other pigs. At low TH concentrations binding of labelled IGF-I to the 150 kDa fraction tended to decrease leading to a significantly different ratio between the two bound fractions compared with the other sera. Thus, the dynamics and/or extent of IGF-I binding in porcine serum may be altered in two different ways depending on TH status, possibly indicating different paths towards lower weight gain associated with separate or combined decreases in serum IGF-I and TH concentrations.

Key words: growth, IGF-I, thyroid hormones, pigs, IGFBP, iodine

INTRODUCTION

Growth of young animals is controlled by a complex interaction of genetic and environmental factors which affect pituitary- target gland hormonal axes by many different mechanisms. Thus, growth rates in pigs have been found to be positively related to somatotroph secretory activity, hepatic growth hormone (GH) receptor mRNA levels and the resulting production of endocrine hepatic IGF-I (Matteri et al., 1994; Weller et al., 1994). This system is regulated by energy status and protein intake directly and also through changes in thyroid hormone (TH) status (Straus, 1993; Dauncey et al., 1994). Thus, significant correlations between height and both IGF-I and thyroxine (T4) levels were found in children growing up in iodine deficient areas (Wan Nazaimoon et al., 1996). However, no simple

correlation between plasma concentrations of T4 and IGF-I occurred in young pigs under conditions of controlled energy intake (Danceyet al., 1990).

While TH do stimulate GH secretion directly, all TH effects on the IGF-I system in rats are not GH mediated (Näntö-Salonen et al., 1993). Renal growth in hypophysectomised rats was promoted additively by T4 and GH(Marshall et al., 1993). Moreover, GH deficient mutant mice were found to exhibit different changes in IGF-binding proteins (IGFBPs) from those in hypothyroid mutant mice(Sugisaki et al., 1993). Differences in the distribution of IGF-I between different binding protein complexes may affect its biological activity because individual binding proteins have very different properties such as susceptibility to the action of various proteases, kinases and other enzymes and the possession or not of motifs allowing binding to integrins, intercellular matrixes etc.(Stewart and Rotwein, 1996). Only the smaller complexes can leave the circulation through capillary walls.

During investigations on the effects of feeding maize based diets with different protein supplements to young pigs, it was noticed that growth rate differed greatly between individual animals within groups and was associated with their serum total IGF-I and TH concentrations. This paper describes an attempt to examine differences in IGF-I distribution in porcine serum in relation to total IGF-I concentration, TH status and weight gain using samples from young gilts and barrows fed on similar diets in four trials performed over a two year period.

MATERIALS AND METHODS

Animals and original trials. Crossbred (Yorkshire x Landrace) female and castrated male pigs (barrows), aged about 9 weeks, were weighed and group fed *ad libitum* on diets balanced for amino acid content in four trials. In Experiment 1 the influence of including a fat supplement or slightly decreasing the protein content of the standard maize/soybean oilmeal diet (20% to 17.5%) was examined in three groups of pigs over a 45 day period (Nikolić et al., 1993). Diets in the subsequent trials which lasted for about 35 days were all isonitrogenous and isoenergetic. In Experimental 2 the effects of replacing some of the maize and soybean oilmeal with peas prepared in three different ways or the fishmeal supplement with yeast were examined in five groups of pigs. The results indicated a possible iodine deficiency in the yeast containing diets. Experiment 3 was a repeat of the first four treatments in Experiment 2 (standard and pea containing diets) with adequate iodine supplementation (Nikolić et al., 1994). In Experiment 4 all four diets examined contained yeast, while the soybean oilmeal supplement was progressively replaced with sunflower oilmeal. Experiments I and 3 were carried out in late autumn and Experiments 2 and 4 in midsummer. The ratio of barrows to gilts was 3:1 in Experiments 1 and 4 but 1:1 in Experiments 2 and 3.

Determination of iodine. Iodine content of the fish meal and yeast incorporated into the diets was determined a redox reaction:

$$2\,Ce^{4+} + As^{3+} \rightarrow 2\,Ce^{3+} + As^{5+}$$

catalysed by iodide (Lauber, 1975). The reaction kinetics is a linear function of iodide concentration upto 6 ng/ml reaction mixture. Using the procedure described, about 1 ng of iodine may be detected in 1 g of dry organic material. The relative standard deviation of the method is 1%.

Serum collection. About 2 days before the end of thetrials blood samples were taken from the retroorbital sinus of four pigs from each group (except for Group 2 in Experiment 1 where three pigs were sampled) between 8:00 and 9:00 h after an overnight fast. After coagulation and centrifugation the serum was aspirated and stored at -20 ^{0}C for later analysis.

Determination of serum hormone concentrations. Triiodothyronine (T3) and T4 were determined by radioimmunoassay with commercial kits in accordance with the instructions (INEP Diagnostics, Zemun). The mean intraassay coefficients of variation of duplicate samples were 2.3% for T4 and 5.7% for T3.

Total IGF-I was determined after separation of binding proteins by acid-ethanol extraction with cryoprecipitation (Daughaday et al., 1982; Breier et al., 1991). The radioimmunoassay has been validated for swine serum (Nikolić et al., 1996). Mean (SD) recovery of reference IGF-I (WHO/518)added to porcine serum samples was 92 (9.5)% (n = 3) and the mean intraassay coefficient of variation of duplicate determinations was 5.9%.

Regrouping the pigs. For the purposes of this investigation the blood serum samples (n = 63) obtained from these pigs were regrouped using the following criteria: Group 1 with IGF-I concentrations >13.1 nmol/1, T3 > 0.5 nmol/l and T4 > 20 nmol/l (9 females and 7 barrows); Group 2 with IGF-I < 13.1 nmol/l and TH concentrations as above (6 females and 5 barrows); Group 3 with IGF-I concentrations as for Group 1 but T3 and/or T4 below 0.5 and 20 nmol/l respectively (4 females and 7 barrows); while Group 4 (5 females and 20 barrows) consisted of those animals with low serum levels of IGF-I and one or both TH. Group 1 contained eight, four, three and one pig respectively from each of the four trials; Group 2 contained one, four and six pigs from the first three trials; Group 3 consisted of two, one, one and seven pigs from all four trials; while Group 4 consisted of eleven, six and eight pigs respectively from the last three trials. One pig assigned to new Group 1, eight pigs placed in Group 3 and eleven pigs in Group 4 had received yeast containing diets. Representative samples from these new groups including twenty serum samples from females and barrows originating from each original trial were examined for their IGF-1 binding profiles.

Examination of IGFBP profiles.

a. *Column chromatography.* A Sephacryl S-200 (Pharmacia Fine Chemicals, Sweden) size exclusion column (0.9 x 62.5 cm) was calibrated with the following molecular mass markers: blue dextran relative molecular mass (Mr) 2000 kDa; imunoglobulin-G (IgG) Mr 150 kDa; bovine serum albumin (BSA) Mr 68 kDa; ovalbumin (Ova) Mr 43 kDa; chymotrypsin (Chy) 25 kDa; ribonuclease (Rib) 14 kDa, applied to the column in 0.1 ml elution buffer (0.05M sodiumphosphate pH 7.4 containing 0.14M NaCl (PBS)) and eluted at 0.2 ml per min at room temperature. The stability and purity of the ^{125}I-IGF-I was examined regularly by passing

an aliquot (about 3 x 10^5 cpm) through the column in the presence of carrier IGF-I (0.36 mg) and BSA (2 mg). The radioactivity of the collected fractions (0.3 ml) was determined in (Gammachem 4800, Italy) at an efficiency of about 70% and their optical density at 280 nm on a spectrophotometer (Cecil, Cambridge). The column was well washed with buffer between elutions.

Determination of labelled serum IGF-I complexes. Serum samples (0.1 ml) were incubated overnight (18 h) at 4^0C with ^{125}I-IGF-I (about 0.3 ng in 20 μl PBS containing 0.25% BSA) alone or together with cold IGF-I (0.35 μg) or sodium ethylenediamine-tetraacetic acid (EDTA; 0.2 mg). The mixtures were applied to the column and eluted as above. The optical density elution profiles served as internal molecular weight markers for each serum. The radioactive elution profile showed three regions (A, B and C). The contribution of each region was determined gravimetrically by plotting net cpm per fraction on graph paper, cutting out each area and weighing it on an analytical balance (Metler, Switzerland). The analytical error, determined by weighing squares of paper from different sheets (n=13) of area similar to the smallest region was 2.6%. An index of the relative amount of bound IGF-I was determined by dividing the weight of the sum of regions A and B by the weight of region C which corresponded to free IGF-1. The relative amount of lower molecular mass complexes was estimated from the ratio B/A.

b. *Gelelectrophoresis.* Sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970) using 12% homogeneous gel under non-reducing conditions (Hossenlopp et al., 1986). Diluted serum samples (1:30 in 0.0625M Tris-HCl buffer pH 6.8 containing 2% SDS, 10% glycerol and 0.01% bromphenol blue (BFB) were boiled for 7 min, quenched on ice for 2 min and then loaded on to the gel. Molecular mass markers (BSA, Ova, Chy and Rib) were run in parallel. Samples were electrophoresed in a Trans-blot cell system (Bio-Rad Laboratories) at constant voltage (80 V) until the BFB marker reached the bottom of the gel (about 2 h). Each gel was run in duplicate. One gel was stained with 0.25% Coomassie Briliant Blue (CBB) and the other was electrotransferred.

Electrotransfer. Electroblotting was performed in 0.015 M Tris-HCl buffer pH 8.3 containing 0.12M glycine and 5% methanol under constant current (0.4 A) at 4^0C for 4 h. Separated proteins were transferred to nitrocellulose membrane (0.45 μm; Schleicher and Schuell). Electrotransfer was confirmed by reversible staining of the membrane with 5%Ponceau S solution (for 15 min), as well as soaking the remaining gel in the CBB reagent. Ponceau stained membranes were photocopied. Blotted membranes were serially washed with the following solutions: (i) 0.01M Tris-HCl buffer pH 7.4 containing 0.15M NaCl (TBS buffer) for 30 min; (ii) TBS buffer supplemented with 3% NP-40 for 30 min; (iii) TBS buffer containing 0.1% Tween 20 for 30 min and (iv) TBS buffer four times for 5 min (Hossenlopp et al., 1986).

Detection of proteins binding IGF - I. Blotted membrane (6.5 x 10 cm) was incubated overnight at 4 ^{0}C with 2 x 10^6 cpm ^{125} I-IGF-I in TBS buffer with 0.1% Tween 20 (20ml). Thereafter, the membrane was washed at 4 ^{0}C, twice for 30 min in the same buffer and then three times for 30 min in TBS buffer alone. Membranes

were allowed to dry for 2 days at 4^{0}C and then exposed to X-ray film (AGFA, Belgium) for 3 to 7 days at -70 ^{0}C using intensifying screens. The positions of darkened bands were assessed from the positions of molecular mass markers on the Ponceau stained photocopies. The intensity of the bands was scored as follows: duplex at 43 kDa dominant (3); band at approximately 34 kDa dominant (1);both regions equal (2).

Statistical analysis. The correlation coefficients of different variables were calculated and groups of data were subjected to analysis of variance (AVNOVA) using a computer programme (MSTATC, USA). When Barlett's test indicated nonhomogeneity of variance between groups AVNOVA was repeated using mathematically transformed data (square root). When the F-test indicated singificant differences ($P< 0.05$), orthogonal contrasts were made between groups using the original and transformed data.

RESULTS

Growth rate and hormone concentrations in the original trials. The results are summarized in Table 1 where in may be seen that mean serum IGF-I concentrations were highest in Experiment 1 and lowest in Experiment 3. Mean TH concentrations, particularly T4, also differed widely between the different trials. In Experiment 1 there was a positive correlation between weight gain in the preceding month and serum T4 concentration ($r = 0.708$; $P = 0.013$), whereas in Experiment 2 daily gain was correlated with T3 concentrations ($r = 0.518$; $P = 0.019$). Serum TH levels found in Experiments 3 and 4 were not significantly associated with preceding rate of weight gain but close positive correlations between gain and serum IGF-I levels emerged (Experiment 3 - $r = 0.806$; $P< 0.001$; Experiment 4 - $r = 0.598$; $P = 0.014$).

Table 1. Average growth rate during the preceding month, final body weight and hormone concentrations in young pigs two or three days before the end of the experiment (Mean (SD))

Experiment	No. pigs	Grain (g/day)	Body wt. (kg)	Fasting serum concentrations (nmol/l)		
				T3	T4	IGF-I
1	11*	434 (47)	27.9 (2.4)	0.80 (0.29)	64.3 (12.2)	21.3 (7.1)
2	20	519 (67)	26.9 (2.9)	0.51 (0.14)	29.3 (8.9)	12.6 (6.0)
3	16	403 (77)	22.8 (2.4)	0.55 (0.28)	73.7 (12.0)	9.7 (5.6)
4	16	300 (100)	20.2 (4.9)	0.42 (0.21)	12.3 (3.8)	14.1 (7.0)
All	63	419 (112)	24.3 (4.5)	0.55 (0.26)	42.5 (26.7)	13.8 (7.3)

*These pigs were about 10 days older than the remainder which were aged 86 - 90 days

In Experiment 2 the much lower mean (SD) T4 concentration in the sera of the four pigs receiving the diet containing yeast (14.7 (1.9) nmol/l) compared with those given fish meal suggested a possible dietary iodine deficiency. Analysis of the iodine content of these two feedstuffs showed that the fishmeal contained 1.46 mg I/kg while the yeast contained only 0.18 mg I/kg. TH status was also very

low in Experiment 4 in which all the pigs received yeast supplemented diets without fishmeal (Table 1). However body mass achieved and rate of gain in Experiment 4 were not correlated with either T3 or T4 concentrations.

Table 2. Correlation coefficients between body weight attained and fasting serum concentrations of T3, T4 and IGF-I in young pigs.

Sex	No.	Variable	T3		T4		IGF-I	
			r	P	r	P	r	P
Barrows	38	Body wt.	0.477	0.002	0.355	0.029	0.402	0.012
		Growth rate*	0.421	0.008	0.308	0.060	0.244	0.139
Females	25	Body wt.	0.076	0.716	0.051	0.810	0.467	0.018
		Growth rate*	0.056	0.791	0.031	0.885	0.212	0.310
Overall	63	Body wt.	0.372	0.003	0.236	0.063	0.414	0.001
		Growth rate*	0.327	0.009	0.199	0.117	0.269	0.033

*Average daily weight gain in the preceding month

Table 3. Mean body weigh, growth rate in the preceding month and hormone status of 3- month-old pigs divided into four categories depending on serum levels of IGF-I and TH

Group	No.	IGF-I (nmol/l)	T3 (nmol/l)	T4 (nmol/l)	Body wt. (kg)	Growth rate (g/day)
1	16	21.8	0.78	57.6	27.4	484
2	11	9.5	0.74	64.0	24.4	439
3	11	18.7	0.42	24.4	23.7	378
4	25	8.4	0.38	31.2	22.6	387
SE	–	0.9	0.03	3.4	0.6	14
Orthogonal comparisons:						
1 > 2	P =	< 0.001	–	–	< 0.001	< 0.001
1 > 3	P =	–	< 0.001	< 0.001	< 0.001	< 0.001
1 > 4	P =	< 0.001*	0.003	0.260	< 0.001*	0.002
1 > 2, 3, 4	P =	–	–	–	< 0.001	0.003

*Large variance in group 4 necessitated mathematical transformation of data whereupon this apparently statistically significant difference disappeared

Although the average metabolic energy intake (about 14 MJ per day) in Experiment 3 was very similar to that in Experiment 2, the weight gain was lower. This may have been influenced by the lower ambient temperature (20^{0}C) compared with that in the previous summer trial(25^{0}C). Taken as a whole (Table 2), the associations of body weight attained (and rate of gain in the preceding month) with both serum IGF-I and T3 concentrations were highly significant. These relationships were particularly marked for the barrows. The trends shown in Table 2 are clearly evident in Table 3. Namely both body weight achieved and rate of gain was higher in the pigs assigned to Group 1 than in each of the other groups.

Validation of the column chromatographic and ligand blot procedures. The elution patterns of proteins of known molecular mass showed adequate separation within the range 7 - 2000 kDa Mr (Fig. 1 a, b. A total of 91.5 ± 1.5% of the added radioactivity was eluted in a single peak at the position expected for 7 kDa molecules such as IGF-I. The small amounts of radioactivity associated with BSA and small molecular weight compounds such as NaI did not increase over a 2 month time interval indicating stability of the radioligand. Incubation of porcine serum with ^{125}I-IGF-I gave an elution profile (Fig. Ic) in which most of the radioligand was associated with lower molecular mass binding proteins (40-45 kDa). Both this peak (region B) and the preceding shoulder (region A) were decreased when the same serum was incubated with added IGF-1 while region C, corresponding to free IGF-I, was increased. This result confirms the specificity of IGF-I binding to swine serum proteins. Overnight incubation of porcine serum and elution in the presence of EDTA also greatly reduced the binding of radioligand to serum proteins (Fig. 1d), suggesting that divalent cations may be required for the association/dissociation reaction. Incubation for longer periods (up to 4 days) did not lead to formation of a larger peakin region A.

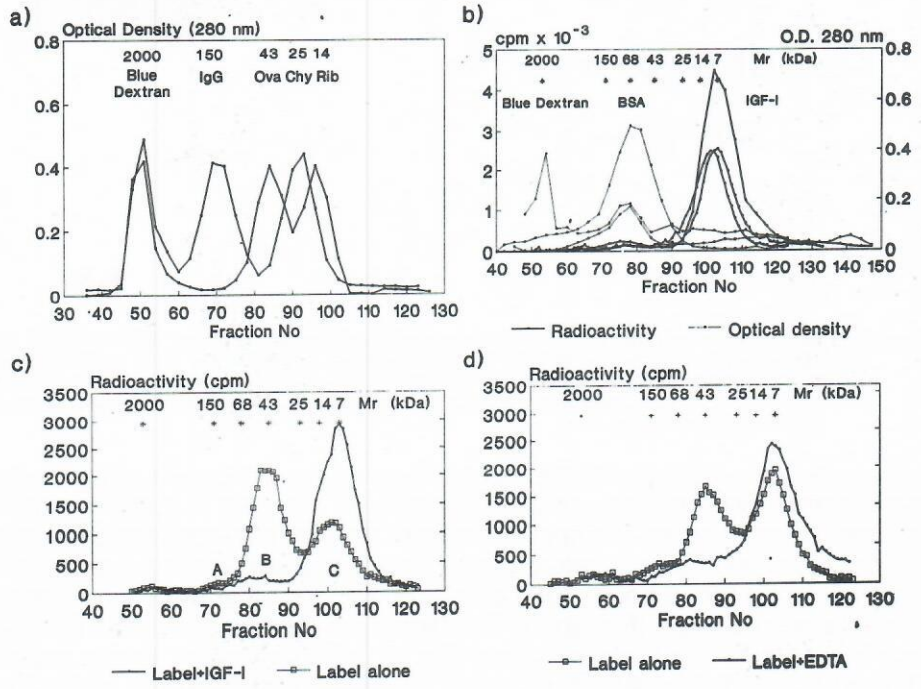

Figure 1. Elution profiles of a) molecular mass markers; b) labelled IGF-I and BSA at fortnightly intervals; c) porcine serum (IGF-I 5. 1 nmol/l) incubated with labelled IGF-I (0.3 ng)in the presence of cold IGF-I (350 ng); d) porcine serum (IGF-I 23.9 nmol/l) incubated with labelled IGF-I (0.3 ng)in the presence and absence of EDTA. Sephacryl S-200 (0.9 x 62.5 cm) was equilibrated and eluted with 0.05M phosphate buffer, pH 7.4, containing 0.14M NaCl at 0.2 ml per min at room temperature and 0.3 ml fractions collected.

The electrophoretic profiles of proteins in four porcine sera from Experiment 4 and the corresponding radioactive ligand blots detected after exposure to X-ray film are shown in Figure 2. The separation resulution was satisfactory allowing the putative IGFBP-3 duplex at 43 kDa Mr to be distinguished from the band supposedly corresponding to IGFBP-2 at about 34 kDa. The relative intensity of these bands differed greatly between individual porcine sera (Fig. 2b), among which the first three (left to right) had been assigned to new Group 3 and the last to Group 4.

Further examples of elution profiles and ligand blots. The optical density and radioactivity elution profiles of sera from three barrows of similar weight (one

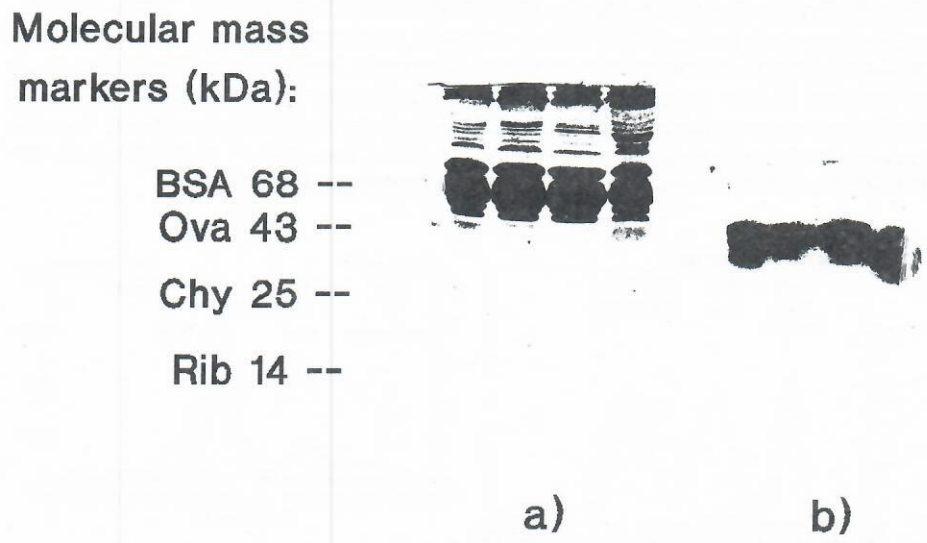

Figure 2. Separation of serum proteins from four pigs from Experiment 4 by SDS-PAGE: a) gel stained with Coomassie briliant blue; b) corresponding ligand blots detected after electrotransfer to nitrocellulose, probing with ^{125}I-IGF-I and autoradiography (scores left to right: 2; 3; 3; 1; from a barrow (11.0 kg), a barrow (19.0 kg), a female (25.0 kg) and a barrow (13.0 kg) respectively).

from each dietary treatment in Experiment 1) are shown in Figure 3, together with their ligand blots. The amount of radioactivity in shoulder A and the relative intensity of the band at 43 kDa were lower for the only pig exhibiting a low total IGF-I concentration and therefore placed in Group 2, suggesting a decreased presence of IGFBP - 3 bound IGF-I compared with the other two animals which were placed in Group1 (Fig. 3b, d). The blot in the putative position of IGFBP-2 was very faint for the heaviest animal (Fig. 3c, d). The optical density profiles showed peaks at the position expected for albumin preceded by a shoulder representing IgG.

IGF-I binding was also examined in the sera from two barrows and two females from Experiment 2, one each with high IGF-I and low IGF-I levels respectively. Elution profiles for the females, which were placed in new Groups 1

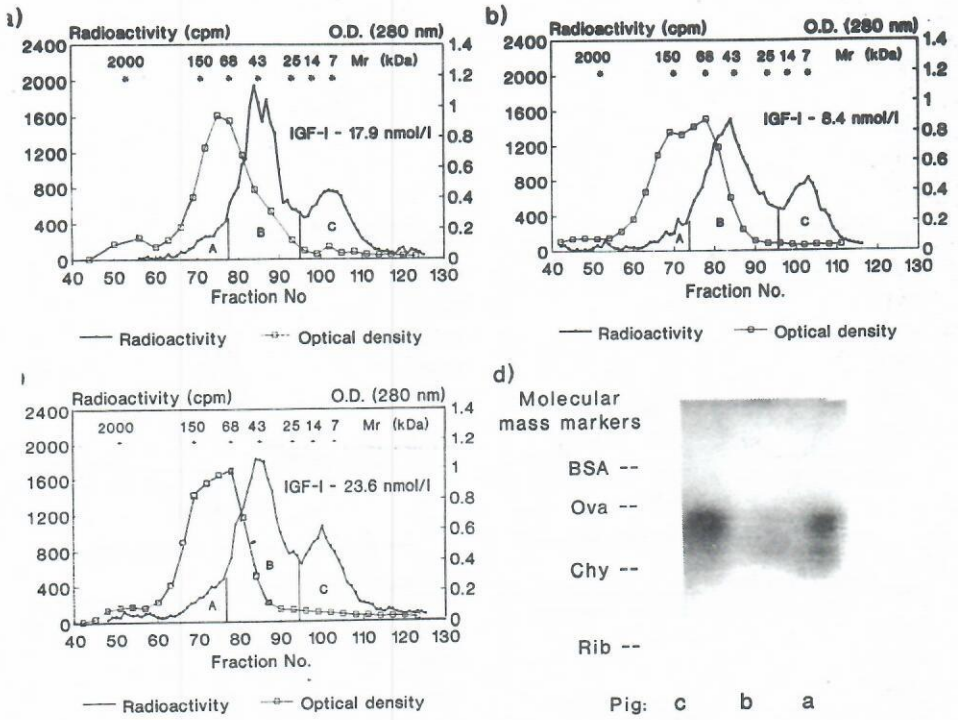

Figure 3. Elution profiles of three porcine sera from Experiment 1 and the corresponding ligand blots: a) barrow (29.0 kg) fed standard diet with 20% protein; b) barrow (28.0 kg) fed standard diet with 17.5% protein; c) barrow (31.8 kg) fed higher energy diet; d) ligand blots. (Conditions as described for Figs 1 and 2)

and 4, respectively are given in Figure 4a. Increases in the area of peak B at the expense of shoulder A and peak C and greater intensity of ligand blots at the position expected for IGFBP-2 were associated with low IGF-I and TH concentrations in both cases. Elution profiles of bound IGF-I and ligand blots for sera from six pigs from Experiment 3 showed the same tendencies as those observed in the first two experiments. However, when low IGF-I was accompanied by low T3 concentrations (new Group 4), the decrease in shoulder A was more marked and the increase in peak B less expressed (Fig. 4c) than when only IGF-I levels were low (new Group 2; Fig. 4b). IGF-I binding characteristics were examined in seven sera with different IGF-I contents from pigs of widely different body weight (11 -

25 kg) from Experiment 4. Alterations in elution profiles (Fig. 4d) and ligand blot patterns (Fig. 2) associated with low IGF-I concentrations corresponded with those encountered in the previous trials (Fig. 4a,c). On the other hand, elution profiles and ligand binding patterns for the five sera with high IGF-I levels and low

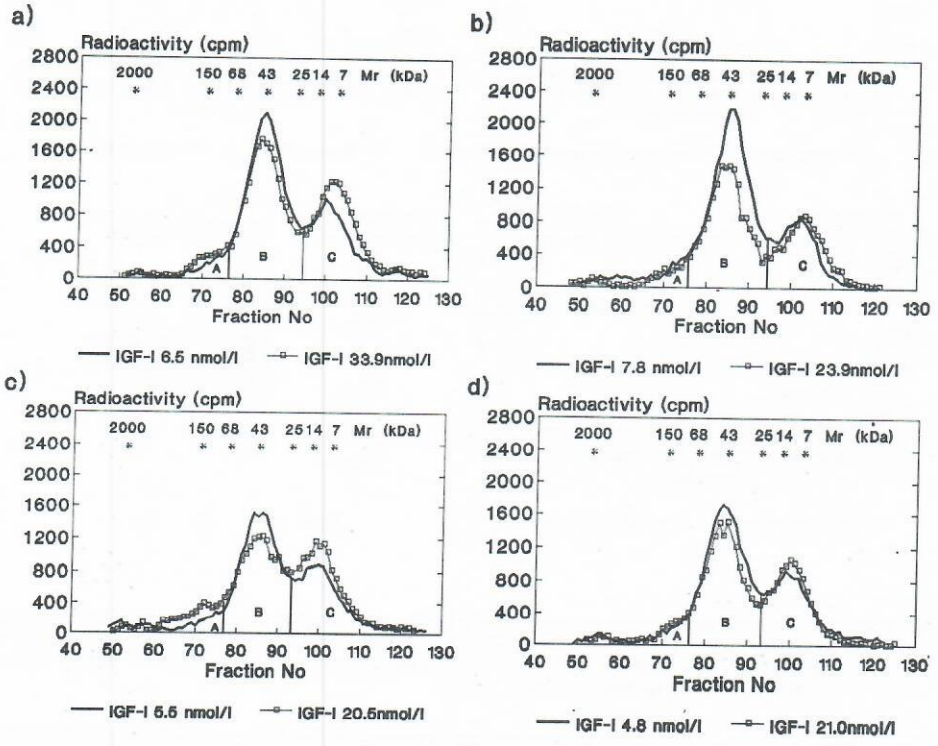

Figure 4. Elution profiles of eight representative porcine sera: a) female (21.6 kg) with low IGF-I and TH (T3 - 0.34 and T4 13.8 nmol/l) and female (26.4 kg) with high high IGF-I and adquate TH (T3 - 0.51 and T4 - 35.8 nmol/l) from Experiment 2; b) barrow (21,8 kg) with low IGF-I and high TH (T3 - 0.70 and T4 - 81.5 nmol/l) and female (27.4 kg) with high IGF-I and TH (T3 -0.93 and T4 - 73.9 nmol/l) from Experiment 3; c) barrow (22.5 kg) with low IGF-I and low T3 (T3 - 0.37 and T4 - 78.2 nmol/l) and female (23.8 kg) with high IGF-I and high TH (T3 - 0.54 and T4 - 55.6 nmol/l from Experiment 3; d) female (15.0 kg) with low IGF-I and T4 (T3 - 0.84 and T4 - 10.8 nmol/l) and barrow (19.0 kg) with high IGF-I and low TH (T3 -0.50 and T4 - 15.2 nmol/l) from Experiment 4. (Conditions as given in Fig. 1).

TH status (new Group 3) were not different from those obtained in the other experiments where TH concentrations were normal.

Table 4. Mean body weight and the relative amount of bound IGF-I fractions in three month old pigs divided into groups depending on their serum TH and IGF-I concentrations

Group	No.	Body wt. (kg)	Bound IGF-I in elution area			Bound free*	Ligand blot score**
			A (%)	B (%)	Ratio B/A		
1	6	28.3	7.1	46.9	5.65	1.73	2.42
2	3	24.5	6.8	55.0	6.44	2.51	1.67
3	5	20.9	6.5	48.7	6.20	1.83	2.80
4	6	20.1	4.2	52.1	8.52	1.82	1.42
SE		1.3	0.5	1.0	0.43	0.09	0.17
Orthogonal comparisons:							
1≠2	P	< 0.001	–	< 0.001	–	0.013	< 0.001
1≠3	P	0.002	–	–	–	–	–
1≠4	P	0.011	–	0.027	0.008	–	0.004
2≠3	P	–	–	< 0.001	–	0.129	< 0.001
2≠4	P	–	–	–	< 0.001	0.005	–
3≠4	P	–	–	< 0.001	0.003	–	0.008
1+3≠2+4	P	–	–	0.019	–	–	< 0.001
1≠2+3+4	P	< 0.001	–	–	–	–	–
2≠1+3+4	P	–	–	–	–	0.006	–
4≠1+2+3	P	–	–	–	< 0.001	–	–

* Elution areas; $\frac{A+B}{C}$
**34 kDa> 43 kDa -1; 34 kDa =43 kDa - 2; 34 kDa < 43 kDa - 3

The results are summarised in Table 4, which shows that when serum IGF-I levels were maintained above 13.1 nmol/l, no differences were detected in binding patterns (Groups 1 and 3), despite differences in TH concentration and body weight. On the other hand at low total IGF-I concentrations, there was a consistent tendency towards predominance of the putative IGFBP-2 blot (ligand blot score < 2). When low IGF-I was accompanied by high TH status, the predominant change in elution profile was an increase in the area of peak B at the expense of peak C, leading to a significantly increased bound/free ratio (Table 4) in comparison with the other groups.

When both IGF-I and TH levels were low (Group 4), the increase in area B was accompanied by a decrease in shoulder A leading to values for the bound/free ratio as in the groups with higher serum IGF-I concentration, but a different distribution (B/A) of bound radioligand (Table 4). Since the time course of radioligand binding was not examined in detail it is not certain that equilibrium had been reached. Thus, the differences found may reflect either a slower rate of exchange or a difference in binding affinity of the IGFBPs at low TH levels.

DISCUSSION

The elution profiles obtained here after molecular sieve chromatography of porcine sera incubated with ^{125}I-IGF-I were very similar to those obtaind for sera from older pigs (80 kg) with similar total IGF-I concentrations (Evock et al., 1990). Since 87.5% of the found ^{125}I-IGF-I eluted with 40 kDa proteins, it could be calculated that the B/A ration was 7.0, which is in the range of the values found here for 3-months-old barrows and females (Table 4). Treatment with exogenous GH increased the proportion of IGF-I bound to 150 kDa complexes resulting in elution profiles similar to those found by others in younger pigs (Lord et al.,1994). Ligand blots for these 3-month-old animals showed absolute dominance of binding in the region characteristic for IGFBP-3. Dominance of IGFBP-3 blots may occur at 6 weeks old (Lee et al., 1993), while in 2-month old pigs Dauncey and coworkers (1993) observed a marked reciprocal relation between the 40-45 and 34 kDa IGFBPs, the former being elevated in the warm and on a high food intake. According to Buonomo and Klindt (1993), serum IGFBP-2 concentrations declined more than two-fold from an apex at 8 weeks of age to a plateau commencing at 14 weeks of age in both genetically lean and obese lines of swine. Total IGF-I concentrations were in the upper range of those found here. It is probable that situations resulting in decreased growth rates delay the decline in IGFBP-2 levels and the increase in IGFBP-3 levels. Thus, intrauterine growth retarded swine exhibited enhanced and prolonged hepatic expression of IGFBP-2 mRNA in comparison with control animals up to 7 weeks of age (Kampman et al., 1993). In the 20 pigs studied here the correlation between body weight and B/A ratio ($r = -0.508$) was closer than for total IGF-I ($r = 0.409$).

Mechanisms leading to prolonged dominance of serum IGFBP-2 probably result in close correlation between total IGF-I concentration and weight gain because these complexes can leave the circulation and act in tissues. When IGFBP-3 is dominant, larger molecular mass complexes are formed which represent a slowly equilibrating reservoir of IGF-I. Since they are formed under favourable conditions of protein and energy nutrition they act permissively allowing other factors to influence growth. Thus, no association between body weight achieved and total IGF-I concentration was observed in the pigs with serum IGF-I above 13.1 nmol/ and putative IGFBP-3 dominant. Since the correlation between body weight and total IGF-I concentrations was closer than the correlation between gain in the preceding month and IGF-1 (Table 2), it appears that the nutritional and environmenatal differences had a smaller on growth than genetic, intrauterine or neonatal factors. Namely, growth of individual animals is partly programmed at very early stages as indicated by the finding that fetal and maternal GH and IGF-I secretion are regulated independently (Bauer et al., 1996).

Changes in IGF-I binding patterns were observed in all pigs with low IGF-I levels regardless of TH status. Low TH status was probably caused by iodine deficiency, because T4 levels were decreased much more than T3 levels. If any contribution from the salt supplement is disregarded (Sinadinović et al., 1991), it

may be calculated that the diets containing fish meal would have supplied about 0.12 mg I/kg which approaches the daily requirement of 0.14 mg I/kg feed for young pigs,, whereas the diets with yeast used in Experiments 2 and 4 would have had only 0.07 mg/kg (Fëdorović-Tome et al., 1970; Lee et al., 1994). It appears that the barrows were much more susceptible to growth restriction associated with low iodine intake and low TH status than the female pigs. This may reflect a possible effect of estrogens on the TH system even in these immature animals. Cromwell and coworkers (1975) observed hypertrophied thyroids and low plasma protein-bound-iodine in pigs fed on diets with 0.055 mg I/kg but no effect on gain. The sex of the animals was not specified. No overt signs of goitre were observed in our pigs.

While hypothyroidism isusually associated with decreased serum IGF-I concentrations (Näntö-Salonen et al., 1993; Sugisaki et al., 1993; Miell et al., 1993), in our studies low TH status was not always accompanied with low IGF-I and putative IGFBP-3 levels. Three-fold higher IGF-I concentrations and faster growth were observed in 8-week-old thyroidectomized pigs compared with euthyroid controls both fed at 30 g feed per kg body weight per day, suggesting a predominant effect of energy balance (Dauncey et al., 1990). Namely, resting metabolic rate is usually low in hypothyroid animals. In Experiment 2 the yeast fed pigs grew slightly faster and more efficiently than the control group. However, permanent negative effects of low TH status in young animals should not be ignored. Even a short period of mild hypothyroidsm induced by consumption of methimazole and iopanoic acid was sufficient to cause changes in myofibre differentiation in the skeletal muscle of piglets with the prospect of muscle weakness but no inhibition of weight gain (Harrison et al., 1996).

When IGF-I concentrations are low with putative IGFBP-2 predominating, it appears that TH status can directly affect the dynamics or extent of exchange between free active IGF-I and complexes. Dissociable free (2%) and ultrafiltered free (1%) IGF-I concentrations were significantly correlated with total IGF-I, IGFBP-3 and the molar ratio between them in growing boys (Juul et al., 1996). Thus, if free IGF-I is taken as about 1% of the total amount, at a total IGF-I concentration of 31.4 nmol/l the standard amount of added label (0.3 ng ^{125}I-IGF-I) would equilibrate with a similar quantity (0.24 ng) of free IGF-I increasing the pool by a factor of less than two. However, at an IGF-I concentration of 5.2 nmol/l, as found in pigs from Groups 2 and 4, there would be only 0.04 ng free IGF-I in the serum sample, which is nearly eight times less than the amount of radioligand added. The sudden increase in concentration of free IGF-I of high specific activity would be expected to push the equilibrium towards increased binding at easily exchangeable sites, as did occur in pigs with high TH status. Namely, the bound/free ratio was significantly higher than in pigs with high IGF-I concentrations. When Takahashi and coworkers (1990) found large increases in the relative amount of ^{125}I-IGF-I bound to 40 kDa plasma proteins in rats fed protein free diets compared to those given casein, they interpreted the results as

evidence of incomplete saturation i. e. excess binding capacity. It has been calculated that there is a 50% molar excess of IGFBPs over IGFs in normal adult human serum (Mohan et al., 1996), which may increase in unfavourable situations.

However, this effect was minimal in pigs with low TH status. Namely, the increased area of 40 kDa complexes was cancelled by the decreased binding to 150 kDa complexes resulting in no overall change in the bound/free ratio. It is therefore possible that low TH status is associated with increased stability of 150 kDa complexes. However, this change was not necessarily related to gain because some pigs placed in the doubly deficient Group 4 did exhibit adequate weight gain. The composition of this gain was not investigated.

Thus, the complex interrelationship between TH and IGF-I actions and their influence on growth needs to be further investigated.

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PROMENA U NAČINU VEZIVANJA INSULINU-SLIČNOG FAKTORA RASTA (IGF-I) U SERUMU SVINJA PRATI SMANJENI PRIRAST USLOVLJEN NISKIM KONCENTRACIJAMA TIREOIDNIH HORMONA I/ILI IGF-I

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SADRŽAJ

Način vezivanja IGF-I iz seruma za vezujuće proteine određivan je kod tromesečne prasadi sa vrlo različitom koncentracijom ukupnog IGF-I i tireoidnih hormona (TH). Obe promenljive su bile u pozitivnoj korelaciji sa brzinom prirasta. Serumi prasadi koji su imali visoku koncentraciju IGF-I (> 13.1 nmol/l), bez obzira na nivo TH, nisu pokazivali različito vezivanje obeleženog IGF-I, što je utvrđeno hromatografskim i elektroforetskim metodama. Kod seruma sa niskom koncentracijom IGF-I intenzivnije je bilo vezivanje za vezujući protein-2 (IGFBP-2), odnosno za serumske proteine od 40 kDa. Pošto je pri visokim TH koncentracijama vezivanje obeleženog IGF-I za 150 kDa frakciju bilo nepromenjeno, odnos ukupno vezanog i slobodnog IGF-I u serumu bio je znatno veći nego kod ostale prasadi. Pri niskim TH koncentracijama vezivanje IGF-I za 150 kDa frakciju je bilo smanjeno, što se odrazilo kao promena u međusobnom odnosu dve vezane frakcije u poređenju sa ostalim serumima. Dinamika i/ili intenzitet vezivanja IGF-I u svinjskom serumu, prema tome, mogu biti promenjeni na dva načina u zavisnosti od TH statusa. Rezultati ukazuju na različite puteve koji dovode do smanjenog težinskog prirasta, a koji su uslovljeni odvojenim ili kombinovanim efektom sniženih koncentracija IGF-I i TH u serumu.