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Nutritional Regulation of GLUT Expression, Glucose Metabolism, and Intramuscular Fat Content in Porcine Muscle*

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ABSTRACT : We conducted a series of investigations in order to elucidate role of nutritional status in regulating GLUT expression and energy metabolism in porcine muscle. Firstly, the role of mild undernutrition in regulating muscle GLUT gene expression and function was studied in growing pigs (3 wk of age) on a high (H) or low (L) food intake (H = 2L) at 35°C or 26°C. Low food intake selectively upregulates GLUT1 and GLUT4 gene expression; mRNA levels were elevated in longissimus dorsi (*L. dorsi*) and *rhomboideus* muscles but not in diaphragm or cardiac muscles. Our next step was to determine whether dietary lysine, a major primary limiting amino acid in diets for pigs, affects muscle GLUT4 expression. Pigs of 6 wk of age were pair-fed a control or low lysine (LL) diet. The control diet contained optimal amounts of all essential amino acids, including 1.15% lysine. The LL diet was similar but contained only 0.70% lysine. GLUT4 mRNA expression was upregulated by the LL diet in *L. dorsi* and *rhomboideus* muscles, whereas that in cardiac muscle was unaffected. GLUT4 protein abundance was also higher in *rhomboideus* muscle of animals on the LL diet. We conducted another investigation in order to elucidate effects of the LL diet on post-GLUT4 glucose metabolism. Activity of hexokinase was unaffected by dietary lysine levels while that of citrate synthase was higher both in *L. dorsi* and *rhomboideus* muscles of pigs fed on the LL diet. Glucose 6-phosphate content was higher in *L. dorsi* msucle in the LL group. Glycogen content was higher both in *L. dorsi* and *rhomboideus* muscles in the LL group. Further, we determined the effects of dietary lysine levels on accumulation of intramuscular fat (IMF) in *L. dorsi* muscle of finishing pigs. A low lysine diet (lysine content was 0.40%) meeting approximately 70% of the requirement of lysine was given to finishing pigs for two months. IMF contents in *L. dorsi* of the pigs given the low lysine diet were twice higher than those of the pigs fed on a control diet (lysine content was 0.65%). Finally, we proved that a well known effect of breadcrumbs feeding to enhance IMF of finishing pigs could be attributed to shortage of amino acids in diets including breadcrumbs. (**Key Words :** GLUT, Intake, Lysine, Glucose Metabolism, Porcine Muscle)

INTRODUCTION

Two major facilitative glucose transporter proteins in muscle, GLUT1 (insulin-independent) and GLUT4 (insulin-dependent) are recognized to play a key role in controlling glucose uptake in muscle. Since muscle is the main peripheral site of nutrient oxidation and insulin action, it is extremely important to elucidate factors regulating expression of these glucose transporter proteins. Prolonged food deprivation, a high fat diet, severe cold exposure, and thyroid hormone administration (Charron et al., 1990; Nikami et al., 1992; Neufer et al., 1993; Kim et al., 1994; Weinstein et al., 1994) have been reported to alter GLUT expression. Most treatments in these studies were extreme. Further, all these results were obtained from investigations using laboratory rodents. No information was available on the influence of postnatal nutritional status in regulating GLUT expression in farm animals. Therefore, we conducted five investigations to determine the role of postnatal nutritional status in regulating GLUT expression and energy metabolism in porcine muscle (Katsumata et al., 1999, 2001, 2003, 2005; Ieiri et al., 2007). The first investigation was undertaken to determine the role of mild postnatal undernutrition, which enables growth to continue but at a reduced rate, in regulating GLUT gene expression during postnatal development. We found that mild postnatal

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Table 1. Influence of food intake and environmental temperature on growth rate and food conversion efficiency during postnatal development

	35H	35L	26H	26L	Pooled SEM
Growth rate (g/d)	323	170	320	146	3
Food conversion ratio (g food intake /g weight gain)	1.05	1.04	1.09	1.24	0.02

The overall effect of food intake on growth rate was significant ($p<0.01$). There were statistically significant interactions between the two environmental temperatures; the effect of food intake on growth rate was greater at 26°C than at 35°C ($p<0.01$). Pigs at 26°C on the low food intake had higher food conversion ratios than the three other groups ($p<0.05$).

undernutrition up-regulated GLUT gene expression in a muscle-specific manner. This suggested that undernutrition was associated with up-regulation of GLUT expression. However, the role of specific nutrients in this response was unknown. Hence, we carried out the second investigation to determine whether dietary amino acids affect GLUT4 gene and protein expression in porcine muscle. The focus was on lysine because it is a major primary limiting amino acid in diets for pigs. The results obtained in the second investigation demonstrated that a low lysine diet selectively up-regulated muscle GLUT4 gene and protein expression in growing pigs. However, the role of dietary lysine level in regulating post-GLUT4 glucose metabolism was unknown. Thus, we decided to conduct a further study to elucidate effects of a low lysine diet on post-GLUT4 glucose metabolism in porcine skeletal muscle. It was demonstrated in the third investigation that post-GLUT4 glucose metabolism in association with oxidative capacity of muscle was affected by dietary lysine levels. Furthermore, positive effects of diets which are deficient in amino acids, including lysine, on intramuscular fat (IMF) content was proposed (Ellis and McKeith, 1999). Hence, we hypothesized that a reduced intake of lysine might promote IMF accumulation in porcine muscle. In order to test this hypothesis, we conducted the fourth investigation with finishing pigs (Katsumata et al., 2005). Finally, to examine our hypothesis that a well known effect of feeding breadcrumbs on IMF accumulation is attributed to shortage of amino acids in breadcrumbs, we conducted a feeding trial with finishing pigs (Ieiri et al., 2007).

MILD UNDER NUTRITION SELECTIVELY UP-REGULATES MUSCLE GLUT EXPRESSION

Six litters each of four male pigs aged 3 weeks were used. The littermates were assigned randomly to one of four treatments, with two levels of food intake: high (H) and low (L), where H = 2L, and two environmental temperatures: a high temperature (35°C) and a temperature close to thermal neutrality (26°C). Thus, there were four treatment groups

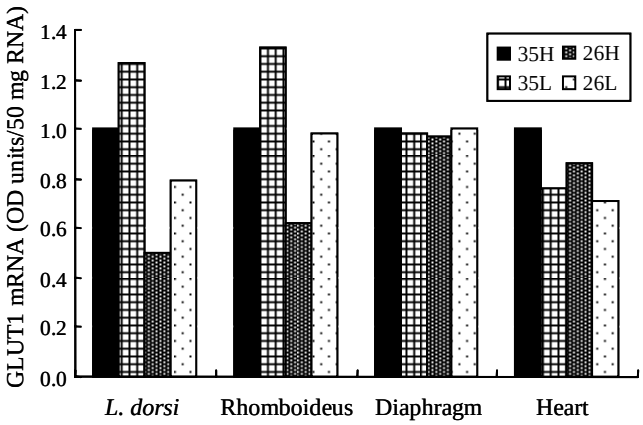

Figure 1. Regulation of GLUT1 gene expression by food intake and environmental temperature. Bars represent mean values. Analysis of variance indicated that the overall effect of food intake was significant in *L. dorsi* and *rhomboideus*: low>high; $p<0.05$ for both muscles. The overall effect of environmental temperature was also significant: 35°C>26°C; $p<0.01$ and $p<0.05$ for *L. dorsi* and *rhomboideus*, respectively. Neither food intake nor environmental temperature affected GLUT1 mRNA abundance in diaphragm and heart.

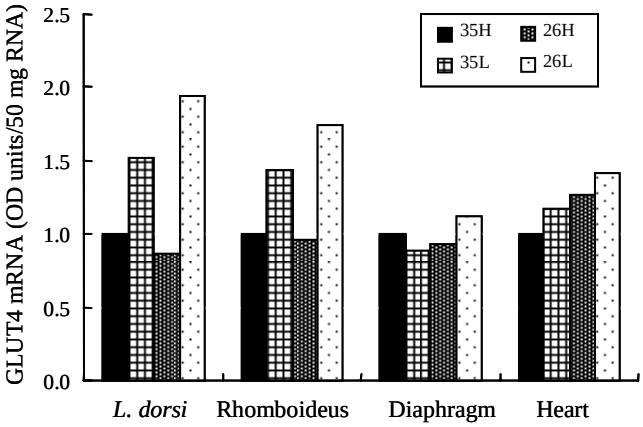

Figure 2. Regulation of GLUT4 gene expression by food intake and environmental temperature. Bars represent mean values. Analysis of variance indicated that the overall effect of food intake was significant in *L. dorsi* and *rhomboideus*: low>high; $p<0.001$ for both muscles. Neither food intake nor environmental temperature affected GLUT4 mRNA abundance in diaphragm and heart.

within each litter: 35H, 35L, 26H, and 26L. The pigs were raised for 4 weeks until assessment of GLUT gene expression and function. A commercially available diet (14 MJ GE/kg and 225 g crude protein/kg) was given daily at 9:30 AM and the amount provided was increased gradually with age. Daily food intakes were 150 g (H) and 75 g (L) at 3 weeks, and increased to 700 g (H) and 350 g (L) at 7 weeks. To determine GLUT gene expression in different skeletal and cardiac muscles, RNase protection assays were carried out using newly constructed species-specific riboprobes (Katsumata et al., 1999). To assess the function

Table 2. Influence of food intake and environmental temperature on *in vitro* uptake of 2-deoxy-glucose (2-DG) in an isolated muscle, and percentage increment of 2-DG uptake stimulated by insulin

	35H	35L	26H	26L	Pooled SEM
Basal (pmol/ml/20 min)	8.6	8.6	8.8	13.6	1.9
Insulin-stimulated (pmol/ml/20 min)	13.8	15.6	14.2	17.0	2.3
Percentage increment (%)	68.3	83.0	74.8	23.5	16.1

Means from four litters each of four animals are presented. P value for the interaction in percentage increment between food intake and environmental temperature = 0.07.

Table 3. Influence of dietary lysine level on growth rate and food conversion ratio in pigs during postnatal development

	Control	LL	Pooled SEM
Growth rate (g/d)	445	336	17
Food conversion ratio (g food intake/g weight gain)	1.43	1.86	0.09

Influence of dietary lysine level was statistically significant for both growth rate and food conversion efficiency ($p<0.01$).

of GLUTs in muscle, we measured *in vitro* 2-deoxy-glucose (2-DG) uptake in an isolated small muscle, flexor carpi radialis, from four litters, by a modification of a previously published method (Henriksen et al., 1990).

Table 1 shows results for the influence of food intake and environmental temperature on growth rate and food conversion ratio. Growth rates were higher in the high intake groups ($p<0.01$), and there were statistically significant interactions between the two environmental temperatures; the effect of food intake was greater at 26°C than at 35°C ($p<0.01$). Food conversion ratios of the 35H, 35L and 26H groups did not differ. Pigs at 26°C on the low food intake had a suboptimal energy balance and lower growth efficiencies than the three other groups ($p<0.05$). Figure 1 and 2 present results for regulation of GLUT gene expression by nutritional status and thermal environment. Analysis of variance indicated that the overall effect of food intake on GLUT1 mRNA levels was significant in *L. dorsi* and *rhomboideus*: low>high; $p<0.05$ for both muscles. The overall effect of environmental temperature on GLUT1 mRNA levels was also significant: 35°C>26°C; $p<0.01$ and $p<0.05$ for *L. dorsi* and *rhomboideus*, respectively. In *L. dorsi* and *rhomboideus*, the low food intake was associated with marked up-regulation in expression of GLUT4 ($p<0.01$). There was no overall effect of environmental temperature on GLUT4 gene expression. Although interactions between the two variables, food intake and environmental temperature, were not statistically significant, the effect of food intake on GLUT4 mRNA levels tended to be greater at 26°C than 35°C. Neither food intake nor environmental temperature affected mRNA levels of GLUT genes in diaphragm and heart. Table 2 shows the results for basal and insulin-stimulated 2-DG uptake in isolated muscle. Basal uptake was nearly identical in the 35H, 35L, and 26H groups, whereas in the 26L group it was about 50% greater. Mean values for insulin-stimulated 2-DG uptake showed a trend similar to that for GLUT4 mRNA. The low food intake resulted in a higher 2-DG uptake than did the high food intake, at both environmental temperatures. Further, the calculated percentage increment in 2-DG uptake after stimulation by insulin suggested that there was an interaction between food intake and environmental temperature ($p = 0.07$). The 26L group had the lowest percentage increment in 2-DG uptake after stimulation by insulin.

Before we conducted this investigation, attention had focused on the influence of extreme conditions on GLUT expression, such as prolonged food deprivation or high fat feeding (Charron et al., 1990; Neufer et al., 1993; Kim et al., 1994). The influence of mild conditions on GLUT expression was still unknown, and this was therefore an especially significant aspect of this investigation. We found that mild undernutrition, which enables growth to continue but at a reduced rate, resulted in muscle-specific up-regulation of GLUT gene expression. Further, the response was dependent not only on food intake but also on energy status. The suboptimal energy balance of pigs on a low food intake at 26°C was associated with further up-regulation of GLUT4 gene expression.

Our results indicate that up-regulation of GLUT4 gene expression is not necessarily associated with an increase in insulin-stimulated glucose uptake. The highest GLUT4 mRNA levels in *L. dorsi* and *rhomboideus* muscles occurred in animals kept at 26°C on a low food intake. Because the zone of thermal neutrality is dependent on energy intake, 26°C may sometimes have been below the critical temperature of the 26L pigs. During these times, energy balance would have been suboptimal, and further up-regulation of GLUT expression thus appears to occur when energy intake is limited in relation to its demand. Although basal glucose uptake was highest in the 26L pigs, they had the smallest increment in glucose uptake after stimulation by insulin and therefore a reduction in insulin sensitivity. This suggests that when energy balance is suboptimal, the population of both GLUT1 and GLUT4 located on the plasma membrane of muscle cells is relatively high, even in the basal state. In addition, the intracellular population of GLUTs, mainly GLUT4, is inadequate to increase glucose uptake in response to insulin when pigs are subjected to suboptimal energy balance. Thus, we suggest that not only GLUT4 gene expression but also the subcellular distribution of GLUTs is affected by energy balance.

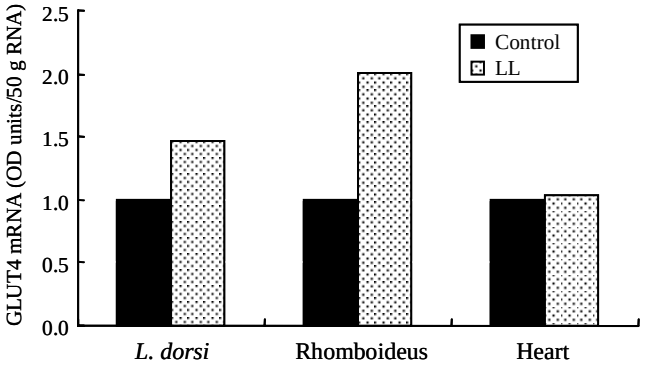

Figure 3. Regulation of GLUT4 gene expression by dietary lysine level. Effect of dietary lysine level was significant in *L. dorsi* and *rhomboideus* muscles: LL>control; $p<0.05$ for *L. dorsi* and $p<0.01$ for *rhomboideus*. Dietary lysine level did not affect GLUT4 gene expression in heart.

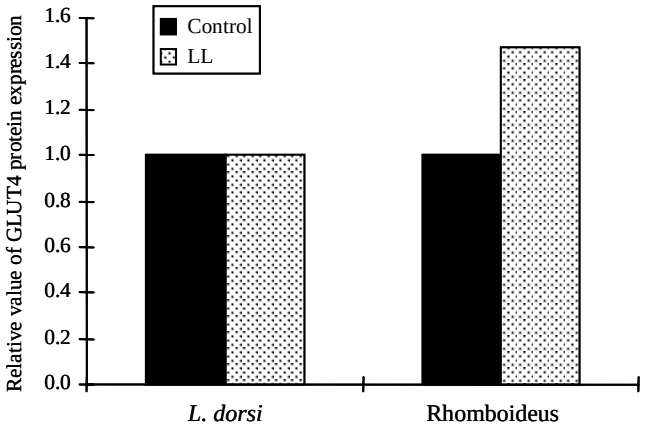

Figure 4. Regulation of GLUT4 protein expression by dietary lysine level. Effect of dietary lysine level was significant in *rhomboideus* muscle: LL>control; $p<0.05$. Dietary lysine level did not affect GLUT4 protein expression in *L. dorsi* muscle.

DIETARY LYSINE LEVEL PLAYS A ROLE IN REGULATING GLUT4 EXPRESSION

Eight litters each of two male pigs aged 6 weeks were used. Each littermate was assigned to one of two diets, low lysine (LL) or control. Details of the diets have been described elsewhere (Katsumata et al., 2002). In brief, the diets were isoenergetic and similar in protein content, providing 14.3 MJ DE/kg for both diets, 185 g protein/kg for the control diet and 180 g protein/kg for the LL diet. The control diet contained all essential amino acids in the recommended amounts, including 1.15% lysine. The LL diet was similar but contained only 0.70% lysine. Pigs were pair-fed these diets for 3 weeks. The amount of food was increased as the animals grew and the final daily intake was 900 g. The diet was provided as two meals per day, at 9 AM and 4 PM. The pigs were housed at an ambient temperature of 26°C, which is close to thermal neutrality. GLUT4 mRNA abundance in *L. dorsi*, *rhomboideus* and cardiac muscles were determined by RNase protection assay. GLUT4 protein level was measured by Western blotting analysis using a commercially available antibody (Biogenesis, Poole, UK).

Table 3 shows results for the influence of dietary lysine level on growth rate and food conversion ratio. Despite identical energy and protein intakes, growth rates were significantly lower in pigs fed on the LL compared with the control diet ($p<0.01$), hence food conversion ratios were significantly greater with the LL than the control diet ($p<0.01$). By contrast with the reduced growth rate, GLUT4 mRNA expression was up-regulated by the LL diet in *L. dorsi* ($p<0.05$) and *rhomboideus* ($p<0.01$) but cardiac muscle was unaffected (Figure 3). The considerable increase in *rhomboideus* muscle GLUT4 gene expression in pigs fed the LL diet was reflected by a 50% increase in GLUT4 protein level ($p<0.05$), whereas there were no effect on GLUT4 protein level in *L. dorsi* muscle (Figure 4).

To our knowledge, the results obtained in this second investigation were the first evidence that amino acid undernutrition results in up-regulation of GLUT4 gene and protein expression. Moreover, we have recently found that young growing pigs fed a low threonine diet have higher GLUT4 gene expression in *L. dorsi* muscle compared with pigs fed a control diet (Katsumata et al., 2004).

An important finding of the present two investigations was that overall food intake and dietary amino acid level affected GLUT gene expression in *L. dorsi* and *rhomboideus* muscles but not in diaphragm and heart. This may be explained in part by the specific functions of different muscles within the body. Diaphragm and heart play essential roles in respiratory and cardiovascular function, and these functions will not be compromised by defective glucose transport due to external factors such as undernutrition. By contrast, *L. dorsi* and *rhomboideus* have important roles in glucose and whole body energy metabolism, and the altered GLUT expression that occurs in response to nutrition will enable modification of metabolic fuel utilization.

In relation to the influence of protein nutrition on glucose uptake, a low protein diet can enhance peripheral insulin sensitivity (Crace et al., 1990; Ozanne et al., 1996; Reis et al., 1997). It is therefore possible that pigs fed on the LL diet, with higher GLUT4 levels in *L. dorsi* and *rhomboideus*, had higher glucose uptake in muscle as compared with pigs fed on the control diet. However, there is evidence indicating that the influences of absolute protein level and protein quality are not equivalent. According to Löhrke et al. (2001), growing pigs fed on a soy-protein based diet had a higher basal glucose uptake but a decrease in response to insulin compared with pigs fed on a casein based diet, suggesting that dietary protein of inferior quality results in desensitization of muscle glucose uptake to insulin. Since the LL diet in our study is comparable with

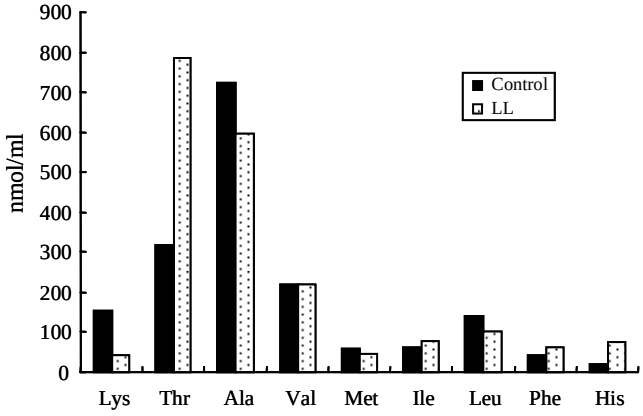

Figure 5. Influence of dietary lysine levels on contentrations of plasma free amino acid. Plasma concentrations of lysine, alanine and leucine were significantly lower in the LL group ($p<0.01$) whereas those of threonine, isoleucine, phenylalanine and histidine were higher in the LL group ($p<0.01$). Dietary lysine levels did not affect concentrations of valine and methionine.

the soy-protein based diet, it is possible that a higher basal glucose uptake and lower response to insulin occur at the same time in muscle from pigs fed on the LL diet. In order to have further insights into influence of dietary lysine levels on glycemia, influence of dietary lysine levels on plasma glucose concentration was determined in the third investigation.

DIETARY LYSINE LEVEL AFFECTS POST-GLUT4 GLUCOSE METABOLISM IN MUSCLE

We conducted a similar feeding trial as the second investigation described above; seven litters each of two barrows aged 6 weeks were used and each littermate was assigned to one of two diets, the LL diet and the control diet. At 9 weeks old, *L. dorsi* and *rhomboideus* muscles were sampled 16-17 h after the last meal. D-glucose (3 g/kg $BW^{0.75}$) was orally given to the pigs 1 h before the samplings in order to make the most of predicted up-regulation of GLUT4 expression. Activities of hexokinase (EC 2.7.1.1) and citrate synthase (EC 4.1.3.7) were

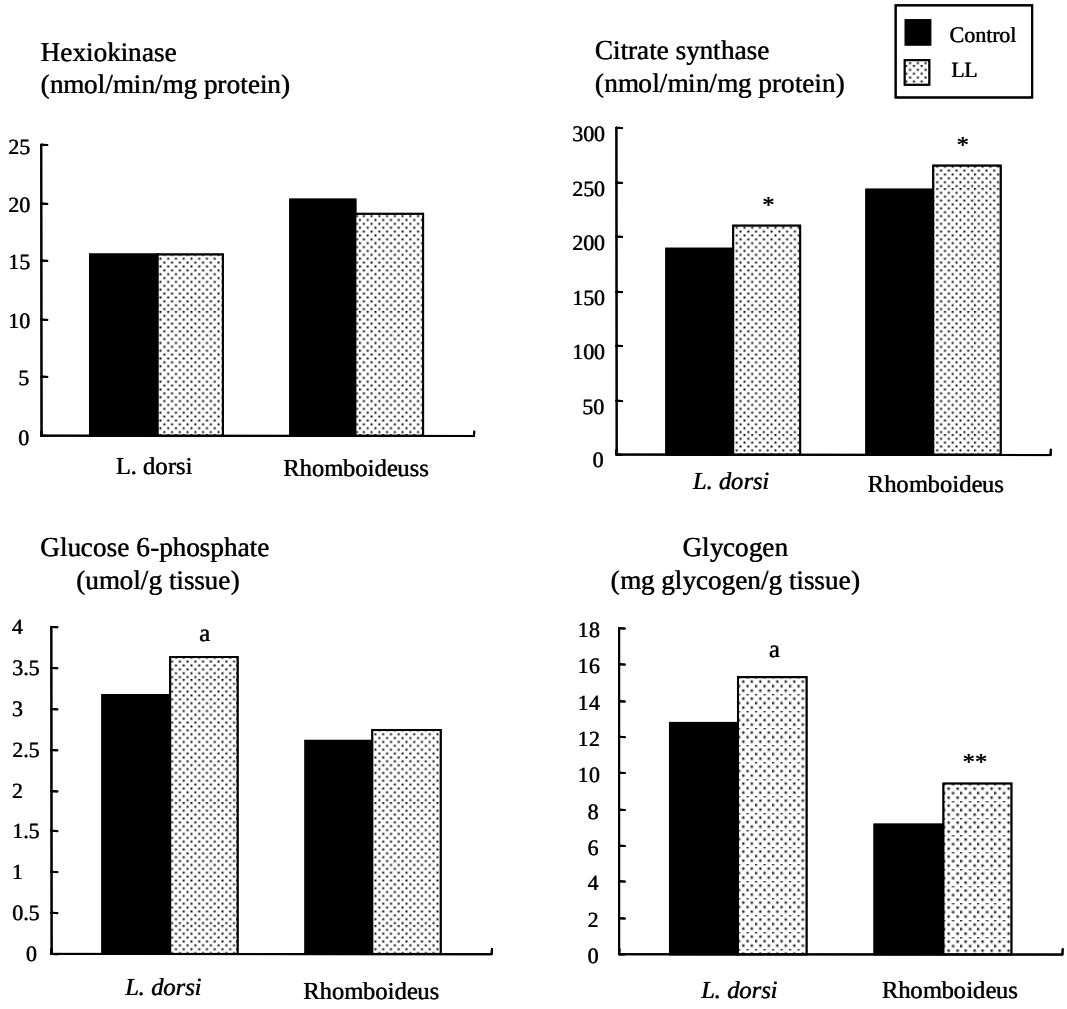

Figure 6. Activities of hexokinase and citrate synthase, and contents of glucose 6-phosphate and glycogen in *L. dorsi* and *rhomboideus* muscles. [a,] * and **; $p<0.10$, $p<0.05$, and $p<0.01$, respectively.

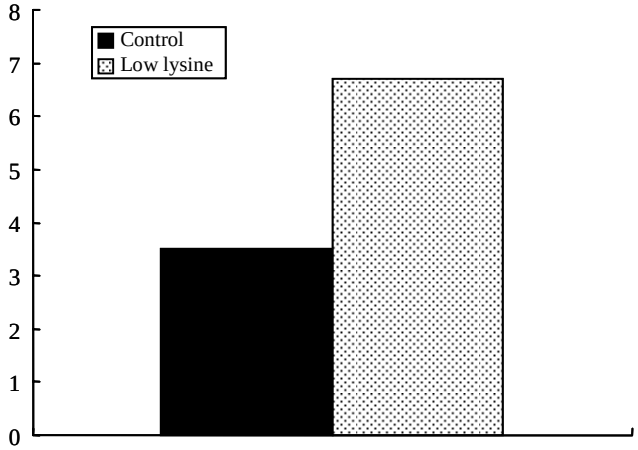

Figure 7. Influence of a low lysine diet on intramuscular fat (IMF) content in *L. dorsi* muscle. The IMF content was significantly higher in the low lysine group ($p<0.01$).

determined spectrophotometrically. The enzymes were selected to be analyzed because the coordinated up-regulation of GLUT4 and these two enzymes had been observed (Hjeltnes et al., 1998; Henriksen and Halseth 1995). Contents of glucose 6-phosphate and glycogen were measured by enzymatic methods.

Although it was confirmed in this investigation again that GLUT4 mRNA abundance in *rhomboideus* muscle was higher in the pigs fed on the LL diet, plasma glucose concentration was not affected (data not shown). Plasma lysine concentration was significantly lower in the pigs fed on the LL diet compared with that of pigs fed on the control diet (Figure 5, $p<0.01$). Interestingly, concentrations of plasma threonine, isoleucine, phenylalaniane, and histidine were higher and those of alanine and leucine were lower in pigs fed on the LL diet (Figure 5, $p<0.01$). Activity of citrate synthase was significantly higher in both *L. dorsi* and *rhomboideus* muscles of pigs fed on the LL diet (Figure 6, $p<0.05$), whereas dietary lysine levels did not affect that of hexokinase. Glucose 6-phosphate content in *L. dorsi* muscle tended to be higher in pigs fed on the LL diet compared with the control diet (Figure 6, $p = 0.0666$). In addition, glycogen content in *L. dorsi* muscle tended to be higher in the LL diet group (Figure 6, $p = 0.0745$), while that in *rhomboideus* muscle was significantly higher in the LL diet group (Figure 6, $p<0.01$).

We predicted that plasma glucose level would be lower in pigs fed on the LL diet because of the up-regulation of muscle GLUT4 expression. Indeed, plasma glucose concentration after 3 h removal of diet tended to increase with increasing dietary lysine level in *ad lib* fed finishing pigs (Goodband et al., 1990). However, plasma glucose concentration of pigs fed on the LL diet did not differ from that of pigs fed on the control diet. As suggested by Löhrke et al. (2001), sensitivity of muscle to insulin might be affected by dietary lysine level. However, no information is currently available on role of dietary amino acid level in controlling sensitivity of porcine muscle to insulin. It is necessary to conduct further investigations to determine the effect of dietary amino acid level on glycemia in relation to peripheral sensitivity to insulin. Although plasma glucose concentration was not affected by the dietary lysine levels, content of glucose 6-phosphate tended to be higher in the muscle samples of pigs fed on the LL diet. In addition, content of glycogen in the muscle samples were higher in pigs fed on the LL diet. These results indicate that the amount of glucose entering the glycolysis and/or glycogen synthesis in skeletal muscle is higher in pigs fed on the LL diet.

Table 4. Influence of dietary lysine levels on growth performance

	Control	Low lysine	
Initial body weight (kg)	62	61	
Final body weight (kg)	110	110	
Feed intake (g/d)	2,494	2,374	
Live weight gain (g/d)	784	715	$p = 0.118$
Feed efficiency	0.32	0.30	$p = 0.052$
Age at slaughter (day)	173	178	$p<0.01$
Back fat depth (cm)	2.35	2.55	

The higher activity of citrate synthase in the muscle sample of pigs fed on the LL diet may be partly explained by higher oxidative capacity of muscle. Indeed, enzyme histochemistry revealed that activities of reduced nicotinic amide adenine dinucleotide dehydrogenase (EC 1.6.5.3), a well known indicator of oxidative capacity of muscle, in 10-μm cross sections of *L. dorsi* and *rhomboideus* muscles were higher in the pigs fed on the LL diet (unpublished observation). Amino acids may play a role. As shown in Figure 5, concentrations of threonine, isoleucine, phenylalanine, and histidine in plasma were higher in pigs fed on the LL diet. A striking effect was observed in the concentration of threonine. Overall protein synthesis rate in pigs fed on the LL diet might be lower due to the shortage of lysine in the diet. Thus, excess amino acids that were not utilized for body protein synthesis kept circulating in the blood stream, and as a consequence, concentrations of these four essential amino acids might be maintained higher. The remaining carbon skeleton of these excess amino acids can be completely oxidized by the combined action of the TCA cycle and oxidative phosphorylation. Thus, the TCA cycle in muscle might be activated to oxidize the carbon skeletons of these excess amino acids.

REDUCED INTAKE OF DIETARY LYSINE PROMOTES ACCUMULATION OF IMF

Eleven gilts aged 110 days were used. The average initial live weight was approximately 60 kg. Six pigs were

assigned to the low lysine diet group (lysine content was 0.40%) and the rests were assigned to the control group (lysine content was 0.65%). The diets were iso-energetic and iso-protein, and contained all essential amino acids apart from lysine in the recommended amounts (Katsumata et al., 2005). The pigs were fed these diets for two months until their live weight reached 110 kg.

As shown in Figure 7, the IMF content in *L. dorsi* muscle of the low lysine group was twice as high as that of the control group ($p<0.01$). However, due to the shortage of lysine in the diet, live weight gain and feed efficiency tended to be lower in the low lysine group (Table 4, $p = 0.118$ and $p = 0.052$, respectively). Further, pigs from the low lysine group took five days longer to reach 110 kg (Table 4, $p<0.01$).

To our knowledge, this investigation demonstrates for the first time that the reduced intake of a single essential amino acid promotes accumulation of IMF in finishing pigs. On the other hand, however, the merit on IMF obtained by lower intake of dietary lysine is counteracted by its negative effects on growth performance. Thus, as far as effects of dietary lysine levels are concerned, there is a trade-off between enhancement in IMF content and growth performance. In order to minimize such negative effects, further studies are required to elucidate the appropriate dietary lysine level enables to enhance accumulation of IMF while its effects on growth performance is limited, and the effective minimum feeding period of low lysine diet.

ENHANCEMENT OF IMF BY BREADCRUMBS FEEDING RELATES TO SHORTAGE OF DIETARY AMINO ACIDS

Current movements towards developing recycling-based societies and recent increase in the price of corn due to promotion of using bioethanol for fuel encourage us to substitute food co-products for cereals as a feed ingredient. The pig industry in Japan have conducted several attempts to use food co-products for feeds of pigs and it is now well recognized that feeding breadcrumbs to finishing pigs enhances IMF content. However, underlying mechanisms of this effect of breadcrumbs feeding on IMF was unknown. Since contents of several amino acids including lysine in food co-products made from cereals seem to be low, we hypothesized that enhancement in IMF content induced by breadcrumbs feeding could be attributed to shortage of amino acids in the diet. We conducted an investigation to test the hypothesis that a shortage of amino acids in breadcrumbs may affect IMF.

We prepared three diets as follows; a control diet, a diet including 30% breadcrumbs (BS diet), and a diet including breadcrumbs supplemented with amino acids, lysine, methionine, threonine, and tryptophan, to meet their requirements (BS+AA diet). The average initial body weight of the pigs was 40 kg. The pigs were fed these diets until their body weights reached to 110 kg.

Daily live weight gain and feed efficiency of the pigs did not differ among the groups. IMF content in the *L. dorsi* muscle of pigs fed on the BS diet was higher than those of the other two groups (3.52% for the BS groups, 2.29% for the control, and 1.98% for the BS+AA, respectively; BS vs. control $p = 0.0515$ and BS vs. BS+AA $p<0.05$, respectively). Thus, the enhanced IMF level induced by breadcrumbs feeding returned to the control level by the supplementation of amino acids to the diet. This result supported our hypothesis that response of IMF to breadcrumbs feeding was due to a shortage of amino acids in breadcrumbs and that the enhancement was not a specific response to breadcrumbs. We infer that feeding other food co-products from cereals enhances IMF because their amino acid contents are similar to that of breadcrumbs. The details of this investigation are reported elsewhere (Ieiri et al., 2007).

SUMMARY

We conclude that undernutrition due to either an overall reduction in food intake or deficiency of a single amino acid induces muscle-specific upregulation of GLUT expression. This muscle-specific response may be related to the physiological function of each muscle in the context of the whole body. Thus, *L. dorsi* and *rhomboideus*, the two muscles affected by nutritional status, may be especially important for whole-body energy metabolism, whereas the essential functions of heart and diaphragm in cardiovascular and respiratory function may protect them from major modification by nutrition. Moreover, in addition to GLUT expression, the subcellular distribution of GLUTs, and therefore glucose uptake both under basal and insulin-stimulated conditions may also be affected by energy and amino acid nutritional status. This in turn suggests the occurrence of important insulin-independent GLUT trafficking pathways, at least in myocytes. Furthermore, not only GLUT4 expression in muscle is regulated by dietary lysine levels but post-GLUT4 glucose metabolism in association with oxidative capacity of muscle is also affected. In particular, an interesting finding is that oxidative capacity of muscle is enhanced by shortage of lysine in diet. We speculate amino acids may play a role. Thus, this model could be a good example of regulation of energy metabolism by nutritional status of amino acids. As reasonable amount of IMF is currently required to improve the quality of pork, we believe that regulation of dietary amino acid levels aiming to control IMF is an important target of applied pig nutrition.

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