FULL PAPER *Biochemistry*

Postnatal Development of Glucose Transporter Proteins in Bovine Skeletal Muscle and Adipose Tissue

Hiroyuki ABE[1)]*, Yumi KAWAKITA[1)], Kyoko HODATE[1)] and Masayuki SAITO[2)]

[1)]National Institute of Livestock and Grassland Sciences, Tsukuba 305–0901 and [2)]Laboratory of Biochemistry, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060–0818, Japan

(Received 11 April 2001/Accepted 18 June 2001)

ABSTRACT. Facilitated diffusion of glucose across the plasma membrane is mediated by a family of glucose transporter (GLUT). GLUT1 is ubiquitously present in all tissues and involved in cellular glucose uptake, while GLUT4 plays a key role in cellular glucose uptake stimulated by insulin in skeletal muscles and adipose tissue. To examine the postnatal change in the GLUTs of ruminants, the protein levels of GLUT1 and GLUT4 were measured by Western blot analysis of skeletal muscles, adipose tissue and brain of Holstein male calves aged from 0 to 12 months. Analysis of rumen short chain volatile fatty acids revealed that rumen fermentation increased around 2–3 months old. The GLUT1 level did not change in all tissues examined during the postnatal period, while the GLUT4 levels in skeletal muscle and subcutaneous adipose tissue decreased gradually, and at 12 month old, it was about 40% of those seen at 0 month old. These results are contrast to those in non-ruminant species, in which GLUT4 increases during postnatal development, and may be related to the insulin-resistance seen in adult ruminants.

KEY WORDS: bovine, glucose, GLUT1, GLUT4, postnatal.

J. Vet. Med. Sci. 63(10): 1071–1075, 2001

Glucose transport across the plasma membrane is largely mediated by specific transporters [7]. Two classes of glucose transporters have been described in mammalian cells, the sodium-glucose co-transporter (SGLT) and the facilitative glucose transporter (GLUT). SGLT is present in epithelial cells of the small intestine and proximal tube of the kidney, and responsible for sodium-dependent active absorption of glucose from the lumen of the intestine or nephron. GLUT is a family of at least 6 isoforms, each of which functions in tissue- and age-specific manners. Among them, GLUT1 is present in all tissues and mediates basal cellular glucose uptake. In contrast, GLUT4 is the major transporter responsible for insulin-stimulated glucose uptake, and is expressed in some limited tissues such as skeletal muscle and adipose tissue.

These informations about GLUTs are largely based on those in human and rodents, whereas only fragmental informations on ruminant GLUTs have been reported so far [1, 2, 4, 6, 14, 15]. One of the marked metabolic characteristics in ruminants is that short-chain volatile fatty acids (VFAs) derived from rumen fermentation, rather than glucose, is the major energy substrate [11]. Moreover, glucose utilization is less sensitive to insulin compared to non-ruminant mammalian species [12]. To examine a possible relation of GLUTs to the features of glucose metabolism specific to ruminants, we previously cloned cDNA of GLUT4 from bovine skeletal muscle [1]. Our result showed that GLUT4 mRNA is detected only in skeletal muscle and adipose tissues in cattle, as in many other non-ruminants. Furthermore, there was one amino acid conversion in the C-terminal region, which is proposed as the targeting signal of GLUT4 relating to the post-receptor events of insulin [8]. Despite of these findings, the reason of insulin resistance in ruminants is still unclear.

In rodents and human, the GLUT4 expression level is known to be rather low at birth, but substantially increased during postnatal development [4]. Such a change in GLUT4 is conceivable to be a response to those in dietary carbohydrate, especially before and after weaning. During postnatal development in bovine, rumen fermentation is low at neonatal period and becomes high after weaning. It might be thus possible that such a postnatal change in rumen fermentation may influence GLUT4 expression. In the present study, we examined the protein levels of GLUT4 as well as GLUT1 in Holsteins at various ages from 0 to 12 months old, during which rumen fermentation dramatically changed. Our results showed a substantial decrease in the GLUT4 level during postnatal development of bovine, in contrast to the increase of GLUT4 in rodents and human.

MATERIALS AND METHODS

Animals and feeding conditions: Twenty-six male Holsteins, from newborn to 12 months of age, were used. The chemical compositions of feeds analyzed by Association of official analytical chemists (AOAC) methods [3] were shown in Table 1. Nineteen calves of 0.5 to 3 months of age, were given whole milk, calf starter, and hay, but four 12-month-old oxen, which had been castrated at 3 month of age, were fed only hay.

Tissue sampling: At appropriate months of age, animals were slaughtered by an overdose administration of sodium pentobarbital, and 2–5 g of tissue samples were taken from skeletal muscles (M. quadriceps, M. longissimus), adipose tissues (perirenal adipose tissue, hind-back subcutaneous

* CORRESPONDENCE TO AND PRESENT ADDRESS: ABE, H., National Institute of Agrobiological Sciences, Tsukuba 305–8602, Japan.

Table 1. Chemical composition of feeds

	Whole milk	Calf starter	Italian Rye Grass hay
Chemical composition (%):			
DM	11.4	87.1	84.2
CP	2.9	13.2	13.8
EE	3.3	2.1	3.1
NFE	4.5	61.6	35.5
CF	0.0	4.0	22.7
Ash	0.7	6.1	9.1

DM: Dry matter, CP: Crude protein, EE: Ether extract, NFE: Nitrogen free extract, CF: Crude fiber

Table 2. Feed intake

Age (month)	n	Intake (g/day) Whole milk	Calf starter	Italian Rye Grass hay
0	3	NF	NF	NF
0.5	6	4,500 ± 0	51 ± 7	11 ± 3
1	4	4,500 ± 0	286 ± 54	36 ± 9
2	4	NF	1,166 ± 114	200 ± 47
3	5	NF	1,748 ± 75	344 ± 68
12	4	NF	NF	5,500 ± 0

NF: Not fed
Values are mean ± S.E.M.

adipose tissue), and brain cortex. They were immediately frozen in liquid nitrogen and kept at –80°C until analysis. In some cases, tissue samples of spleen, liver, and lung were also collected. Rumen fluid of each animal, except newborns, was weighed and its aliquot was stored for VFA analysis. Adult Holstein's (2 years old) tissue, were used for the GLUT1 and GLUT4 tissue distribution study.

VFA analysis: The concentrations of acetic acid, propionic acid, and butyric acid in rumen fluid were determined by gas chromatography (model 5890, Hewlet-Packard, Avondale, PA, U.S.A.) using a 1.2 m glass column (inner diameter 2 mm) with 5% Thermon1000 and 0.5% phosphoric acid on 80/100 mesh Chromosorb-W (Wako Pure Chemical Industries Ltd., Osaka, Japan). Injection and detection temperature are 250°C, and column temperature 140°C. Nitrogen gas was used as a carrier gas [10].

Western blot analysis of GLUTs: Tissue GLUT1 and GLUT4 were analyzed by Western blotting as reported previously [2, 10]. Tissue specimen (0.5–1 g) was homogenized in phosphate-buffered saline (10 m*l*) using Polytron (Kinematica GMBH, Luzern, Switzerland), and centrifuged at 1,000 × g for 10 min. The resulting infranant (6.6 μg of protein) was subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to a nitro cellulose filter, and incubated with diluted GLUT4 antiserum (1/500) [13] or GLUT1 antiserum (1/500) (East Acres Biological, Southbridge MA, U.S.A.) The protein blots were luminescent visualized using an ECL-detection kit (Amersham, Buckinghamshire, UK), exposing to X-ray film, and quantified using a scanning densitometer.

Statistical Analysis: All values were expressed as means ± S.E.M. for 3–6 animals at each age, and statistically analyzed by the Fisher's test after analysis of variance (ANOVA).

RESULTS

In order to confirm the postnatal developmental pattern of rumen fermentation, first, the VFA content in rumen fluid was examined in male Holsteins of various ages. The major feed source was whole milk, calf starter and hay at ages of 0.5–1, 2–3, and 12 months old, respectively (Table 2). Small amount of calf starter and hay were also taken even in 0.5–1 months old calves. As shown in Table 3, although VFA was already present in the rumen even at 0.5 month old, the total amount was only about 1% of that at 12 months old. This was largely due to the extremely small amount of rumen content in the early suckling period, which was negligible compared to the post-weaning periods (12 months old).

It has been established in many mammalian species that GLUT1 is expressed ubiquitously in all tissues, while GLUT4 in some limited tissues such as skeletal muscle and adipose tissue. To determine whether this is also the case in bovine, next, crude homogenate of various tissues of adult Holstein was subjected to Western blot analysis using respective antibodies raised against GLUT1 and GLUT4. As shown in Fig. 1, a protein band corresponding to GLUT1

Table 3. Rumen VFA concentration, content and total amount of VFA

Age	0.5	1	2	3	12
VFA (mmol/kg)					
Acetate	47.1 ± 6.0	67.2 ± 7.0	71.8 ± 6.5	61.7 ± 8.2	87.1 ± 4.3
Propionate	12.1 ± 2.0	39.1 ± 8.1	43.8 ± 2.8	28.3 ± 6.2	23.7 ± 1.5
Butyrate	6.6 ± 1.2	4.0 ± 1.1	10.2 ± 0.3	12.1 ± 2.7	14.8 ± 1.0
Total VFA	68.9 ± 8.0	115.7 ± 13.4	133.4 ± 5.4	108.4 ± 16.9	132.7 ± 6.2
Rumen contents (kg)	0.56 ± 0.09	1.53 ± 0.19	4.82 ± 0.66	12.00 ± 2.64	30.38 ± 2.67
Total amount of VFA (mmole)	40 ± 9	183 ± 37	653 ± 115	1,416 ± 477	4,008 ± 332

Values are mean ± S.E.M.

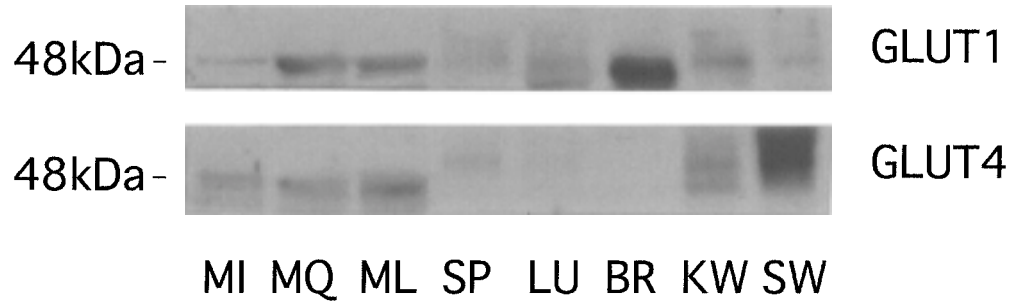

Fig. 1. Tissue distribution of GLUT1 and GLUT4 proteins in adult Holstein (2 years old). MI: intercostale muscle, MQ: quadriceps muscle, ML: longissimus muscle, SP: spleen, LU: lung, BR: cerebral cortex, KW: perirenal adipose tissue, SW: subcutaneous adipose tissue.

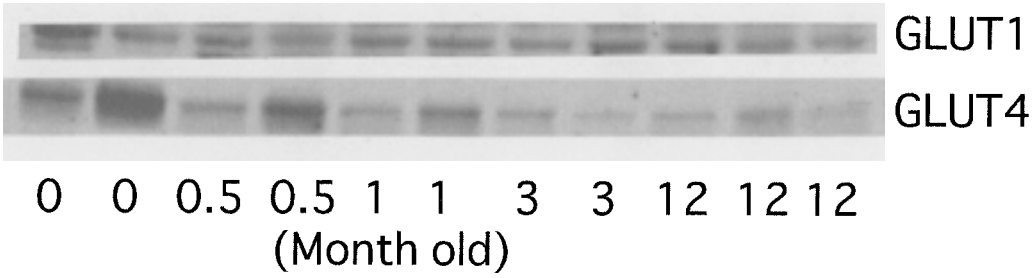

Fig. 2. Western blots of GLUT1 and GLUT4 proteins in quadriceps muscle of 0–12 months old Holsteins.

was found in the extracts from all tissues examined (skeletal muscle, spleen, lung, brain and adipose tissue), giving the strongest signal in the brain. In contrast, a GLUT4 band was detected only in skeletal muscle and adipose tissue. Both bands of GLUT1 and GLUT4 were at the expected size of 48 kDa in skeletal muscle, while those in adipose tissue were slightly larger (53–55 kDa), probably due to different post-translational modifications such as glycosylation [15]. These results are essentially the same as those reported in other mammals [7].

To examine the postnatal changes in the amounts of GLUT1 and GLUT4, crude tissue homogenates were prepared from skeletal muscle and adipose tissue of 0–12 months old, and analyzed by Western blot, followed by quantification of the density of corresponding bands. Figure 2 shows typical Western blots of quadriceps. As summarized in Fig. 3, the GLUT1 content in brain homogenate was fairly unchanged at 0 to 12 months after birth. Similarly, there was no significant difference in the GLUT1 content in two types of skeletal muscle (quadriceps and longissimus muscles) and also in perirenal adipose tissue, although the GLUT1 content tended to be slightly higher at 12 months old. In contrast to GLUT1, substantial postnatal changes were found in GLUT4 (Fig. 4): that is, the GLUT4 content in both types of skeletal muscle decreased gradually after birth and was about one-third levels at 12 months old. The GLUT4 contents in adipose tissue also decreased postnatally, but the decrement pattern between perirenal adipose tissue and subcutaneous adipose tissue is different: that is, it decreased slowly and slightly in perirenal adipose tissue, but more promptly and markedly in subcutaneous adipose tissue. Collectively, the tissue content of GLUT4 decreased in both skeletal muscle and adipose tissue from 0 to 12 months after birth, while that of GLUT1 did not change or slightly increased.

DISCUSSION

There have been some reports describing postnatal development of glucose transporters in ruminants, most of which focus on intestinal SGLT [5]. In the present study, we first depicted the postnatal changes in tissue contents of GLUT1 and GLUT4 in bovine. Our results clearly showed that GLUT4 content decreased gradually during the 12-month postnatal period, while GLUT1 content did not change significantly. These results are quite contrast to the postnatal increase in GLUT4 expression in non-ruminants such as rodents [4]. In rat skeletal muscle and adipose tissue, for example, GLUT1 is the predominant isoform during fetal and early neonatal life, and it progressively decreases during the suckling period and is replaced by GLUT4. Such opposite changes in bovine and rat during postnatal life seem to

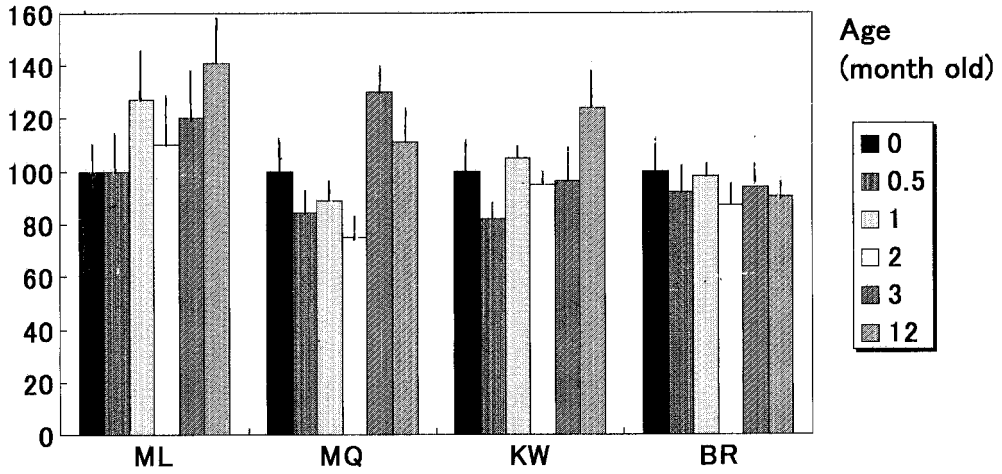

Fig. 3. Postnatal changes of GLUT1 protein in skeletal muscle, adipose tissue and cerebral cortex. Values are mean ± S.E.M. for 3–6 animals and express as relative to those of newborn (0 month old). There was no significant difference between the age groups ($p>0.05$). ML: longissimus muscle, MQ: quadriceps muscle, KW: perirenal adipose tissue, BR: cerebral cortex.

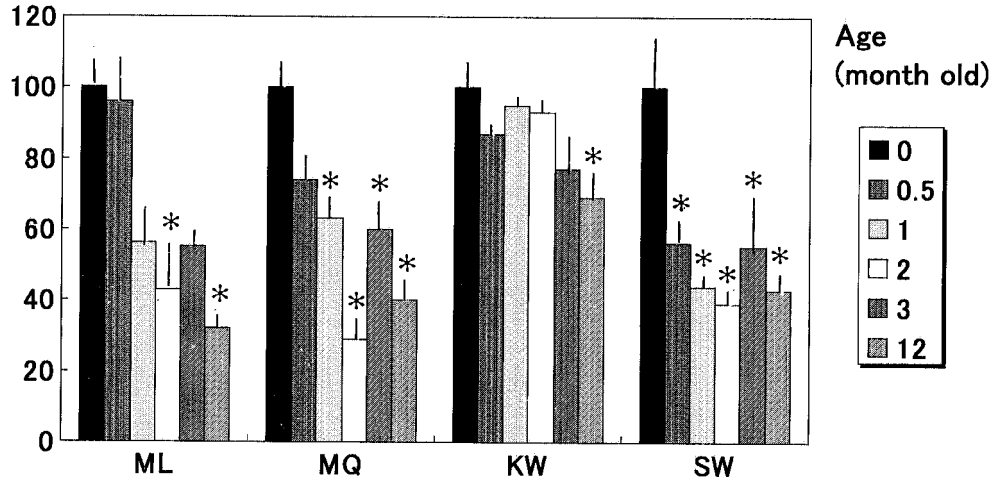

Fig. 4. Postnatal changes of GLUT4 protein in skeletal muscle, adipose tissue. Values are mean ± S.E.M. for 3–6 animals and express as relative to those of newborn (0 month old). * $p<0.05$ vs newborns (0 month old). ML: longissimus muscle, MQ: quadriceps muscle, KW: perirenal adipose tissue, SW: subcutaneous adipose tissue.

be related to the different dietary condition between the two species: that is, the major energy source in the rat is changed from milk triglyceride (long chain fatty acid) to starch (glucose) around weaning, whereas in the bovine from milk triglyceride to VFA derived from rumen fermentation [10]. In fact, in the present study, we confirmed that rumen fermentation estimated by VFA production was very low during the neonatal periods when whole milk was given, but increased dramatically as hay was given as the main feed. It is also well established in ruminants that the glucose concentrations in intestinal lumen and blood are low in adults compared to those in neonatal infants. This is also true for non-ruminant species. Thus, it seems likely that the substantial decrease in bovine GLUT4 after weaning is an adaptive response and attributable to the decreased supply of dietary glucose. A similar decline in intestinal SGLT was also reported during the postnatal development of lambs. Interestingly, the SGLT decline was restored by intra-luminal infusion of glucose, confirming a critical role of dietary glucose for SGLT expression [6].

Unlike GLUT4, the tissue content of GLUT1 showed no significant change during the postnatal period from 0 to 12

months old. This is also a different result from those of rodents, in which muscle GLUT1 decreased much after weaning [4]. Although the mechanism for such isoform-specific regulation of GLUT expression is not known at present, these results reflect different roles of GLUT1 and GLUT4 in glucose metabolism: that is, GLUT1 is a principal glucose transporter in ruminant during the whole postnatal life, and thereby is little influenced by nutritional challenges. On the other hand, the low level of GLUT4 after weaning and adults may be a cause of insulin-resistance specifically seen in ruminants [12]. It is to be noted, however, that GLUT4 is not a sole target for insulin action: for example, the insulin receptor and the post-receptor machinery also influence the cell sensitivity to insulin. Further studies on the molecular mechanisms of insulin-signaling, in connection with regulation of GLUTs, are needed in ruminants.

REFERENCES

1. Abe, H., Morimatsu, M., Nikami, H., Miyashige, T. and Saito, M. 1997. Molecular cloning and mRNA expression of the bovine insulin-responsive glucose transporter (GLUT4). *J. Anim. Sci.* **75**: 182–188.
2. Abe, H., Kawakita, Y., Miyashige, T., Morimatsu, M. and Saito, M. 1998. Comparison of amino acid sequence of the C-terminal domain of insulin-responsive glucose transporter (GLUT4) in livestock mammals. *J. Vet. Med. Sci.* **60**: 769–771.
3. AOAC. 1995. Official methods of analysis (16th ed.). Association of Official Analytical Chemists. Washington, D.C.
4. Castello, A., Rodriguez-Manzaneque, J. C., Camps, M., Perez-Castillo A., Testar, X., Palacin, M., Santos, A. and Zorzano, A. 1994. Perinatal hypothyroidism impairs the normal transition of GLUT4 and GLUT1 glucose transporters from fetal to neonatal levels in heart and brown adipose tissue. *J. Biol. Chem.* **269**: 5905–5912.
5. Dyer, J., Barker, P. J. and Shirazi-Beechey, S. P. 1997. Nutrient regulation of the intestinal Na+/glucose co-transporter(SGLT1) gene expression. *Biochem. Biophys. Res. Commun.* **230**: 624–629.
6. Freeman, T. C., Wood, I. S., Sirinathsinghji, D. J., Beechey, R. B. and Shirazi-Beechey, S. P. 1993. The expression of the Na+/glucose cotransporter (SGLT1) gene in lamb small intestine during postnatal development. *Biochim. Biophys. Acta* **1146**: 203–212.
7. Gould, G. W. and Holman, G. D. 1993. The glucose transporter family: structure, function and tissue-specific expression. *Biochem. J.* **295**: 329–341.
8. Haney, D. M., Levy, M. A., Strube, M. S. and Mueckler, M. 1995. Insulin-sensitive targeting of the GLUT4 glucose transporter in L6 myoblasts is conferred by its COOH-terminal cytoplasmic tail. *J. Cell Biol.* **129**: 641–658.
9. Kajikawa, H., Odai, M., Saitoh, M., Abe, H., Tano, R., Takahashi, T. and Abe, A. 1990. Effects of sugar-beet pulp on ruminal and lactation performances of cow lactating different fermentation patterns. 1990. *Anim. Feed Sci. Tec.* **31**: 91–104.
10. Kawakita, Y., Abe, H., Takada, R., Saitoh, M. and Miyashige, T. 1998. Effects of dietary γ-linolenic acid on insulin responsive glucose transporter (GLUT4) protein expression in pigs. *Anim. Sci. Technol.(Jpn.)* **69**: 347–354.
11. Leng, R. A. 1970. Fermentation and production of volatile fatty acid in the rumen. pp. 406–421. *In*: Physiology of Digestion and Metabolism in the Ruminant (Phillipson, A.T. ed.), Oriel Press Ltd., Newcastle upon Tyne, England.
12. Sasaki, S. 1990. Mechanisms of insulin resistance in the post receptor events in sheep: 3-O-methylglucose transport in ovine adipocytes. *Hormon Metab. Res.* **22**: 457–461.
13. Shimizu, Y., Nikami, H., Tsukazaki, K., Machado, U. F., Yano, H., Seino, Y. and Saito, M. 1993. Increased expression of glucose transporter GLUT4 in brown adipose tissue of fasted rat after cold exposure. *Am. J. Physiol.* **264**: E890–895.
14. Trayhurn, P., Thomas, M. E. and Keith, J. S. 1993. Postnatal development of uncoupling protein, uncoupling protein mRNA, and GLUT4 in adipose tissues of goats. *Am. J. Physiol.* **265**: R676–682.
15. Zhao, F. Q., Glimm, D. R. and Kennelly, J. J. 1993. Distribution of mammalian facilitative glucose transporter messenger RNA in bovine tissues. *Int. J. Biochem.* **25**: 1897–1903.