

Weaning marginally affects glucose transporter (GLUT4) expression in calf muscles and adipose tissues

BY JEAN-FRANÇOIS HOCQUETTE¹, CAROLE CASTIGLIA-DELAUVAUD¹,
BENOÎT GRAULET¹, PASCAL FERRÉ^{2*}, BRIGITTE PICARD¹
AND MICHEL VERMOREL¹

¹INRA, Laboratoire Croissance et Métabolismes des Herbivores, Theix, 63122 Saint-Genès-Champanelle, France

²INSERM U342, Hôpital Saint Vincent de Paul, 75014 Paris, France

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The nutritional regulation of glucose transporter GLUT4 was studied in eight muscles and four adipose tissues from two groups of preruminant (PR) or ruminant (R) calves of similar age (170 d), empty body weight (194 kg) at slaughter, and level of net energy intake from birth onwards. Isocitrate dehydrogenase (EC 1.1.1.41) activity in muscles was not different between PR and R except in *masseter* muscle from the cheek (+71 % in R; $P < 0.003$), which becomes almost constantly active at weaning for food chewing. Basal and maximally-insulin-stimulated glucose transport rate (GTR) per g tissue wet weight in *rectus abdominis* muscle were significantly higher in R calves (+31 and 41 % respectively; $P < 0.05$). GLUT4 protein contents did not differ in muscles from PR and R except in *masseter* (+74 % in R; $P < 0.05$) indicating that the increased GTR in *rectus abdominis* cannot be accounted for by an enhanced GLUT4 expression. GLUT4 mRNA levels did not differ between the two groups of animals in all muscles suggesting a regulation of GLUT4 at the protein level in *masseter*. GLUT4 number expressed on a per cell basis was lower in adipose tissue from R calves (–39 %; $P < 0.05$) and higher in internal than in peripheral adipose tissues. In summary, the regulation of GLUT4 in calves at weaning differs markedly from that previously described in rodents (for review, see Girard *et al.* 1992). Furthermore, significant inter-individual variations were shown for metabolic activities in muscle and for biochemical variables in adipose tissue.

Glucose transport rate: Glucose transporter expression: Insulin-sensitive tissues

The suckling–weaning transition is characterized by profound changes in nutrition which in turn induce metabolic adaptations of glucose metabolism. For instance, in rats at weaning, the fat-rich milk is gradually replaced by the high-carbohydrate diet of the adult. This is associated with an enhanced overall insulin responsiveness of glucose utilization and of glucose transport rate (GTR) in adipose tissue and skeletal muscles (for review, see Girard *et al.* 1992), the latter being the rate-limiting step of glucose utilization (for review, see Kahn 1992; Hocquette *et al.* 1996a). These regulatory changes are related to an increased expression of the insulin-regulatable glucose transporter (GLUT4) in muscles and adipose tissues (for review, see Girard *et al.* 1992).

The changes in nutrition at weaning differ markedly between rodents and ruminants. Indeed, unlike the rat, the contribution of carbohydrates to total nutrition decreases from 35–45 % of total energy absorption to less than 5 % as calves become ruminant. However, much less is known about the regulation of glucose metabolism in bovines than in rats at

* Present address: INSERM U465, Centre Biomédical des Cordeliers, 75270 Paris Cedex 06, France.

weaning. This lack of knowledge results in part from the lack of available information about the regulation of glucose utilization and transport in large species such as cattle, and also from the limited information on glucose transporters in ruminants (for review, see Hocquette *et al.* 1996a). Nevertheless, it is well known that young suckling calves (Kouame *et al.* 1984) or calves fed on milk-substitutes (Doppenberg & Palmquist, 1991) have higher levels of circulating blood glucose and insulin than ruminant (R) animals, and it has been previously reported that glucose clearance rate *in vivo* was higher in R calves than in milk-fed calves at least in the postprandial state (Palmquist *et al.* 1992).

We have previously described a method to measure GTR into incubated fibre strips of bovine muscle (Hocquette *et al.* 1995) similar to that used in human subjects (for review, see Zierath, 1995), and we have also demonstrated that GLUT4 was expressed in bovine muscles and adipose tissues at both the protein (Hocquette *et al.* 1995) and the mRNA levels (Hocquette *et al.* 1996b). However, direct comparison of GTR in preruminant (PR) or R calves had never been reported. The aim of the present study, therefore, was to investigate the regulation of glucose transport and glucose transporter expression at weaning in bovine tissues. We thus examined the expression of GLUT4 in muscles from PR and R calves in relation to putative variations in GTR in the muscle tissue. In addition, the expression of GLUT4 was also studied in four adipose tissues in the same PR and R calves.

MATERIALS AND METHODS

Reagents

A polyclonal antibody raised against rat GLUT4 was supplied by East-Acres Biologicals (Southbridge, MA, USA). The synthetic peptide, to which the antibody was raised, was prepared by Neosystem Laboratoire (Strasbourg, France). Endoglycosidase F-N-glycosidase F was obtained from Boehringer-Mannheim (Meylan, France). The chemicals for immunoblotting were supplied by Bio-Rad (Munich, Germany), except membranes (Immobilon-P) which were obtained from Millipore (Bedford, MA, USA). ^{125}I -labelled protein A (>1.1 GBq/mg), ECL Western blotting kit and Hyperfilms MP were supplied by Amersham International (Amersham, Bucks.). 2-Deoxy[1,2- ^3H]glucose (970 GBq/mmol) was purchased from New England Nuclear Life Science Products (Boston, MA, USA). [U- ^{14}C]sucrose (20 GBq/mmol), [α - ^{32}P]dCTP (>111 TBq/mmol) and [γ - ^{32}P]dATP (>111 TBq/mmol) were purchased from ICN Biochemicals (Irvine, CA, USA). Guanidium thiocyanate was obtained from Fluka (Ronkonkoma, NY, USA). Genescreen membranes were obtained from New England Nuclear Life Science Products. T4 polynucleotide kinase was supplied by New England Biolabs Inc. (Beverly, MA, USA). Saturated phenol, chloroform-isoamyl alcohol (49:1, v/v), agarose and nonprimer labelling kit with reagents for probe purification were purchased from Appligène (Illkirch, France). RNA molecular-weight markers were from Bethesda Research Laboratories (Bethesda, MD, USA). Other reagents were from Sigma (St Louis, MO, USA).

Animals and experimental design

Fourteen Montbéliard male calves, tied and housed in individual stalls in a room with natural lighting were used. Two homogeneous groups were formed at the age of 88 d on the basis of live weight, average daily weight gain and average nutritional efficiency. From birth to 107 d of age, animals were maintained on a standard milk-substitute according to a

feeding pattern designed to allow an average daily weight gain of 1000 g. From 107 d to slaughter, PR calves of the first group were fed individually on a commercial milk-substitute containing approximately (g/kg DM) 200 fat, 550 carbohydrates and 250 protein. The amount of lactose in this diet was higher than that in classical milk-substitutes for veal-calf production in France in order to exacerbate the differences in dietary carbohydrate supply between the two groups of calves. Animals in the second group were progressively weaned from 107 to 128 d of age and became R calves. These calves were fed individually on a mixed diet composed of (g/kg) 800 concentrate and 200 hay, according to a feeding pattern designed to allow an average daily gain of 1300 g. Composition (g/kg) of the concentrate was: 300 dehydrated lucerne (*Medicago sativa*), 400 sugarbeet pulp, 140 barley, 110 soyabean meal and 50 mineral compound. The concentrate contained (g/kg) 162 protein and 18 fat. Body weight was measured weekly and the rate of feeding was adjusted accordingly so that net energy intake from birth onwards was similar for the two groups of animals.

Calves were slaughtered at 170 d of age (Picard *et al.* 1995). Slaughtering was done at 09.00 hours after an overnight fast by stunning (captive-bolt pistol) and exsanguination. Tissue samples (50–100 g) from the following muscles were always taken at the same site for all animals to minimize sampling error (Johnson *et al.* 1973; Hocquette *et al.* 1995): heart (H), *masseter* (MA) *diaphragma* (D), *musculus rectus abdominis* (RA), *musculus longissimus dorsi* (LD) at the level of the 6th rib, *musculus semitendinosus* (ST) from the medial portion, *musculus tensor fasciae latae* (TFL), *musculus cutaneus trunci* (CT) from the thick part, perirenal adipose tissue (PAT), intermuscular adipose tissue (IMAT), omental adipose tissue (OAT) and subcutaneous adipose tissue (SCAT).

Samples from calf muscles were quickly trimmed of visible fat and connective tissue. Then, samples of muscles or white adipose tissues were cut into pieces, which were then divided into two parts as soon as they were obtained. One part (six to seven small pieces within the sample) was immediately homogenized at 4° in buffer containing peptidase inhibitors (chymostatin, pepstatin A, leupeptin, antipain (5 µg/ml each), aprotinin (0.17 trypsin inhibitor units/ml) and 1 mM-phenylmethylsulphonyl fluoride which was added extemporaneously) as previously described (Hocquette *et al.* 1995). The second part was frozen in less than 10 min post exsanguination in liquid N₂ and stored at –80° for subsequent analyses. Samples were always pulverized in liquid N₂ to obtain an homogeneous powder before any treatment in order to avoid any problem of sampling error.

2-Deoxyglucose transport

A sample of muscle RA was taken with a 30 mm clamp from each calf at slaughter. Six muscle-fibre strips weighing 70–120 mg were isolated from each mounted muscle sample in less than 10 min. The rest of the sample was frozen in liquid N₂ or homogenized as described previously for other analyses. The muscle strips were incubated in 4.4 ml oxygenated Krebs-Henseleit buffer containing 2 mM-pyruvate and 5 mM-2-deoxy[1,2-³H]-glucose (18.5 kBq/ml). Basal and maximally-stimulated GTR measurement was performed without or with insulin (10^{–6} M) respectively (Hocquette *et al.* 1995). Preliminary experiments showed that maximal stimulation of GTR was observed beyond 10^{–8} M-insulin (data not shown). Tissue concentrations of labelled 2-deoxyglucose and 2-deoxyglucose-6-phosphate were determined as previously described (Hocquette *et al.* 1995).

Analytical techniques

Packed cell volume was measured after centrifugation of blood samples. Commercial kits were used to determine plasma insulin (INSIK-5 P2796; Sorin Biomedica, Saluggia, Italy) and glucose concentrations (PAP 250 ref 61271; BioMérieux, Marcy-l'Etoile, France). Protein and DNA contents in muscles and adipose tissues and activities of isocitrate dehydrogenase (*EC* 1.1.1.41; ICDH), characteristic of muscle oxidative metabolism, and of lactate dehydrogenase (*EC* 1.1.1.27; LDH), characteristic of muscle glycolytic metabolism, were measured as previously described (Hocquette *et al.* 1995). Concentrations of volatile fatty acids in the rumen content of R calves were determined according to the method of Jouany (1982).

Quantification of GLUT4 protein by immunoblotting

Tissue samples homogenized in sucrose buffer containing peptidase inhibitors were used and crude membranes were prepared as described previously (Hocquette *et al.* 1995). In some experiments, crude membranes were treated with endoglycosidase F-N-glycosidase F for complete removal of the oligosaccharides on the proteins (Kaestner *et al.* 1991). Subcellular fractions were stored at -80° until required for immunoblot analyses (Hocquette *et al.* 1995). The samples were not routinely heated before electrophoresis but incubated at room temperature overnight in Laemmli buffer containing 2-mercaptoethanol (50 ml/l) to avoid the formation of aggregates. Samples were then subjected to SDS-PAGE (12% gels). Immunoblot was performed using polyclonal antibody against rat GLUT4 and using ^{125}I -labelled protein A or a horseradish peroxidase (*EC* 1.11.1.7)-linked anti-rabbit immunoglobulin detected by a chemiluminescent reaction (ECL Western blotting kit). To test the specificity of the detected bands, the blots were incubated either with a non-immune serum or with diluted immune serum in the presence of the peptide (0.5 $\mu\text{g/ml}$) to which the antibody was raised (Hocquette *et al.* 1996b). Hyperfilm MP were exposed to membranes, and GLUT4 amounts were quantified by scanning densitometry (Hoeffer, San Francisco, CA, USA) of the autoradiograms (Hocquette *et al.* 1995).

Quantification of mRNA coding for GLUT4

Total RNA were isolated as previously described (Hocquette *et al.* 1996b). The concentration of RNA was determined by absorbance at 260 nm. All samples had a 260:280 nm absorbance ratio of approximately 2.0. The RNA samples were stored precipitated by 0.1 vol. sodium acetate (2 M, pH 5.0) and 2.5 vol. ethanol until required for use. For Northern analyses, RNA portions (40 μg) were washed by diethylpyrocarbonate-treated water (250 ml/l) and ethanol (750 ml/l), then denatured in a solution containing 2.2 M-formaldehyde and formamide (500 ml/l) by heating at 65° for 10 min, size fractionated by 1.5% agarose gel electrophoresis, and electrophoretically transferred to Genescreen membranes. The integrity of RNA was assessed by ethidium bromide staining before transfer. The bovine GLUT4 probe previously cloned in our laboratory (Hocquette *et al.* 1996b) was labelled by random priming with ^{32}P and free nucleotides removed using the nonaprimer labelling kit (Appligène, Illkirch, France) following the recommendations of the suppliers. Prehybridizations and hybridizations to the GLUT4 probe were performed at 42° for 2–4 h and 16–20 h respectively in solutions containing deionized formamide (450 ml/l). The membranes were washed twice for 5 min in twice the standard concentration of saline-sodium phosphate-EDTA buffer (SSPE) at room temperature

(standard SSPE 0.15 M-NaCl, 10 M-NaH₂PO₄ (pH 7.4) and 1 mM-EDTA), once for 30 min in twice the standard concentration of SSPE containing SDS (20 ml/l) at 55°, once for 15 min in standard SSPE containing SDS (20 ml/l) at 55° and once for 15 min in tenfold dilution of standard SSPE containing SDS (20 ml/l) at 55°. The membranes were exposed to Hyperfilm MP for 2–10 d at –80° with two intensifying screens. Quantification was performed using scanning densitometry (Hoeffler, San Francisco, CA, USA). Results were corrected for variations in the amount of RNA loaded by using values of hybridization to a 18S rRNA probe (Hocquette *et al.* 1996b).

Statistical analyses

ANOVA of the data was done using the GLM procedure of Statistical Analysis Systems (1987). For GTR, the effects tested in the model included group (G) of calves (PR, R), calf (C) nested within treatment group, insulin (I); i.e. absence or presence of insulin for basal and maximally-stimulated GTR respectively), and the interaction group \times insulin (G \times I). For biochemical variables and GLUT4 protein contents in tissues, the effects tested in the model included G of calves (PR, R), C nested within groups, tissue (i.e. muscle (M) or adipose tissues (AT)) and the interaction group \times tissue (G \times M or G \times AT). The G factor was tested against C within groups. The residual mean square was used as the error term for other effects. Results are expressed as means and standard errors of the means ($SEM = \sqrt{V}$; residual mean squares/number of observations per treatment). If a statistically significant difference was found among tissues, a paired Student's *t* test was used to determine the site of significance. If a statistically significant difference was found among groups, a paired Student's *t* test was used for comparisons between groups, tissue per tissue. $P < 0.05$ was considered statistically significant for Student's *t* tests.

RESULTS

Experimental conditions and animal characteristics

Body weight of the animals as well as average daily weight gain between 18 and 118 d of age (beginning of the experiment and mid period of weaning respectively) were similar for the two groups of PR or R calves (Table 1). However, average daily weight gain from 118 d of age onwards and live body weight at slaughter were higher for R calves than for PR calves because of a higher digesta content after weaning. Nevertheless, empty body weight at slaughter did not differ between the two groups of animals. Carcass weight tended to be lower for R than for PR calves (–6%; $P < 0.10$; Table 1).

According to the composition of the lactose-rich milk-substitute used in the present experiment, protein, carbohydrates and fat provided approximately 25, 40 and 35% of absorbed energy respectively in PR calves. In contrast, in R calves, concentrate and hay are converted in the rumen into volatile fatty acids by micro-organisms. Therefore, protein, volatile fatty acids, fat and carbohydrates provided approximately 25, 65–70, 8 and 2% of absorbed energy respectively, according to the composition of the diet and the estimations of Vermorel (1978). As the relative amounts of acetate and butyrate in the volatile fatty acid mixture in the rumen content of the R calves were high (69 (SE 4.2)% and 6 (SE 1.7)% respectively, $n = 7$) and the relative amount of propionate (the main gluconeogenic precursor) was low (19 (SE 1.9)%, $n = 7$), it was calculated, with the assumptions of Vermorel (1978), that the contribution of glucose (mainly produced by the gluconeogenic process) to total energy supply was less than 23% for R calves.

Table 1. *Animal characteristics and metabolic variables at slaughter for preruminant (PR) and ruminant (R) calves*†

(Mean values with their standard errors for seven calves per group)

	PR calves		R calves	
	Mean	SE	Mean	SE
Live body wt (kg) at: 18 d of age	57	1.3	58	1.2
118 d of age	156	3.4	153	4.2
Average daily wt gain (g/d):				
Between 18 and 118 d of age	1040	28	990	32
Between 118 d of age and slaughter	950	36	1280***	52
Total net energy intake (MJ):				
Between 18 and 118 d of age	1757	54	1757	54
Between 118 d of age and slaughter:				
For the milk-fed calves	1423	33	–	–
For the weaned calves	–	–	1339	25
Age at slaughter (d)	170	4	170	3
Live body wt at slaughter (kg)	202	4.3	217*	2.7
Empty body wt at slaughter (kg)	195	5.0	193	2.8
Carcass wt (kg)	121	3.0	114†	1.8
Packed cell volume (%)	39	1.1	39	1.7
Plasma glucose (mg/l)	997	25.0	947	26.6
Plasma insulin (μ U/ml)	14.5	1.67	11.2†	0.74

Mean values were significantly different from those for PR calves: * $P < 0.05$, *** $P < 0.001$.

† The difference between mean values for PR and R calves showed a tendency towards significance ($P < 0.10$).

‡ For details of animals and procedures, see pp. 252–253.

As net energy intake from birth onwards, age and empty body weight at slaughter were similar for the two groups (Table 1), only the effects of changes in energy-yielding substrates on glucose transport and glucose transporter expression were studied.

No significant differences between groups were found in packed cell volume or in plasma glucose levels at slaughter, i.e. 16 h after the last meal. However, the plasma insulin level tended to be lower in R calves (-23% ; $P < 0.10$) which may be related to the high lactose content of the diet of the PR calves.

Muscle metabolic and biochemical characteristics

Muscle metabolic type was assessed using ICDH and LDH activities, characteristic of oxidative and glycolytic metabolisms respectively (Fig. 1). These two enzyme activities were found to be inversely related in muscles of both PR and R calves, as previously described for the weaned animals (Hocquette *et al.* 1995). On the basis of these enzyme activities, H, MA and D were classified as oxidative muscles whereas other skeletal muscles from the carcass were considered as oxido-glycolytic. As previously described for the R calves (Hocquette *et al.* 1995, 1996b), the most oxidative muscle, H, was characterized by the highest DNA and extractable RNA contents (1939 (SE 31.7) and 607 (SE 62.0) μ g/g wet tissue respectively; $P < 0.05$). DNA and RNA contents were also higher in the two other oxidative muscles (MA and D) than in mixed or glycolytic skeletal muscles of the carcass (1405–1428 v. 897–1141 μ g/g and 405–413 v. 234–293 μ g/g for DNA and RNA contents respectively; $P < 0.05$). The most glycolytic muscle was LD for the two groups of calves since it exhibited the highest LDH activity (Fig. 1) and the lowest DNA content.

A significant effect of calf was demonstrated for metabolic variables (ICDH and LDH activities; $P < 0.05$; Fig. 1) but not for biochemical variables (protein, DNA and RNA contents), providing evidence that muscle metabolic type depends largely on physiological characteristics inherent in each animal (genetic, hormonal status, etc.). In addition, ICDH activity in LD from the fourteen calves was positively correlated with ICDH activity in D and MA ($r 0.66$; $P < 0.01$) and LDH activity in LD was positively correlated with LDH activities in MA, ST and RA ($r 0.52, 0.61$ and 0.69 respectively; $P < 0.05$). Taken together, these results suggest that some calves are characterized by particularly high (or low) ICDH or LDH activities whichever muscle is studied.

ICDH activity was not different between the two groups of animals except in the MA and LD muscles; ICDH activity was 71% higher in MA ($P < 0.003$) and 30% lower

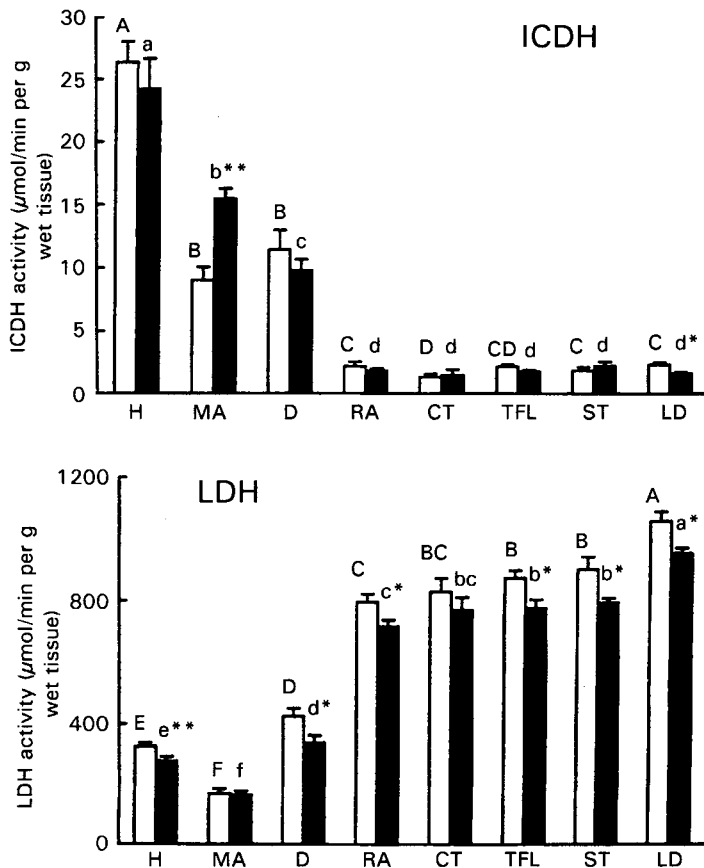


Fig. 1. Isocitrate dehydrogenase (*EC* 1.1.1.41; ICDH) and lactate dehydrogenase (*EC* 1.1.1.27; LDH) activities in heart and various skeletal muscles from preruminant (PR; □) and ruminant (R; ■) calves. Enzyme activities were measured spectrophotometrically in tissue homogenates. Values are means with their standard errors for seven animals per group. Significant effects of calf, of muscle and of the group \times muscle interaction were observed for ICDH activity ($P < 0.007$, $P < 0.0001$ and $P < 0.0002$ respectively). Significant effects of group (tested against calves within group), of calf and of muscle were observed for LDH activity ($P < 0.0025$, $P < 0.014$ and $P < 0.0001$ respectively). ^{A,B,C,D,E,F,a,b,c,d,e,f} Means within a group with different superscript letters were significantly different ($P < 0.05$). Mean values were significantly different from those for PR calves: * $P < 0.05$, ** $P < 0.01$. H, heart; MA, masseter; D, diaphragma; RA, rectus abdominis; TFL, tensor fasciae latae; ST, semitendinosus; CT, cutaneous trunci; LD, longissimus dorsi. For details of animals and procedures, see pp. 252–254.

($P < 0.01$) in LD from R calves (Fig. 1). LDH activity was slightly lower in oxidative or mixed muscles (except MA and CT) from R calves (from -10 to -21% ; $P < 0.05$; Fig. 1). Total DNA content was lower in muscles from R calves (from -0.7% in MA to -6.3% in ST muscles; $P < 0.05$; data not shown). In contrast, RNA contents did not differ between the two groups of animals except in the MA and CT muscles: RNA content was 23% higher in MA ($P < 0.05$) and 17% lower ($P < 0.05$) in CT from R calves (data not shown).

Rate of glucose transport into muscle

GTR into incubated muscle-fibre strips was measured as previously described (Hocquette *et al.* 1995) for RA muscle only, since it is not possible to isolate and incubate properly muscle-fibre strips from other muscles (Hocquette *et al.* 1995). Basal and maximally-insulin-stimulated GTR per g tissue wet weight were 31 and 41% higher respectively for R than for PR calves ($P < 0.001$; Table 2). Moreover, the stimulation of GTR by insulin was significantly lower in PR calves than in R animals ($+210$ v. $+326$ nmol/20 min per g tissue wet weight, i.e. $+70$ v. $+83\%$ over basal GTR; $P < 0.005$; Table 2). Data were also expressed per mg DNA or protein assuming that equal concentrations of DNA or protein reflect the same number of multinucleated muscle cells. Similar significant differences were observed regardless of the method of expression of the results (Table 2). A significant effect of calf was demonstrated for GTR, especially when results were expressed per mg protein (Table 2), indicating that glucose uptake potential by the muscle tissues varies greatly among individuals. Most of the intracellular 2-deoxyglucose transported into bovine muscle (more than 90%) was phosphorylated for both PR and R calves (Table 2) suggesting that hexokinase (*EC* 2.7.1.1) activity was not rate-limiting. However, when GTR was increased from the lowest value (basal GTR for PR calves) to the highest one (stimulated GTR for R calves), the relative amount of 2-deoxyglucose which was phosphorylated decreased from 94.0 to 90.8% ($P < 0.05$; Table 2). In addition, values for basal or stimulated GTR were negatively correlated with the proportion of phosphorylated 2-deoxyglucose ($r -0.45$; $n 26$; $P < 0.05$). This might indicate that glucose transport tended to become no longer rate-limiting at the highest GTR.

GLUT4 protein contents and mRNA levels in muscles from preruminant and weaned calves

In order to identify the molecular mechanisms which could explain the higher basal and insulin-stimulated GTR in R calves, we assessed GLUT4 amount in muscle tissues. Our previous studies have shown that the detection and, therefore, the quantification of GLUT4 in whole homogenates or Triton extracts of muscles was difficult because non-specific bands could appear which correspond to the binding of the primary antibody to major proteins (myosin, actin; Hocquette *et al.* 1996a,b). Moreover, we also showed that the total content of GLUT4 protein in oxidative muscles was harder to measure using Triton extracts because of its scarcity (Hocquette *et al.* 1995). Consequently, it was necessary to prepare crude membranes to be sure of detecting possible alterations in the abundance of GLUT4 in muscles between PR and R calves. Protein yields from crude membrane preparations per g tissue wet weight or relative to total protein content in homogenates did not differ between PR and R calves but were higher in oxidative (H, MA) than in glycolytic muscles (e.g. RA; Table 3). However, when expressed relative to DNA contents, protein yields from crude membrane preparations did not differ between muscles (Table 3).

Table 2. 2-Deoxyglucose transport into incubated fibre strips of musculus rectus abdominis from preruminant (PR) and ruminant (R) calves*
(Values are means for the no. of animals shown)

Study group . . . n . . .	Basal transport		Insulin-stimulated transport			Statistical significance (<i>P</i> <) of effect of:				
	PR 7	R 6	PR 7	R 6	SEM†	G	C	I	G × I	
Transport rate (nmol/20 min):										
per g tissue wet wt	301 ^a	394 ^b	511 ^c	720 ^d	14.0	0.0001	0.05	0.0001	0.002	
per mg DNA	275 ^a	377 ^b	469 ^c	688 ^d	15.0	0.0001	0.03	0.0001	0.002	
per mg protein	1.33 ^a	1.60 ^b	2.25 ^c	2.92 ^d	0.074	0.05	0.0008	0.0001	0.02	
Percentage of intracellular 2-deoxyglucose which was phosphorylated	94.0 ^a	92.4 ^{a,b}	92.6 ^{a,b}	90.8 ^b	0.90	0.19	0.13	0.11	0.90	

^{a,b,c,d}Means within a horizontal row with different superscript letters were significantly different (*P* < 0.05).

G, C, I, G × I, significant effects of group (G) tested against calves within group, of calf (C), of insulin (I) and of the group × insulin interaction (G × I).

† Calculated for six animals.

* For details of animals and procedures, see pp. 252–253.

As shown in a representative experiment (Fig. 2(A)), the amount of GLUT4 in crude membranes from RA muscle did not differ significantly between PR and R calves (1.00 (SE 0.210) and 1.25 (SE 0.289) arbitrary densitometric units respectively). Similar results were observed in H (1.00 (SE 0.091) and 0.87 (SE 0.087) units in PR and R calves respectively) and in TFL muscle (1.00 (SE 0.059) and 0.89 (SE 0.107) units respectively). In contrast, the amount of GLUT4 was 74 % higher in crude membranes from MA in R calves than in PR calves ($P < 0.05$; Fig. 2(B)).

GLUT4 mRNA levels were assessed using a species-specific probe as previously described (Hocquette *et al.* 1996b). GLUT4 mRNA levels were similar for PR and R calves in H, D, RA, ST and MA muscles (Table 4), although GLUT4 protein content was higher in MA from R calves.

Adipose tissue biochemical characteristics

Four adipose tissues were also studied in both PR and R calves. Biochemical variables varied greatly among adipose tissues ($P < 0.0001$; Table 5). DNA contents were lower in internal adipose tissues (541 (SE 51.7) and 377 (SE 51.1) $\mu\text{g/g}$ tissue wet weight for PAT and OAT respectively) than in peripheral adipose tissues (856 (SE 58.8) and 694 (SE 38.2) $\mu\text{g/g}$ tissue wet weight for IMAT and SCAT respectively). The highest protein content was observed for SCAT (28.5 (SE 3.56) v. 10.4–15.9 mg/g tissue wet weight for other tissues). Protein yields (mg/g tissue wet weight) from crude membrane preparations were the highest in PAT (1.98 (SE 0.219)) and the lowest in IMAT (0.32 (SE 0.042)) when compared with other adipose tissues (0.63–0.78). When expressed relative to total protein content in crude homogenates, protein yields from crude membrane preparations were, in decreasing order: PAT (13.0 (SE 1.30) %), OAT (6.1 (SE 0.68) %), peripheral adipose tissues (3.6 (SE 0.62) and 2.7 (SE 0.29) % for IMAT and SCAT respectively).

In addition, differences were also observed between PR and R calves: protein content in homogenates tended to be higher for internal adipose tissues from the R group whereas DNA contents tended to be higher for OAT and SCAT from R calves (Table 5). All these differences are likely to be explained by differences in adipocyte sizes between adipose tissues and groups of animals since total DNA and protein in homogenates on a per g tissue wet weight basis were shown to be decreased in obesity (Pénicaud *et al.* 1991; Fabres-Machado & Saito, 1995) as adipocytes became larger.

Finally, in contrast to observations in muscles, a significant effect of calf was also demonstrated for all these biochemical variables, providing evidence that fat deposition in adipocytes was largely determined by animal characteristics.

GLUT4 protein contents in adipose tissues

As shown in a representative autoradiogram (Fig. 3(A)), GLUT4 content assessed by Western-blot experiment in whole-tissue homogenates was, in decreasing order: (1) PAT, (2) OAT and IMAT, (3) SCAT. Similar results were observed when crude membranes were used: the mean levels of GLUT4 protein relative to that for OAT were 1.50 (SE 0.209), 1.00 (SE 0.098), 0.85 (SE 0.191), 0.18 (SE 0.028) arbitrary densitometric units for PAT, OAT, IMAT and SCAT respectively. In addition, GLUT4 concentration was higher in crude membranes than in homogenates, and even undetectable in homogenates from SCAT (Fig. 3(A)). Finally, GLUT4 was undetectable in the first pellet and the supernatant fraction discarded during preparation of crude membranes (data not shown). These results indicate that GLUT4 protein content in crude membranes is representative of total GLUT4 protein

Table 3. Protein yields of crude membrane preparations from heart and different muscles for preruminant (PR) and ruminant (R) calves†
(Values are means for seven animals per group)

Study group . . .	PR			R			Statistical significance ($P < $) of effect of:				
	H	MA	RA	H	MA	RA	SEM	G	C	M	G × M
Protein yields (mg/g tissue wet wt)	9.34 ^A	5.75 ^B	4.38 ^B	7.65 ^{A*}	5.65 ^B	4.83 ^B	0.477	0.20	0.73	0.0001	0.09
Protein yields (mg/g total protein)	55.8 ^A	31.5 ^B	18.8 ^C	48.3 ^A	31.9 ^B	20.0 ^C	3.12	0.37	0.75	0.0001	0.33
Protein yields (mg/mg DNA)	4.71 ^A	4.05 ^A	4.00 ^A	4.05 ^A	4.03 ^A	4.62 ^A	0.335	0.94	0.57	0.57	0.19

A,B,C,a,b,c Means within a group (PR or R) with different superscript letters were significantly different ($P < 0.05$).
H, heart; MA, *masseter*; RA, *rectus abdominis*; G, C, M, G × M, significant effects of group (G) tested against calves within group, of calf (C), of muscle (M) and of the group × muscle interaction (G × M).

* The difference between mean values for PR and R calves showed a tendency towards significance ($P < 0.10$).

† For details of animals and procedures, see pp. 252–254.

‡ Results obtained with *tensor fasciae latae* and *longissimus dorsi* muscles were similar to those with *rectus abdominis*.

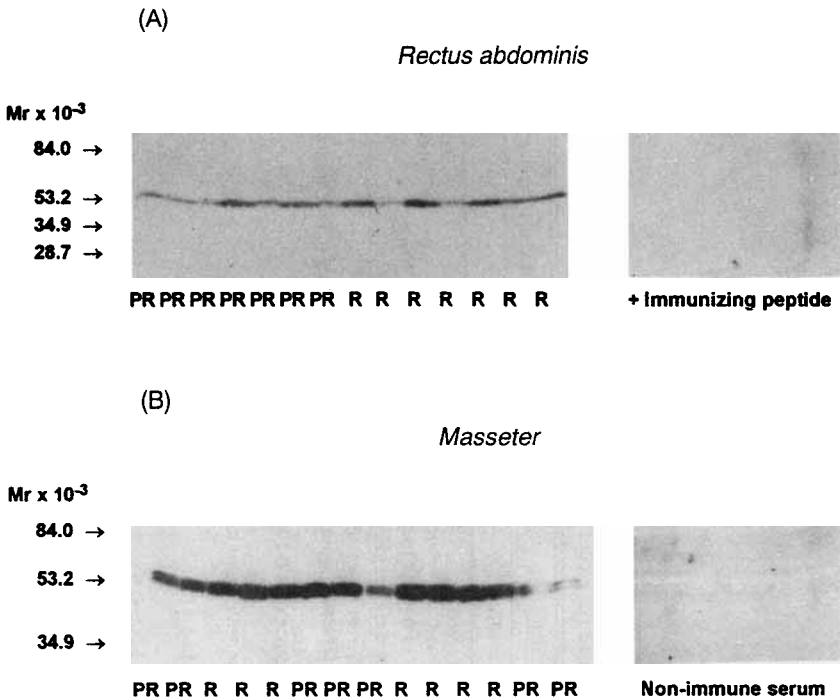


Fig. 2. Western-blot analyses of glucose transporter (GLUT4) protein in crude membranes of muscles from preruminant (PR) and ruminant (R) calves. Tissue samples were homogenized in sucrose buffer containing peptidase inhibitors and crude membranes were prepared by differential centrifugation. Different Western-blot experiments were done with equal amounts of protein for each sample (50–100 μ g depending on the experiment) and using the highly-sensitive enhanced chemiluminescence system. Representative autoradiograms are shown for *rectus abdominis* (A) and *masseter* (B) muscles. Control autoradiograms were overexposed compared with the representative autoradiograms to check the absence of the specific immunoreactive bands by pre-absorbing the primary antibody with the immunizing peptide (+immunizing peptide) or with a non-immune serum. →, The position of relative molecular mass (M_r) standards. For details of animals and procedures, see pp. 252–254.

in homogenates, as previously described for rodents (Fabres-Machado & Saito, 1995). It seemed to us, therefore, more appropriate to use membranes rather than homogenates to be sure of detecting any possible alterations in the abundance of GLUT4 between PR and R calves. However, since the amount of GLUT4 depends on the basis on which it is expressed, i.e. per cell or per cell surface area (Fabres-Machado & Saito, 1995), GLUT4 protein contents were expressed in different ways to take into account variations in protein or DNA contents and in protein yields from crude membrane preparations among animals, adipose tissues and groups of calves (Table 5).

Regardless of the method of expression of the results, GLUT4 protein content relative to that in OAT was the highest in PAT and the lowest in SCAT (Table 5). The mean GLUT4 protein content in all the calves was in decreasing order: PAT, OAT, IMAT and SCAT ($P < 0.05$) when the results were expressed per mg total protein in homogenates (3.24 (SE 0.517), 1.00 (SE 0.081), 0.43 (SE 0.056), 0.09 (SE 0.014) units respectively) or per g tissue wet weight (4.75 (SE 0.681), 1.00 (SE 0.103), 0.41 (SE 0.077), 0.25 (SE 0.052) units respectively). However, when the results were expressed per mg DNA, the mean GLUT4 protein content in IMAT (0.17 (SE 0.033) units) was as low as that in SCAT (0.12 (SE

Table 4. *Relative levels between preruminant (PR) and ruminant (R) calves of glucose transport protein GLUT4 mRNA in heart and various skeletal muscles**

(Mean values with their standard errors for five to seven calves per group. Northern-blot experiments were done using total RNA to quantify relative levels of GLUT4 mRNA levels between PR and R calves. Values are expressed in arbitrary densitometric units per mg RNA loaded on the gel (relative to the mean value in PR calves). No significant differences were observed between the two groups of animals)

Muscle . . .	H		MA		D		RA		ST	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
PR calves	1.00	0.148	1.00	0.269	1.00	0.171	1.00	0.061	1.00	0.229
R calves	1.22	0.152	1.06	0.097	1.14	0.126	1.32	0.199	0.95	0.178

H, heart; MA, *masseter*; D, *diaphragma*; RA, *rectus abdominis*; ST, *semitendinosus*.

* For details of animals and procedures, see pp. 252–255.

0.025) units), but higher in PAT and OAT (3.09 (SE 0.524) and 1.00 (SE 0.132) units respectively).

As shown in a representative experiment for OAT (Fig. 3(B)), GLUT4 protein content per 100 μ g protein in crude membranes loaded on the gels did not significantly differ between PR and R calves for the four different adipose tissues studied (Table 5). Similar results were observed when the data were expressed per g tissue wet weight (Table 5). However, mean GLUT4 protein content was lower in R calves than in PR calves (–39%; $P < 0.05$) when results were expressed on a per cell basis, i.e. per mg DNA or per mg protein in homogenates. This difference was apparent in internal adipose tissues and SCAT but not in IMAT (Table 5).

Attempts were made to detect GLUT4 mRNA in adipose tissues in order to quantify relative levels of GLUT4 transcripts between PR and R calves. Unfortunately, GLUT4 mRNA levels were too low to be detected with confidence even by using a species-specific probe as described previously for muscle tissues. Therefore, data on the GLUT4 gene expression in adipose tissues could not be presented.

Differential glycosylation of the GLUT4 protein between muscle and adipose tissues

Initial Western-blot experiments revealed that the GLUT4 protein from white adipose tissues had a slightly reduced mobility on SDS-PAGE compared with that from bovine skeletal muscle or from rat heart (Fig. 3(C)). The possibility was considered that these different electrophoretic patterns were due to heterogeneity of glycosylation of the GLUT4 proteins. Therefore, membrane samples were pretreated with endoglycosidase F–N-glycosidase F before Western-blot experiments. After complete removal of the oligosaccharides, the GLUT4 protein had a reduced apparent molecular weight (approximately 20% less for adipose tissue and 10% less for MA) which was identical for the three tissues examined (rat heart and bovine adipose tissues or skeletal muscle; Fig. 3(C)).

DISCUSSION

Bovine muscle characteristics

Studies on cattle musculature have concentrated on muscle-fibre-type composition or meat-quality variables and generally only one or two muscles have been studied (Picard *et al.* 1995). Therefore, several muscles were included in the present study to be more confident

Table 5. Protein contents, DNA contents, protein yields from crude membrane preparations and glucose transport (GLUT4) protein contents for perirenal, omental, intermuscular and subcutaneous adipose tissues from preruminant (PR) and ruminant (R) calves.†
(Values are means for the no. of animals shown)

Study group . . .	PR (n 6)			R (n 7)			Statistical significance ($P < $) of effect of:						
	PAT	OAT	IMAT	SCAT	PAT	OAT	IMAT	SCAT	SEM§	G	C	AT	G × AT
Total protein in homogenates (mg/g tissue wet wt)	13.3 ^B	9.2 ^{BC}	9.3 ^C	28.8 ^A	18.2 [*]	12.4 [†]	11.4 ^b	28.3 ^a	2.58	0.41	0.02	0.0001	0.74
DNA (μ g/tissue wet wt)	475 ^B	281 ^C	886 ^A	608 ^B	597 ^b	458 [†]	830 ^a	768 ^{**}	58.2	0.17	0.008	0.0001	0.17
Protein yield of crude membrane preparations: (mg/g tissue wet wt)	2.03 ^A	0.64 ^B	0.37 ^C	0.97 ^{BC}	1.94 ^a	0.62 ^{bc}	0.28 ^c	0.61 ^b	0.180	0.45	0.05	0.0001	0.78
(mg/g total protein)	148 ^A	73 ^B	41 ^C	31 ^C	115 ^a	50 [†]	32 ^{bc}	23 ^c	10.2	0.10	0.05	0.0001	0.55
GLUT4 level:													
ADU/mg protein in crude membranes	1.64 ^A	0.94 ^A	0.72 ^A	0.17 ^B	1.39 ^a	1.06 ^b	0.95 ^{ab}	0.20 ^b	0.195	0.88	0.03	0.0001	0.63
ADU/mg protein in homogenates	4.31 ^A	1.13 ^B	0.44 ^C	0.10 ^D	2.32 [†]	0.89 ^b	0.41 ^c	0.07 ^d	0.340	0.04	0.49	0.0001	0.02
ADU/mg DNA	4.04 ^A	1.22 ^B	0.15 ^C	0.16 ^C	2.29 ^a	0.81 ^b	0.19 ^c	0.09 ^d	0.368	0.05	0.51	0.0001	0.07
ADU/g tissue wet wt	5.73 ^A	0.98 ^B	0.39 ^C	0.30 ^C	3.92 ^a	1.02 ^b	0.43 ^c	0.21 ^d	0.499	0.20	0.51	0.0001	0.18

PAT, perirenal adipose tissue; OAT, omental adipose tissue; IMAT, intermuscular adipose tissue; SCAT, subcutaneous adipose tissue; ADU, arbitrary densitometric units; G, C, AT, G × AT, significant effects of group (G) tested against calves within group, of calf (C), of adipose tissue (AT) and of the group × adipose tissue interaction ($G \times AT$).

A,B,C,D,a,b,c,d Means within a group (PR or R) with different superscript letters were significantly different ($P < 0.05$).

† Mean values were significantly different from those for PR calves: * $P < 0.05$.

‡ The difference between mean values for PR and R calves showed a tendency towards significance ($P < 0.10$).

§ For details of animals and procedures, see pp. 252–254.

§ Calculated for six animals.

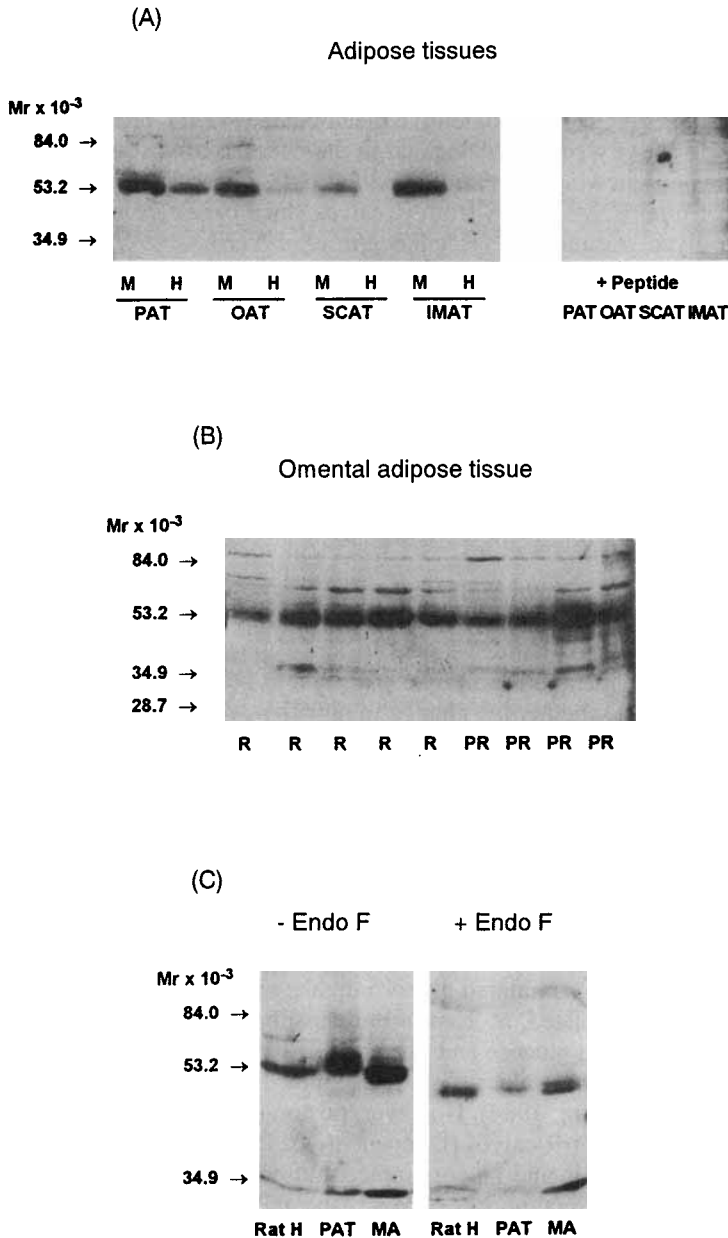


Fig. 3. Western-blot analyses of glucose transporter (GLUT4) protein in bovine adipose tissues. Tissue samples of perirenal (PAT), omental (OAT), intermuscular (IMAT) and subcutaneous (SCAT) adipose tissues were homogenized in sucrose buffer containing peptidase inhibitors and crude membranes were prepared by differential centrifugation. Western-blot experiments were done using the highly-sensitive enhanced chemiluminescence system. →, The position of relative molecular mass (M_r) standards. (A) Analyses were done with 50 μ g protein from homogenates (H) and crude membranes (M) from the four adipose tissues. Control autoradiograms were done to check the absence of the specific immunoreactive bands by pre-absorbing the primary antibody with the immunizing peptide (+peptide). (B) Analyses were done with 30 μ g protein from crude membranes of OAT from preruminant (PR) and ruminant (R) calves. (C) Analyses were done with 30 μ g crude membranes of bovine *masseter* muscle (M) and PAT before (-endo F) and after (+endo F) deglycosylation by endoglycosidase F-N-glycosidase F. A control sample of rat heart (rat H) was added to the experiment.

of the results and/or to detect any muscle-specific response to changes in nutrition at weaning. The decrease in dietary carbohydrate supply from weaning induced a small, but significant, decrease in LDH activity in almost all studied muscles (Fig. 1). In MA, weaning induces large increases in oxidative metabolism (Fig. 1), probably associated with changes in muscle activity since MA located in the cheek is almost constantly active in R calves for solid food chewing (Karlstrom *et al.* 1994). These changes might explain the higher content of total RNA in MA from R calves since oxidative muscles contain more total RNA than glycolytic muscles (Hocquette *et al.* 1996b).

Glucose uptake by bovine muscles

GTR is considered as the rate-limiting step of glucose metabolism in muscles since intracellular free glucose concentrations are low (Table 2; for review, see Kahn, 1992; Hocquette *et al.* 1996a). However, when GTR is excessively elevated by either over-expression of GLUT4, exercise, hyperglycaemia or hyperinsulinaemia, free glucose accumulates in the cells (Manchester *et al.* 1994) and intracellular steps of glucose metabolism other than GTR become rate-limiting (for review, see Zierath, 1995; Hocquette *et al.* 1996a).

Glucose tolerance was shown to decrease with increasing age, either for PR (Webb *et al.* 1969; Doppenberg & Palmquist, 1991) or R (Jarrett *et al.* 1964; Colvin *et al.* 1967; Webb *et al.* 1969) calves or lambs. However, since glucose metabolism is affected by various physiological or metabolic variables (for review, see Kahn, 1992) especially age, it was difficult to conclude whether the change in nutrition which occurs at weaning induces a change in insulin sensitivity in ruminants. Our study addresses this question by measuring *in vitro* GTR (Hocquette *et al.* 1995) under conditions where most of the factors influencing glucose uptake were similar except the change in nutrition induced by weaning. This approach led us to demonstrate (1) that insulin increased GTR in calf RA muscle, but to a lower extent than that in rats or in human subjects (Hocquette *et al.* 1995) and (2) that maximal response to insulin was lower in PR than in age-matched R calves (Table 2) in agreement with other data *in vivo* (Palmquist *et al.* 1992). Thus, these differences in GTR for one muscle are likely to reflect differences in the whole-body response to insulin, since *in vivo* whole-body insulin-stimulated glucose uptake and *in vitro* GTR in *vastus lateralis* muscle are positively correlated, at least in human subjects (Zierath, 1995).

Our findings for plasma glucose and insulin levels 16 h after the last meal (Table 1) are in agreement with preprandial values observed in milk-fed (Hostettler-Allen *et al.* 1994) or weaned calves (Kouame *et al.* 1984). However, postprandial glucose and insulin levels are much higher for PR than for R calves (Doppenberg & Palmquist, 1991). *In vitro* GTR was measured at the same insulin and glucose concentrations in the incubation medium. Thus, the higher *in vivo* glycaemia in PR calves, especially in the postprandial state, may compensate for the lower preprandial GTR by increasing the non-insulin-mediated rate of glucose uptake as in type II diabetic patients (Zierath, 1995).

Regulation of GTR in muscles

Long-chain fatty acids and carbohydrates supplied by the diet decrease as young calves are weaned, since forage is degraded into volatile fatty acids by micro-organisms present in the rumen after weaning. In contrast, rats change from a high-fat diet to a high-carbohydrate diet during the suckling–weaning transition. These changes in the rat were shown to be associated with an increase in insulin responsiveness of glucose uptake by muscles (for

review, see Girard *et al.* 1992). *In vivo* experiments 4 h post feeding (Palmquist *et al.* 1992) and *in vitro* studies in the preprandial state (Table 2) indicate that a similar change occurs in the calf despite great differences between species in the changes of nutrition at weaning. However, unlike the rat (for review, see Girard *et al.* 1992), GLUT4 protein content did not increase in adipose tissues and muscles from the weaned calf except in MA muscle. This clearly demonstrates (1) that the regulation of GTR is under species- and muscle-specific control mechanisms and (2) that GLUT4 expression is poorly regulated at weaning in the calf despite profound changes in nutrition.

The low ability of insulin to stimulate GTR in PR calves in the preprandial state (Table 2) was shown to be exaggerated after feed consumption (Hostettler-Allen *et al.* 1994), providing evidence for the involvement of nutritional factors. The high amount of fat in the milk-substitute might induce an insulin resistance as shown in the rat (Girard *et al.* 1992). However, this seems unlikely since the dietary fat content did not modify the action of insulin on glucose metabolism in veal calves (Palmquist *et al.* 1992). The lactose content of the diet of PR calves, which was particularly high in the present study (40 % of energy intake), may decrease the ability of insulin to stimulate glucose uptake by muscles (Palmquist, *et al.* 1992; for review, see Bauchart *et al.* 1996) leading to the concept of glucose toxicity (for review, see Yki-Järvinen, 1992; Zierath, 1995). The desensitization of the glucose transport system to insulin by chronic hyperglycaemia can be mediated by an increase in glucose flux through the glucosamine pathway without any change in the amount of GLUT4 (Robinson *et al.* 1993) as in our calves (Fig. 2). Veal calves intensively fed on milk-substitutes, especially lactose-rich milk-substitutes, develop glucose intolerance and sometimes glycosuria (Palmquist, *et al.* 1992; Hostettler-Allen *et al.* 1994; for review, see Bauchart *et al.* 1996). This may indicate a saturated capacity for glucose use which would be expected to reduce feed efficiency and, therefore, growth performance (Hostettler-Allen *et al.* 1994).

GLUT4 expression in bovine muscles

GLUT4 protein content is much higher in oxidative muscles than glycolytic muscles in the rat (for review, see Kahn, 1992) but not in human subjects (Andersen *et al.* 1993a; Houmard *et al.* 1995) and in ruminant species (Hocquette *et al.* 1995). This may be related to species-specific muscle characteristics rather than to muscle metabolism itself since, in contrast to rodents, the fibre-type composition of the major muscle groups of human subjects (Johnson *et al.* 1973) and bovines (except H and MA; Karlstrom *et al.* 1994) differs relatively little among different muscle groups. Difference in physical activity may also influence GLUT4 protein content, as shown in rats (Etgen *et al.* 1993; Megeney *et al.* 1993) and in human subjects (Houmard *et al.* 1995). Therefore, it is not surprising that, in ruminants, active locomotor mixed muscles (LD, TFL, ST) contain a relatively high amount of GLUT4 (Hocquette *et al.* 1995). The higher GLUT4 content in the weaned calf only in MA muscle (Fig. 2(B)) results from a specific change in the physiological function of this muscle (the development of food chewing).

Although GLUT4 was initially known to be regulated at the pretranslational level, especially in adipose tissues (for review, see Klip *et al.* 1994; McGowan *et al.* 1995), it is now accepted that GLUT4 expression may be achieved by modulating the translation efficiency or the protein turnover (Tordjman *et al.* 1990; Hocquette *et al.* 1996b; for review, see Klip *et al.* 1994). The small increase in the amount of GLUT4 protein in MA at weaning (Fig. 2(B)) with no significant difference in GLUT4 mRNA levels (Table 4) suggests the existence of a translational or post-translational mechanism of GLUT4

regulation, as in muscles from glucose-infused rats (Klip *et al.* 1994) or from human subjects after 4 h of hyperinsulinaemia (Anderson *et al.* 1993a,b).

Biochemical variables and GLUT4 protein content of adipose tissues

Total protein and DNA contents per g tissue wet weight were reduced in white adipose tissues of obese animals in which size of adipocytes was increased (Pénicaud *et al.* 1991; Fabres-Machado & Saito, 1995). Thus, our results (Table 5) suggest that adipocytes were larger in PR than in R calves and also larger in internal adipose tissues (OAT and PAT) than in peripheral adipose tissues (IMAT and SCAT), as previously described (Robelin, 1986; De la Hoz & Vernon, 1993). This is the reason why it is preferable to express GLUT4 content per mg DNA or per mg total protein, i.e. on a per cell basis.

Unlike in rats, acetate rather than glucose is the major precursor for fatty acid synthesis in ruminants, but glucose is required for lipogenesis since it is a precursor for acylglycerol-glycerol and the major source for NADPH (Smith, 1983). The lower GLUT4 content in peripheral adipose tissue compared with internal adipose tissues is in agreement with the low ability of insulin to stimulate GTR in isolated adipocytes from ovine SCAT (Sasaki, 1990) and with insulin resistance in peripheral adipose tissues compared with OAT (De la Hoz & Vernon, 1993). Similar differences in GLUT4 contents were observed between rat peripheral and internal adipose tissues (Cousin *et al.* 1993).

The lower GLUT4 content on a per cell basis in R calves (Table 5) suggests that glucose uptake by adipose tissues may be lower in R calves than in PR calves, whereas GTR was enhanced in RA muscle from R calves (Table 2). In contrast, a 5-fold increase in GLUT4 content was observed in rat adipose cells at weaning (for review, see Girard *et al.* 1992). These differences among species are likely to be explained by differential variations in insulinaemia; the low insulin level in the weaned calf was associated with a decrease in GLUT4 content whereas the high blood insulin levels in the weaned rat (Girard *et al.* 1992) and in the hyperinsulinaemic obese mouse (Fabres-Machado & Saito, 1995) were associated with increases in GLUT4 content per cell.

Characterization of a GLUT4-like protein in ruminant tissues has been performed by several authors and some conflicting results were observed in relation to its apparent molecular weight (Hocquette *et al.* 1996b). Some of these discrepancies may be explained by a higher molecular weight of the GLUT4 protein in adipose tissues (Fig. 3(C)), as previously observed in the mouse (Le Marchand-Brustel *et al.* 1990). This was shown to be due to a differential glycosylation of the GLUT4 protein (Fig. 3(C)). Such a heterogeneity of glycosylation has been previously observed within the same tissue in mice (Kaestner *et al.* 1991). An altered glycosylation may affect glucose transporter function and, in some cases, gene expression as has been shown for GLUT1; inhibitors of glycosylation induced an enhanced GLUT1 mRNA and protein expression but did not increase GTR, suggesting that the deglycosylated form of GLUT1 is less functional (Maher & Harrison, 1991).

Large inter-individual variability of metabolic and biochemical variables

The statistical analysis allowed us to test the effect of animal for all variables studied. In muscles, no significant effect was demonstrated for protein, DNA and RNA contents, suggesting that the muscle structure did not differ between animals. On the contrary, for adipose tissues, there were highly significant differences between animals for DNA and protein contents and for protein yields from crude membrane preparations (Table 5). This

may be due to great differences in adipocyte size among animals which itself depends on the amount of triacylglycerols stored within each adipocyte.

All metabolic variables examined in muscles (ICDH and LDH activities as well as GTR) varied greatly among calves (Fig. 1 and Table 2) as in steers for muscle enzyme activities (Karlstrom *et al.* 1994) and in human subjects for fibre-type proportion (Johnson *et al.* 1973), GLUT4 content and GTR (for review, see Zierath, 1995). This large inter-individual variability in muscle metabolism is dependent, at least in part, on genetic inheritance and, to some extent, physical activity, at least in human subjects (for review, see Zierath, 1995).

In conclusion, in the case of prolongation of milk-feeding, especially with lactose-rich milk-substitutes for veal calf production (Hostettler-Allen *et al.* 1994), PR calves seem to be less sensitive to insulin than age-matched weaned calves both *in vivo* (Palmquist *et al.* 1992) and *in vitro* as shown in the present study. In contrast to the situation in suckling and weaned rats (for review, see Girard, 1992), GLUT4 amount was approximately the same in skeletal muscles from PR or R calves except in MA muscle which is more oxidative and more active after weaning. Finally, unlike the rat, GLUT4 protein expressed on a per cell basis is lower in adipose tissues in the weaned calf, in agreement with concomitant variations in insulin levels.

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