Animal Science 2004, 79: 85-92

1357-7298/04/30440085$20·00

Circulating levels of insulin-like growth factor-1 and associated binding proteins in plasma and mRNA expression in tissues of growing pigs on a low threonine diet

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Abstract

The aim was to determine whether dietary threonine levels affected hepatic insulin-like growth factor-1 (IGF-1) mRNA expression as well as plasma IGF-1 concentration and IGF binding protein (IGFBP) profile in growing pigs. Two male 6-week-old pigs from each of seven litters were used. Each littermate was assigned to one of two diets, control or low threonine (LT), providing per kg 14·3 MJ digestible energy in both diets, 170 g protein in the control diet and 167 g protein in the LT diet. The control diet contained all essential amino acids in the recommended amounts, including 8·2 g threonine per kg. The LT diet was similar but contained only 5·1 g threonine per kg. Pigs were pair-fed these diets for 3 weeks. Growth rate and food efficiency of pigs given the LT diet were significantly lower than those of pigs given the control diet ($P < 0·001$). Plasma IGF-1 concentration of pigs given the LT diet was proportionately 0·44 lower than that of pigs given the control diet ($P < 0·01$). Plasma free threonine concentration of pigs given the LT diet was lower than that of the pigs given the control diet ($P < 0·001$). Plasma IGFBP2 level of pigs given the LT diet was significantly higher than that of pigs given the control diet ($P < 0·05$). Pigs given the LT diet had a significantly lower plasma IGFBP3 level compared with their littermates given the control diet ($P < 0·05$) suggesting that clearance rate of circulating IGF-1 was higher in the LT group. Dietary threonine level did not affect IGF-1 mRNA abundance in the liver. It is concluded that lower plasma IGF-1 level caused by reduced dietary threonine level may have been partly due to increased clearance rate of circulating IGF-1 but not due to IGF-1 gene expression in the liver.

Keywords: *insulin-like growth factor, pigs, threonine.*

Introduction

Nutritional status is known to be a key factor regulating circulating level of insulin-like growth factor-1 (IGF-1) and its hepatic messenger RNA (mRNA) expression. Takenaka *et al.* (2000) showed that restriction in a single amino acid in the diet significantly reduced plasma IGF-1 in rats. More recently, it was reported that plasma IGF-1 concentration was consistently lower in growing chickens subjected to methionine deficiency (Carew *et al.*, 2003). However, the influence of dietary amino acid level on the growth hormone-insulin-like growth factor (GH-IGF) axis in pigs has not been well understood. Therefore, we conducted an experiment to elucidate the influence of dietary lysine, a major limiting amino acid in pig diets, on the activity of the GH-IGF axis (Katsumata *et al.*, 2002). In that previous study, we found that a low-lysine diet significantly reduced plasma IGF-1 concentration whereas it did not affect IGF-1 mRNA expression in the liver, which is likely to be the most

significant organ producing the circulating IGF-1 (Murphy *et al.*, 1987). From these observations, we concluded that the reduction in plasma IGF-1 caused by reduced dietary lysine might have been partly due to suppression of post-transcriptional events of IGF-1 expression. We also observed that pigs given a low-lysine diet had lower plasma IGFBP3, a significant function of which is to prolong the half-life of circulating IGF-1 (Guler *et al.*, 1989). This suggests that increased clearance rates of circulating IGF-1 in pigs given the low-lysine diet might have been involved in the response.

Our knowledge of the influence of other amino acids on IGF-1 mRNA expression in animals is limited. Although Takenaka *et al.* (2000) determined effects of single amino acids in diets on IGFBP1 mRNA expression in the liver of rats, they did not determine those on IGF-1 mRNA expression. As far as we know, only *in vitro* studies have been conducted. Limitation of methionine level in culture media to 0·20 of its physiological concentration in sheep plasma did not affect basal IGF-1 mRNA expression in ovine hepatocytes (Stubbs *et al.*, 2002). Only a very low concentration of lysine in cultured media of porcine hepatocytes, that was 0·25 of normal concentration found in rat plasma, was necessary for maximum gene expression of IGF-1, whereas the effects of some other amino acids including threonine, which is also a major limiting amino acid in pig diets, were dose dependent (Brameld *et al.*, 1999). A response observed in an *in vitro* cultured cell study may be different from that observed in *in vivo* study. However, we could not deny a possibility that differences in responses of IGF-1 mRNA expression in the liver to lysine and threonine would be found *in vivo* as well.

Evidence suggests that IGF-1 up-regulates glucose transporter-4 (GLUT4) expression in various tissues (Valverde *et al.*, 1999; Maor and Karnieli, 1999; Cheng *et al.*, 2000). We found that expression of GLUT4 in porcine skeletal muscle was up-regulated both in mRNA and protein levels by a low-lysine diet (Katsumata *et al.*, 2001). Plasma IGF-1 concentration was considerably lower in these pigs as previously reported (Katsumata *et al.*, 2002). Thus, the enhancement in GLUT4 mRNA expression caused by reduced dietary lysine seems to contradict a postulated function of IGF-1 in up-regulating GLUT4 expression. Factors other than IGF-1 may play a rôle in up-regulation of GLUT4 expression caused by reduced dietary lysine. We may be able to give further insights into this alteration in GLUT4 expression by dietary lysine through determining influence of other amino acids in diet on GLUT4 expression in porcine muscle.

The aim of present study was therefore to elucidate the influence of dietary threonine on hepatic IGF-1 mRNA expression as well as on plasma IGF-1 concentration and IGFBP profile. In addition, apart from studying components of the GH-IGF axis, we decided to determine expression of GLUT4 mRNA in *longissimus dorsi* (*l. dorsi*) muscle in order to extend our findings about the influence of dietary lysine on GLUT4 to dietary threonine.

Material and methods

Animals and design

Animals used and design of the experiment were similar to our previous study (Katsumata *et al.*, 2002). Seven litters of pigs, Duroc × (Large White × Landrace), were investigated. Pigs were weaned at 4 weeks of age, and two males of similar body weight were then selected from each litter. The pigs were transferred to an air-conditioned room 1

Table 1 *Composition of the control and the low-threonine (LT) diets (g/kg)*

	Diet	
	Control	LT
Ingredients		
Maize	620	620
Soya-bean meal	10	10
Wheat protein	85	85
Skim milk	100	100
Maize starch	73·1	76·0
Sugar	20	20
Soya-bean oil	42·5	42·5
Isoleucine	0·73	0·73
HCl-lysine	8·8	8·8
DL-Methionine	1·65	1·65
Threonine	3·1	0
Tryptophan	0·54	0·54
Valine	1·15	1·15
Tricalcium phosphate	20	20
Sodium chloride	5	5
Vitamin and mineral mix†	4·5	4·5
Antibiotics‡	4	4
Chemical composition		
Crude protein	170	167
Digestible energy (MJ/kg diet)	14·3	14·3
Lysine	12·5	12·5
Threonine	8·2	5·1

† Providing (mg/kg diet): retinyl acetate 5·2, cholecalciferol 0·1, DL-α-tochopheryl acetate 15·0, thiamin nitrate 1·5, riboflavin 10·5, pyridoxine hydrochloride 1·5, calcium pantothenic acid 16·4, nicotinamide 9·0, choline chloride 86·4, Mn 75·0 (manganese sulphate), Zn 75·0 (zinc carbonate), Cu 75·0 (copper sulphate), Fe 75·0 (iron sulphate), I 1·5 (calcium iodate).

‡ Providing 100 mg tyrocin and 100 mg spiramycin per kg diet.

week before the study and housed in pairs for the first 1 or 2 days and then kept in separate pens to allow careful control of food intake. At 6 weeks of age, each littermate was assigned to one of two diets (Table 1), control or low threonine (LT). Average initial live weights of pigs were 11·9 and 11·6 kg for the control and the LT groups, respectively. A basal diet containing 167 g protein per kg diet was first formulated. For the control diet, additional threonine was then added to give a level of 8·7 g/kg diet to meet the requirement for threonine. This added threonine was replaced by added maize starch in the LT diet. Thus, the crude protein contents were 170 and 167 g/kg for the control and the LT diets, respectively. The control diet contained all essential amino acids in the recommended amounts including threonine (National Research Council, 1998). The LT diet was similar to the control diet but contained only 5·1 g threonine per kg diet. The diets were isoenergetic, providing 14·3 MJ digestible energy per kg diet. The pigs were housed at an ambient temperature of 26°C for the 3-week experimental period. A pair-feeding protocol was used. The amount of dietary allowance was increased as the pigs grew, and final daily intake was 1000 g. The food was provided as three meals per day, at 09 : 00, 13:00 and 16:00 h, and water was always available. Average final body weights were 22·5 and 20·4 kg for the control and the LT groups, respectively.

At the end of experiment, pigs were sedated with an intramuscular injection of ketamine hydrochloride (2 ml Ketalar 50 per animal, 57·6 g/l; Sankyo, Tokyo, Japan), and then deeply anaesthetized by intracardiac injection of pentobarbital sodium (4 ml Nembutal per animal, 50 g/l; Dinabot, Osaka, Japan). Blood sampling was carried out by cardiac puncture and pigs were killed by exsanguinations from the carotid artery. Samples of liver and *l. dorsi* muscle were dissected rapidly, divided into 5-g portions, frozen in liquid nitrogen and stored at –80°C until analysis for IGF-1, type I IGF-1 receptor, growth hormone receptor (GHR), and GLUT4 mRNA expression. Plasma was stored at -20°C until analysis for glucose, free amino acids, IGF-1, and IGFBP. All of the tissue and plasma samplings were carried out 18 to 20 h after the last meal. All procedures were examined and approved by the annual research project examination committee of National Agricultural Research Centre for Kyushu Okinawa Region. The examination committee of the Bio-Design Project of the Ministry of Agriculture, Fisheries, and Forestry, Japan carried out further assessment of the experimental procedure.

Assessment of plasma IGF-1 concentrations and IGFBP profile

Details of the assessment have been described elsewhere (Katsumata *et al.*, 2002). Plasma IGF-1 concentration was measured by radio-immuno-assay (RIA) with an IGF-1 reagent pack (Amersham Pharmacia Biotech, Little Chalfont, UK). IGFBP profile was quantified by densitometry after SDS-PAGE separation, Western blot analysis and probing with ^{125}I-IGF–1.

Assessment of plasma glucose and free amino acid concentrations

Plasma glucose concentrations were assessed using a commercial kit (Glucose Test Wako, Wako Pure Chemical Industries Ltd, Osaka, Japan). In order to determine plasma free amino acid concentrations, plasma samples were deproteinized with one volume of 6% 5-sulphosalicylic acid. Supernatant fluids were filtered through a 0·45-μm syringe filter unit (ADVANTEC TOYO Ltd, Tokyo, Japan) to remove the traces of protein. Chromatographic separation of the amino acids was carried out on a Hitachi automatic amino acid analyser (L-8800, Hitachi Ltd, Tokyo, Japan).

Assessment of mRNA expression of IGF-1, type I IGF-1 receptor, GHR and GLUT4

Total RNA was extracted by the guanidine thiocyanate method (Chomczynski and Sacchi, 1987), and quantified by measuring absorbance at 260 nm as described previously (Katsumata *et al.*, 1999). RNase protection assay were carried out with 50 μg total RNA extracted from each tissue. Samples were hybridized with a small molar excess of the radiolabelled antisense class 2 IGF-1, type I IGF-1 receptor, GHR or GLUT4 (Weller *et al.*, 1993 and 1994; Urban *et al.*, 1994; Katsumata *et al.*, 1999) to ensure linearity of the assay with respect to RNA.

Statistical analysis

Statistical significance between the two treatment groups were assessed by analysis of variance for a randomized-block design, where litter was block and dietary threonine level was main effect, using the GLM procedures of Statistical Analysis Systems Institute (1988). Results are presented as means ± pooled s.e. Differences with probabilities of $< 0{\cdot}05$ were considered significant.

Results

Growth rates, food intake and plasma concentrations of IGF-1, glucose and free amino acids

Growth rate was slower in pigs given the LT compared with the control diet ($P < 0{\cdot}001$; Table 2). Food efficiency was also lower in pigs given the LT

Table 2 *Effects of a low-threonine (LT) diet on growth performance and plasma insulin-like growth factor-1 (IGF-1) and glucose concentrations in pigs (no. = 7) (values are means ± pooled s.e.)*

	Diet		
	Control	LT	s.e.
Daily gain (g)	514	424***	8
Food efficiency	0·71	0·59***	0·01
IGF-1 (ng/ml)	122	68**	7
Glucose (mmol/l)	7·6	7·0	0·3

diet ($P < 0·001$; Table 2). Plasma IGF-1 concentration was lower in pigs given the LT diet ($P < 0·01$; Table 2). Although average plasma glucose concentration of pigs given the LT diet tended to be lower than that of pigs given the control diet, the difference was not significant ($P = 0·166$, Table 2). Plasma free threonine concentration of pigs given the LT diet was greatly lower than that of the pigs given the control diet ($P < 0·001$, Table 3). Interestingly, concentrations of valine, isoleucine, phenylalanine, lysine, and histidine were significantly higher in the pigs given the LT compared with the control diet ($P < 0·01$, Table 3).

Table 3 *Effects of a low-threonine (LT) diet on plasma free amino acid concentration (μmol/l) in pigs (no. = 7) (values are means ± pooled s.e.)*

	Diet		
	Control	LT	s.e.
Threonine	358	52***	27
Valine	140	196***	6
Methionine	38	42	4
Isoleucine	65	90**	4
Leucine	143	143	1
Phenylalanine	52	67**	3
Lysine	76	191**	18
Histidine	19	46**	3

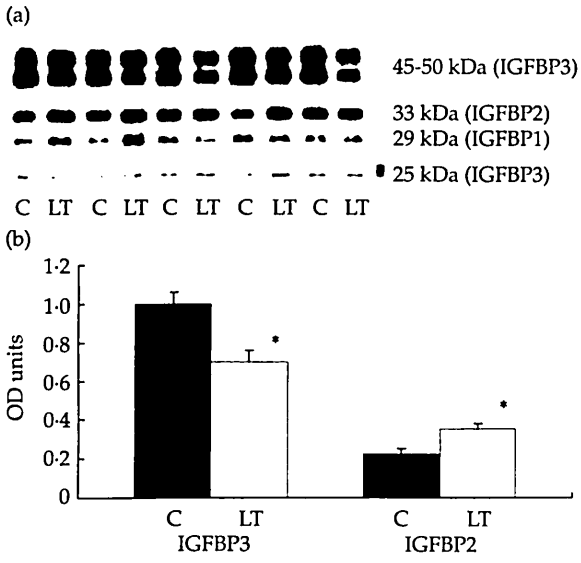

Figure 1 Plasma insulin-like growth factor binding proteins (IGFBPs). (a) Autoradiograph from Western ligland blotting analysis with ^{125}I-IGF-1 as the probe, illustrating plasma IGFBP profiles from five littermate pairs of pigs given the control (C) or the low-threonine (LT) diet. The 25-, 29-, and 33-kDa bands are referred to as IGFBP4, IGFBP1, and IGFBP2, respectively. The doublet bands at 45 to 50 kDa are referred to as IGFBP3. (b) Plasma IGFBP3 and IGFBP2 concentrations from pigs given the control (C) or the low-threonine (LT) diet. Bars are means ± pooled s.e. (no. = 5). The mean IGFBP3 of the control diet group is expressed as 1·0. Asterisks indicate a significant difference from the control group for each IGFBP: *P<0·05.

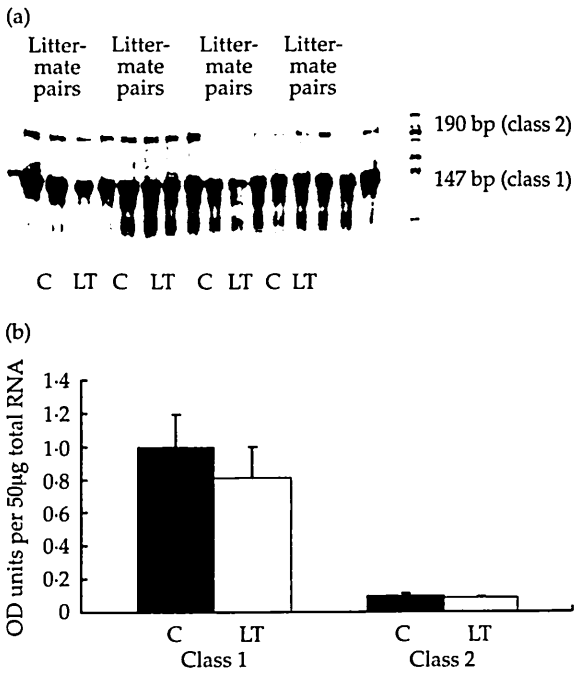

Figure 2 Hepatic insulin-like growth factor-1 (IGF-1) mRNA expression. (a) Autoradiograph from Rnase protection assay illustrating hepatic IGF-1 mRNA expression in four littermate pairs of pigs given the control (C) or the low-threonine (LT) diet. The 190-base band arises due to protection of IGF-1 mRNAs containing exon 2 and 3 and represents class 2 transcripts. The 147-base band is due to protection by exon 3 and represents class 1 transcripts. (b) Hepatic IGF-1 mRNA expression in pigs given control (C) or the low-threonine (LT) diet. Bars are means ± s.e. (no. = 7). The least-square mean of class 1 transcripts from control diet group is expressed as 1·0. All measurements were carried out in duplicate.

Plasma IGFBP concentration

A representative autoradiograph of plasma IGFBP profile obtained from five littermate pairs is presented in Figure 1a. As shown by Dauncey *et al.* (1993), distinct bands were observed with molecular weights of 25, 29, 33 and a doublet at 45 to 50 kDa, corresponding to IGFBP4, IGFBP1, IGFBP2 and IGFBP3, respectively. No clear-cut effects due to dietary threonine were observed in plasma levels of IGFBP4 and IGFBP1. However, plasma IGFBP2 level of pigs given the LT diet was significantly higher than that of pigs given the control diet ($P < 0.05$; Figure 1b). Pigs given the LT diet had lower plasma IGFBP3 level than their littermates given the control diet ($P < 0.05$; Figure 1b), which is consistent with lower plasma IGFBP3 level of pigs given a low-lysine diet in our previous study.

Messenger RNA expression of IGF-1, type I IGF-1 receptor, GHR and GLUT4

Figure 2a represents an autoradiograph obtained using the class 2-specific riboprobe for porcine hepatic IGF-1, showing protected bands of 147 and 190 bp representing class 1 and 2 transcripts, respectively (Weller *et al.*, 1993 and 1994). Dietary threonine levels did not affect any of these estimates of IGF-1 mRNA expression (Figure 2b). Dietary threonine levels did not affect type I IGF-1 receptor mRNA abundance in *l. dorsi* muscle (Figure 3). Pigs given the LT diet tended to have higher GHR mRNA abundance in *l. dorsi* muscle ($P = 0.1034$, Figure 3). GLUT4 mRNA abundance in *l. dorsi* muscle of pigs given the LT diet was twice as high as that of the control pigs ($P < 0.01$, Figure 3).

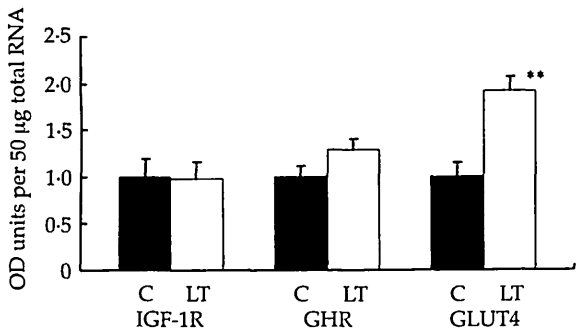

Figure 3 Type I IGF-1 receptor (IGF-1R), growth hormone receptor (GHR), and glucose transporter 4 (GLUT4) mRNA expression in *l. dorsi* muscle of pigs given the control (C) or the low-threonine (LT) diet. Bars are means ± pooled s.e. (no. = 7). The least-square means from the control diet groups are expressed as 1·0. Asterisks indicate a significant difference from the control group: $^{**}P<0.01$.

Discussion

Very low concentrations of lysine in the culture media, specifically 0·25 of the normal concentration found in rat plasma, were sufficient for maximal expression of IGF-1 mRNA in porcine hepatocytes, whereas the effect of threonine in culture media on class 1 IGF-1 mRNA expression in porcine hepatocytes was dose dependent (Brameld *et al.*, 1999). These results suggested that responses of IGF-1 gene expression to lysine and threonine may be different; low levels of threonine in the pig diet may cause lower hepatic IGF-1 mRNA expression. However, in the present study, the LT diet did not alter hepatic IGF-1 mRNA expression. One possible explanation for this is the difference in the range of threonine tested. Brameld *et al.* (1999) tested five dose levels of threonine from 0 to twice the normal concentration found in rat plasma, whereas we tested only two concentrations of dietary threonine, the one was 8·2 g threonine per kg diet which met the amount of threonine (7·4 g/kg diet) recommended by NRC (1998) and the other was 5·1 g threonine per kg diet which was 0·68 of the recommendation. The other possibility that we have to consider is the magnitude of deficiency in threonine of the LT diet since it might not be large enough to affect hepatic IGF-1 mRNA expression. Thus, we measured plasma free amino acids concentration as an indicator. Indeed, plasma threonine concentration of pigs given the LT diet was only about 50 µmol/l, which was lower than those with which Brameld *et al.* (1999) observed dose dependency of IGF-1 mRNA expression to threonine concentrations. Therefore, in terms of IGF-1 mRNA expression, it seems that responses of porcine hepatocytes to threonine concentrations are different between *in vivo* and *in vitro*. Interestingly, plasma concentrations of some other essential amino acids were higher in the LT diet group. The higher plasma concentrations of these amino acids might have been due to the reduced requirements of muscle protein synthesis caused by the inadequate dietary level of threonine. However, it is not known whether these higher concentrations of some amino acids in the plasma play a rôle in maintaining IGF-1 mRNA expression in the liver.

Although the LT diet did not affect hepatic IGF-1 mRNA expression, plasma concentrations of IGF-1 and IGFBP3 were lower in pigs given the LT diet than those in the pigs given the control diet. IGF-IGFBP3-acid labile subunit complex is thought to act as a storage pool for circulating IGF-1 (Guler *et al.*, 1989). This suggests that increased clearance rate of circulating IGF-1 causes the lower plasma IGF-1 concentration in pigs given the LT diet as we suggested in the previous study (Katsumata *et al.*,

2002). However, as it is well established that IGF-1 mRNA is found in most tissues and IGF-1 synthesis is ubiquitous (Pell and Glassford, 1996), we cannot rule out the possibility that a reduction in IGF-1 mRNA expression in tissues other than the liver contributed to the lower plasma IGF-1 concentration caused by the low-threonine diet.

There are already several reports on nutritional regulation of circulating IGFBP2 or IGFBP2 mRNA expression in tissues. Food restriction resulted in a high concentration of plasma IGFBP2 in rats and guinea pigs (Donovan *et al.*, 1991; Sohlström *et al.*, 1998). Donovan *et al.* (1991) observed up-regulation of hepatic IGFBP2 mRNA expression due to food restriction. In a recent study on IGFBP2 mRNA expression in the chicken, it was observed that food deprivation caused high IGFBP2 mRNA abundance in the gizzard and liver (Kita *et al.*, 2002). Although IGF-1 IGFBP3-acid labile subunit complex is thought to function as a storage pool of circulating IGF-1 thus prolonging its half-life, the rôle of IGFBP2 in controlling concentration of circulating IGF-1 is still not clear. Serum IGFBP2 concentration in transgenic mice over-expressing IGFBP2 was increased three-fold compared with control animals, whereas serum concentrations of IGF-1 and IGF-2 were not affected (Hoeflich *et al.*, 1999), suggesting that effects of IGFBP2 on concentration or half life of circulating IGFs are limited. However, a rôle for IGFBP2 as a negative regulator of postnatal growth was suggested by the same author because postnatal live-weight gain of mice over-expressing IGFBP2 was smaller than that of control animals (Hoeflich *et al.*, 1999). Food restriction reduced plasma IGF-1 concentration, and also a negative correlation between weight gain and plasma IGFBP2 concentration was observed in guinea pigs (Sohlström *et al.*, 1998). Pigs with lower live-weight gain due to low dietary threonine level had lower plasma IGF-1 and higher plasma IGFBP2 concentration in the present study. Therefore, although its function still remains unclear, it seems that higher circulating concentration of IGFBP2 is associated with at least lower live-weight gain in young growing animals.

Regulation of IGF-1 receptor mRNA expression and its binding capacity in response to nutritional status varies among animal species and tissues. Food deprivation for 5 days increased expression of IGF-1 receptor mRNA in muscle, kidney and liver in growing chickens (Matsumura *et al.*, 1996). Specific binding of ^{125}I-IGF-1 in skeletal muscle increased with decreasing milk intake in piglets of 7 days of age whereas levels of milk intake did not affect the binding in liver (Louveau and Le Dividich, 2002). Specific binding of ^{125}I-IGF-1 to liver membrane was higher in food-restricted gilts compared with well fed gilts, whereas it did not differ between the two levels of nutrition in other tissues including skeletal muscle (Louveau *et al.*, 2000). Effect of protein nutrition was also reported. IGF-1 receptor mRNA expression and the number of receptors in various tissues of rats were differently regulated by quality of dietary protein when comparison was made between a casein based diet and a gluten based diet, the latter being marginally deficient in lysine and threonine (Takenaka *et al.*, 1996). Further, the amount of IGF-1 receptor was relatively constant in most tissues (Takenaka *et al.*, 1996). Based on these observations, Takenaka *et al.* (1996) concluded that it might be plasma IGF-1 and IGFBPs mainly regulating the action of IGF-1 in tissues in response to protein nutrition rather than IGF-1 receptor. Dietary threonine level did not affect type I IGF-1 receptor mRNA abundance in *l. dorsi* muscle in the present study. Hence, as was suggested by Takenaka *et al.* (1996), it seems that plasma IGF-1 and IGFBPs mainly regulate growth-promoting action of IGF-1 at least in skeletal muscle responding to dietary threonine levels.

GLUT4 mRNA expression in *l. dorsi* muscle was up-regulated by the low-threonine diet in the present study. Thus, we can extend our findings about the influence of dietary lysine on GLUT4 mRNA expression (Katsumata *et al.*, 2001) to dietary threonine. This effect of amino acids is unlikely to be mediated by IGF-1 because this growth factor up-regulates GLUT4 expression in various tissues (Valverde *et al.*, 1999; Maor and Karnieli, 1999; Cheng *et al.*, 2000). A possible explanation for the up-regulation of GLUT4 caused by reduced dietary amino acid level may be oxidative capacity of muscle. Kong *et al.* (1994) reported that the level of GLUT4 expression varied among muscle fibres; slow oxidative fibre > fast oxidative glycolytic fibre > fast glycolytic fibre. It was suggested in our recent study that muscle from pigs given a low-lysine diet had higher oxidative capacity compared with that from pigs given a diet meeting requirements for all essential amino acids (Katsumata *et al.*, 2003b). Higher concentrations of five essential amino acids in plasma from pigs given the low-threonine diet in the present study might have contributed to enhancement of the oxidative capacity of tissues because the carbon skeleton of these excess amino acids would be oxidized by the combined action of the tricarboxylic acid cycle and oxidative phosphorylation (Rawn, 1989). These observations lead us to assume that higher GLUT4 mRNA expression in muscle caused by the reduced level of dietary amino acid might have been partly due to

higher oxidative capacity of the muscle. Consistently higher mRNA expression of GHR in muscle due to lower dietary lysine level in our previous study (Katsumata *et al.*, 2002) and threonine level in the present study may be similarly explained because muscle with higher oxidative capacity has higher expression of GHR mRNA in the pig (Katsumata *et al.*, 2000). However, direct evidence supporting this explanation has not been available so far. Underlying mechanisms for the up-regulation of mRNA expression of GLUT4 and GHR in muscle by reduced dietary amino acid level now need to be established.

In conclusion, a low-threonine diet significantly reduced plasma IGF-1 and IGFBP3 levels whereas it did not affect hepatic IGF-1 mRNA expression. These findings are in accordance with our previous findings on a low-lysine diet. An approximately 0·30 to 0·40 proportional shortage in a single amino acid in a pig diet compared with its requirement does not seems to be severe enough to suppress IGF-1 mRNA expression in the liver. Increased clearance rate of circulating IGF-1, indicated by lower IGFBP3 level, may be a reason for the lower plasma IGF-1 caused by restriction of a single amino acid in a pig diet.

Acknowledgements

We thank Dr M. Matsuzaki, National Agricultural Research Center for Kyushu Okinawa Region, for supporting the RIA of IGF-1 and the analysis of IGFBP profiles. The cDNA for constructing the riboprobe used for RNase protection assay analysing IGF-1 receptor was kindly provided by Dr R. J. Urban, University of Texas Medical Branch, Galveston, Texas, USA. This work was supported by a research grant from Bio-design Project of the Ministry of Agriculture, Fisheries and Forestry, Japan.

Part of this work was previously published in abstract form and presented in oral and poster forms (Katsumata *et al.*, 2003a).

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(*Received* 10 *December* 2003—*Accepted* 2 *March* 2004)