Glucose-transporter (GLUT4) protein content in oxidative and glycolytic skeletal muscles from calf and goat

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It is well accepted that skeletal muscle is a major glucose-utilizing tissue and that insulin is able to stimulate *in vivo* glucose utilization in ruminants as in monogastrics. In order to determine precisely how glucose uptake is controlled in various ruminant muscles, particularly by insulin, this study was designed to investigate *in vitro* glucose transport and insulin-regulatable glucose-transporter protein (GLUT4) in muscle from calf and goat. Our data demonstrate that glucose transport is the rate-limiting step for glucose uptake in bovine fibre strips, as in rat muscle. Insulin increases the rate of *in vitro* glucose transport in bovine muscle, but to a lower extent than in rat muscle. A GLUT4-like protein was detected by immunoblot assay in all insulin-responsive tissues from calf and goat (heart, skeletal muscle, adipose tissue) but not in liver, brain, erythrocytes and intestine. Unlike the rat, bovine and goat GLUT4 content is higher in glycolytic and oxido-glycolytic muscles than in oxidative muscles. In conclusion, using both a functional test (insulin stimulation of glucose transport) and an immunological approach, this study demonstrates that ruminant muscles express GLUT4 protein. Our data also suggest that, in ruminants, glucose is the main energy-yielding substrate for glycolytic but not for oxidative muscles, and that insulin responsiveness may be lower in oxidative than in other skeletal muscles.

INTRODUCTION

In roughage-fed ruminants, most of the carbohydrate from the diet is fermented in the rumen to volatile fatty acids, mainly acetic, propionic and butyric acids. Consequently, only small amounts of glucose are usually absorbed. Blood glucose concentration in ruminants (3–3.8 mM) is lower than in other mammals (4.2–4.8 mM) and gluconeogenesis is of primary importance to provide glucose for glucose-utilizing tissues even in the fed state. Moreover, glucose makes a small contribution to the total CO_2 production of the whole body compared with acetate and ketone bodies (4–11% versus 70%). This can be explained by a low rate of glucose utilization in the central nervous system, liver (which lacks glucokinase) and adipose tissues (which relies more on acetate for lipogenesis) [1,2].

However, glucose metabolism is of importance for ruminant muscles. In fact, 30–60% of CO_2 produced by muscle may be derived from glucose [1]. Muscle cells, especially glycolytic fibres, are absolutely dependent on a regular supply of glucose. Muscle glycogen stores derived from glucose are quantitatively important. Consequently, as much as 40–50% of the glucose cleared daily is disposed of in muscles of normally fed ruminants [1].

Insulin stimulation of glucose utilization has been well documented in sheep [3], cattle [4,5] and goat [6]. Specific characteristics of insulin binding and insulin receptors have been described in sheep and goat skeletal muscles [7,8].

Glucose transport across membranes of muscle cells is the rate-limiting step of glucose utilization by muscle, at least in rat [9,10]. Moreover, this step is acutely regulated by insulin. Human and rat muscle cells express two main glucose-transporter isoforms, GLUT1 and GLUT4. In rat muscle, the amount of GLUT4 is 10–20 times higher than that of GLUT1, and GLUT4 is the isoform primarily responsible for acute insulin-stimulated glucose transport [11]. In cattle, GLUT1 has been reported in brain but not in muscle [12]. To our knowledge, insulin-responsive glucose transport and GLUT4 protein have never been found in ruminant skeletal muscles, only in adipose tissue from sheep and goat [13,14]. Therefore the aim of the present work was to characterize GLUT4 in different calf and goat muscles with various oxidative and glycolytic activities.

EXPERIMENTAL

Reagents

Polyclonal antibody raised against rat GLUT4 [15] was supplied by East-Acres Biologicals (Southbridge, MA, U.S.A.). The chemicals for immunoblot assay were supplied by Bio-Rad (Munich, Germany) except membranes (Immobilon-P), which were from Millipore (Bedford, MA, U.S.A.). ^{125}I-labelled Protein A and Hyperfilms MP were supplied by Amersham International (Amersham, Bucks., U.K.). 2-Deoxy-[1,2-^{3}H]glucose (970 GBq/mmol) and [U-^{14}C]sucrose (20 GBq/mmol) were purchased from New England Nuclear and ICN Biochemicals (Irvine, CA, U.S.A.) respectively. Other reagents were from Sigma (St. Louis, MO, U.S.A.).

Animals and experimental design

Seven Montbeliard calves, housed in individual stalls in a room with natural lighting, were used. Animals were tied up and fed

Abbreviations used: GLUT4, insulin-responsive glucose transporter; 2-dGlc, 2-deoxyglucose; ICDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase.

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individually on concentrate (80 %) and hay (20 %) according to a feeding pattern designed to allow an average daily gain of 1300 g. The composition of the concentrate was dehydrated alfalfa (30 %), sugar beet pulp (40 %), barley (14 %), soya-bean meal (11 %) and mineral compound (5 %). Body weight was measured weekly and the rate of feeding for the entire group was adjusted accordingly. Calves were slaughtered at 170 ± 2.6 days of age (body weight 216.7 ± 2.7 kg). This was carried out at 09:00 h after an overnight fast by stunning (captive-blot pistol) and exsanguination. Tissue samples (50–100 g) were always taken at the same place for all animals less than 10 min post mortem, in the following order: erythrocytes, heart, masseter, musculus rectus abdominis, diaphragma, musculus longissimus dorsi at the level of the 6th rib, musculus semitendinosus from the medial portion, musculus tensor fasciae latae, musculus cutaneus trunci from the thick part, perirenal adipose tissue, small intestine, liver and brain. Samples from calf tissues were cut into pieces and then divided into two as soon as they were obtained: one-half was frozen in liquid nitrogen less than 10 min after exsanguination and stored at −80 °C for subsequent analysis. Samples were always pulverized in liquid nitrogen to obtain a homogeneous powder before any treatment. The second half (six to seven small pieces within the sample) was immediately homogenized at 4 °C in an equal volume of 70 mM sucrose buffer containing 210 mM mannitol, 20 % (w/v) dimethyl sulphoxide and peptidase inhibitors [chymostatin, pepstatin A, leupeptin, antipain (5 μg/ml each), aprotinin (0.17 trypsin inhibitor unit/ml) and 1 mM phenylmethanesulphonyl fluoride which was added extemporaneously]; frozen homogenate was stored at −80 °C for subsequent subcellular preparations.

Six 5–7-year-old alpine dry goats were also used. Animals were fed and slaughtered as previously described [6]. The following tissues were quickly excised and frozen in liquid nitrogen within 15 min: masseter, diaphragma, musculus longissimus dorsi, musculus tensor fasciae latae, musculus anconeus, heart, perirenal adipose tissue, liver and brain.

Adult (4–6 month-old) male Sprague–Dawley rats were from Iffa-Credo (L'Arbresle, France). They were housed, fed and killed as described previously [16]. Epithrochlearis muscles were dissected intact for incubation [16].

2-Deoxyglucose (2-dGlc) transport

A sample of rectus abdominis muscle was taken with a 3 cm clamp from each calf at slaughter. Six muscle fibres strips weighing 70–120 mg were isolated from each mounted muscle sample in less than 10 min and smaller clamps were placed on the muscle strips before they were cut free [17]. The rest of the sample was frozen in liquid nitrogen or homogenized as described above for other analyses. The muscle strips were incubated in oxygenated Krebs–Henseleit buffer containing 2 mM pyruvate [17] and the rate of 2-dGlc transport was measured, as described previously [16,17], for 20 min [the rate of 2-dGlc uptake was linear for 30 min ($r = 0.99$ for four observations at different incubation times; $P < 0.01$; results not shown)]. Tissue concentrations of 2-dGlc and 2-dGlc 6-phosphate were determined as previously described [18].

Analytical techniques

Protein, DNA, isocitrate dehydrogenase (ICDH) activity (characteristic of oxidative metabolism) and lactate dehydrogenase (LDH) activity (characteristic of glycolytic metabolism) were measured spectrophotometrically [19–22]. Tissue concentrations of ATP, phosphocreatine and lactate were measured by enzymic assays [23,24] (Kit Sigma, Ref 826-A).

Western-blot analysis

Tissue samples were homogenized with a Polytron in 10 vol. of PBS containing 1 % Triton X-100 and peptidase inhibitors as above. Insoluble material was removed by centrifugation at 1000 *g* for 10 min at 4 °C [25].

For crude membrane preparations, frozen homogenates in sucrose buffer containing mannitol and dimethyl sulphoxide were used as starting material and treated as described previously [26].

Triton extracts or subcellular fractions were stored at −80 °C until use. Equal amounts of Triton extract or membrane protein (50–100 μg) were solubilized in Laemmli buffer and subjected to SDS/PAGE (12 % gels). Immunoblot analysis was performed using polyclonal antibody against rat GLUT4 (final dilution 1:250–1:500) [15] and using ^{125}I-Protein A as previously described [25]. In some experiments, GLUT4 was detected using the same antibody against rat GLUT4 (final dilution 1:2500–1:5000), a horseradish peroxidase-labelled second antibody and a chemiluminescent reaction (ECL Western blotting kit from Amersham International). Hyperfilm MP was exposed to membranes, and GLUT4 was quantified by scanning densitometry (Hoeffer, San Francisco, CA, U.S.A.) of the autoradiograms [25].

Muscle contents of GLUT4 were routinely determined using Triton extracts and not crude membranes as significant amounts of GLUT4 were lost during membrane preparation. GLUT4 content was linearly related to the amount of protein loaded on the gel ($r = 0.988$ for seven measurements; $P < 0.01$; results not shown). No GLUT4 transporters were detected in the unsolubilized material and GLUT4 concentration (expressed per mg of Triton X-100-solubilized protein) was 1.6–2.5 times higher in Triton extracts than in whole homogenates for musculus tensor fasciae latae and musculus longissimus dorsi respectively, which indicated that Triton X-100 solubilized all GLUT4 transporters.

Statistical analysis

Results were expressed as means ± S.E.M. Significant difference between biochemical parameters of two muscles from the seven calves or from the six goats was evaluated by paired Student's *t* test. Significant difference between 2-dGlc-transport rates was evaluated by a non-parametric test (Mann–Whitney *U* test).

RESULTS

Characterization of GLUT4 in bovine and goat tissues

Characterization of a GLUT4-like protein in ruminant tissues was performed by immunoblot analysis using crude membranes because GLUT4 concentration was higher in crude membranes than in Triton extracts. A band with a molecular mass of around 42 kDa was observed in muscle from calves and goats (Figures 1a and 1b) as described previously for goat adipose tissue [14]. The size of this protein band was similar to that observed for rat GLUT4 (Figure 1) [25]. This band was absent when a non-immune serum was used (results not shown). It was observed in all insulin-responsive tissues [adipose tissue, skeletal muscles (musculus cutaneus trunci, musculus semitendinosus, musculus longissimus dorsi, musculus anconeus, musculus rectus abdominis, musculus tensor fasciae latae, diaphragma and masseter) and heart] but not in brain, small intestine, liver and erythrocytes (Figure 1) as also reported for rat GLUT4 [15]. However, variations in GLUT4 content in crude membrane preparations of various muscles do not necessarily reflect physiological

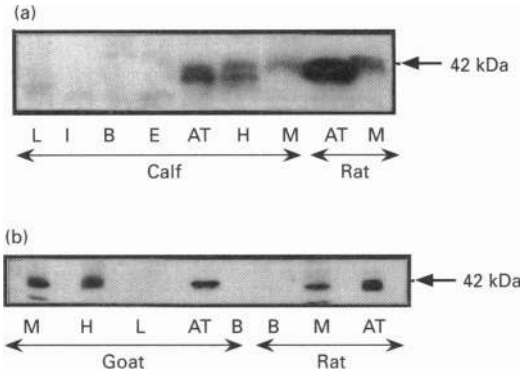

Figure 1 Immunodetection of GLUT4 protein in various tissues from calf, goat and rat

Crude membranes of liver (L), small intestine (I), brain (B), erythrocytes (E), adipose tissue (AT), heart (H) and skeletal muscle (musculus tensor fasciae latae) (M) were prepared and analysed by Western blot as described in the Experimental section. Antigen–antibody complexes were detected using ^{125}I-Protein A (**a**) or the highly sensitive enhanced chemiluminescence system (**b**).

variations in GLUT4 content of the cells of those muscles, as differences between protein yields from crude membrane preparations from oxidative and glycolytic muscles were observed (results not shown). This is why GLUT4 content of the various muscles was precisely determined using the soluble fraction of Triton X-100 preparations.

GLUT4 protein content of oxidative and glycolytic muscles from calves and goats

Muscle metabolic activity was characterized using ICDH and LDH assays [21,22]. These two enzyme activities were found to be inversely related in both bovine and goat muscles (Tables 1 and 2) and the oxidative potential of the muscles was, in decreasing order, heart, masseter, diaphragma, rectus abdominis, tensor fasciae latae, semitendinosus, cutaneus trunci and longissimus dorsi. DNA contents and yields of Triton X-100-solubilized protein (when expressed per total protein or per wet weight) in the various muscles are also indicated in Tables 1 and 2. As previously reported for bovine muscles [22], DNA content and LDH activity were inversely related.

As shown in a representative autoradiogram (Figures 2a and 2b), GLUT4 protein content measured in Triton extracts was low in heart and oxidative muscle (masseter, diaphragma) and high in other skeletal muscles (rectus abdominis, semitendinosus, tensor fasciae latae, longissimus dorsi, anconeus, cutaneus trunci) from calf and goat, whereas, in rat, it was high in oxidative muscles (heart and soleus) and low in glycolytic muscles (extensor digitorum longus and epitrochlearis) (Figure 2c) [15,27,28].

GLUT4 contents expressed in arbitrary densitometric units/mg of solubilized protein were normalized to the mean value in musculus longissimus dorsi. The results show that GLUT4 was present at the lowest concentration in heart, masseter and diaphragma and at the highest concentration in other skeletal muscles, for both calf (Table 1) and goat (Table 2). These data were also expressed in different ways to take into account variations in the content of solubilized protein per total muscle protein and per tissue wet weight and variations in DNA content among the different muscles (Tables 1 and 2). The results were similar regardless of the method of expression (Tables 1 and 2).

Table 1 Biochemical characteristics (in absolute values) and GLUT4 protein content (relative to mean values observed in musculus longissimus dorsi) of eight bovine muscles

Data are means ± S.E.M. of seven animals. Muscle tissues were weighed and homogenized in PBS containing 1% Triton X-100. Total tissue protein and DNA contents were measured in the homogenates. Total cellular GLUT4 was analysed by Western blot and quantified by densitometry of autoradiograms. Results were expressed in arbitrary densitometric units per mg of solubilized protein and then normalized per mg of total muscle protein, per mg of DNA or per g tissue wet weight. In each case, results were divided by the mean GLUT4 content in musculus longissimus dorsi; –, not detectable. A value followed by a superscript differs significantly ($P < 0.05$) from all other values in the same line not followed by the same superscript.

	Heart	Masseter	Diaphragma	Rectus abdominis	Tensor fasciae latae	Semintendinosus	Cutaneus trunci	Longissimus dorsi
ICDH activity (nkat/g)	2.511 ± 0.252^{a}	1.600 ± 0.083^{b}	1.013 ± 0.090^{c}	0.184 ± 0.023^{d}	0.181 ± 0.012^{d}	0.227 ± 0.035^{d}	0.150 ± 0.046^{d}	0.164 ± 0.012^{d}
LDH activity (nkat/g)	28.45 ± 1.49^{g}	17.22 ± 1.30^{f}	35.51 ± 2.59^{d}	73.69 ± 2.10^{c}	79.86 ± 3.01^{b}	81.67 ± 1.52^{b}	79.10 ± 4.39^{bc}	98.40 ± 1.84^{a}
DNA (μg/g tissue wet weight)	1903 ± 43^{a}	1423 ± 57^{b}	1367 ± 41^{b}	1050 ± 14^{c}	1080 ± 35^{c}	1073 ± 33^{c}	1115 ± 37^{c}	871 ± 9^{d}
Triton X-100-solubilized protein (% of total protein)	60.1 ± 2.1^{a}	65.7 ± 2.9^{a}	45.3 ± 1.4^{bc}	40.0 ± 2.1^{c}	54.4 ± 4.2^{ab}	56.2 ± 1.7^{a}	48.4 ± 2.0^{b}	54.4 ± 4.6^{ab}
Triton X-100-solubilized protein (mg/g tissue wet weight)	95.3 ± 3.3^{b}	115.8 ± 3.6^{a}	77.0 ± 2.7^{c}	97.0 ± 2.0^{b}	119.3 ± 8.7^{a}	93.6 ± 3.2^{b}	109.4 ± 4.7^{a}	92.3 ± 4.4^{b}
GLUT4 (arbitrary units/mg of Triton X-100-solubilized protein)	–	–	0.111 ± 0.018^{d}	0.634 ± 0.093^{bc}	0.443 ± 0.043^{c}	0.623 ± 0.059^{b}	0.582 ± 0.099^{bc}	1.000 ± 0.135^{a}
GLUT4 (arbitrary units/mg of total protein)	–	–	0.088 ± 0.012^{d}	0.462 ± 0.072^{c}	0.424 ± 0.037^{c}	0.628 ± 0.063^{b}	0.506 ± 0.094^{bc}	1.000 ± 0.185^{a}
GLUT4 (arbitrary units/mg of DNA)	–	–	0.058 ± 0.009^{c}	0.558 ± 0.087^{b}	0.456 ± 0.043^{b}	0.520 ± 0.062^{b}	0.557 ± 0.116^{b}	1.000 ± 0.148^{a}
GLUT4 (arbitrary units/g tissue wet weight)	–	–	0.091 ± 0.014^{c}	0.667 ± 0.096^{ab}	0.564 ± 0.050^{b}	0.630 ± 0.056^{b}	0.700 ± 0.135^{b}	1.000 ± 0.144^{a}

Table 2 Biochemical characteristics (in absolute values) and GLUT4 protein content (relative to mean values in musculus longissimus dorsi) of five goat muscles

Data are means ± S.E.M. of six animals. Muscle tissues were treated and results expressed as described in Table 1. A value followed by a superscript differs significantly ($P < 0.05$) from all other values in the same line not followed by the same superscript.

	Masseter	Diaphragma	Anconeus	Tensor fasciae latae	Longissimus dorsi
ICHD activity (nkat/g)	2.661 ± 0.127^{d}	1.217 ± 0.035^{c}	0.536 ± 0.097^{b}	0.328 ± 0.044^{a}	0.362 ± 0.025^{ab}
LDH activity (nkat/g)	26.20 ± 1.12^{b}	19.50 ± 1.26^{b}	41.94 ± 2.18^{a}	50.70 ± 1.17^{a}	57.39 ± 2.66^{a}
DNA (μg/g tissue wet weight)	1333 ± 53^{a}	1189 ± 39^{a}	1046 ± 101^{a}	1000 ± 30^{a}	1128 ± 42^{a}
Triton X-100-solubilized protein (% of total protein)	56.2 ± 3.0^{a}	56.2 ± 3.0^{a}	58.3 ± 2.2^{a}	55.3 ± 2.6^{a}	55.6 ± 2.3^{a}
Triton X-100-solubilized protein (mg/g tissue wet weight)	138.4 ± 6.6^{ac}	142.3 ± 6.3^{abc}	142.8 ± 2.3^{c}	157.5 ± 3.2^{b}	132.2 ± 3.8^{a}
GLUT4 (arbitrary units/mg of Triton X-100-solubilized protein)	0.234 ± 0.034^{c}	0.291 ± 0.067^{c}	0.691 ± 0.075^{b}	0.869 ± 0.074^{a}	1.000 ± 0.090^{a}
GLUT4 (arbitrary units/mg of total protein)	0.216 ± 0.016^{b}	0.283 ± 0.051^{b}	0.756 ± 0.089^{a}	0.863 ± 0.079^{a}	1.000 ± 0.106^{a}
GLUT4 (arbitrary units/mg of DNA)	0.198 ± 0.024^{b}	0.294 ± 0.064^{b}	0.926 ± 0.196^{a}	1.176 ± 0.134^{a}	1.000 ± 0.118^{a}
GLUT4 (arbitrary units/g tissue wet weight)	0.229 ± 0.017^{b}	0.306 ± 0.064^{b}	0.811 ± 0.108^{a}	1.033 ± 0.085^{a}	1.000 ± 0.118^{a}

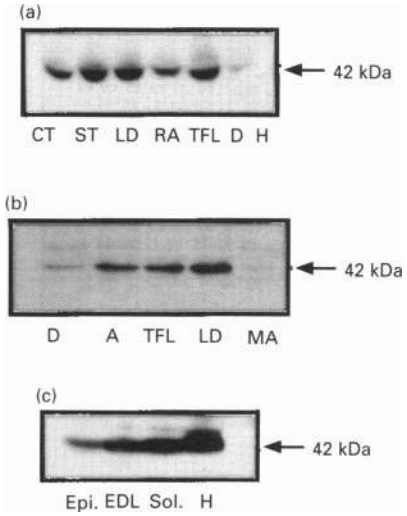

Figure 2 Western-blot analysis of glucose-transporter (GLUT4) protein in Triton extracts of oxidative muscles [heart (H), soleus (Sol.), diaphragma (D), masseter (MA)] and oxidoglycolytic muscles [musculus rectus abdominis (RA), musculus tensor fasciae latae (TFL), musculus semitendinosus (ST), musculus cutaneus trunci (CT), musculus longissimus dorsi (LD), musculus anconeus (A), epitrochlearis (Epi.) and extensor digitorum longus (EDL)] from calf (a), goat (b) and rat (c)

The results are representative of five to seven separate experiments. Western-blot experiments were performed as described in the Experimental section with 100 (**a**) or 50 (**b** and **c**) μg of protein and using ^{125}I-Protein A (**a**) or the highly sensitive enhanced chemiluminescence system (**b** and **c**).

Moreover, mean GLUT4 content in the six bovine muscles in which it was detected and in the five goat muscles showed a negative correlation with mean DNA content and mean ICDH activity and a positive correlation with mean LDH activity (Tables 1 and 2; $r > 0.83$; $n = 6$; $P < 0.05$).

Muscle viability *in vitro*

In order to demonstrate that the GLUT4-like protein identified was functional, we measured the rate of glucose transport in incubated fibres from musculus rectus abdominis. This muscle was chosen because its GLUT4 content was high and because it was easier to isolate muscle fibre strips from it than from other muscles such as musculus semitendinosus (results not shown) [17]. To establish that our muscle preparation was appropriate for the planned metabolic studies, concentrations of ATP, phosphocreatine and lactate were determined in fresh (frozen immediately after excision) and incubated muscle fibre strips. ATP concentration remained in the physiological range but was significantly reduced after 90 min of incubation (Table 3) as previously observed for human muscle strips [17]. Concentrations of phosphocreatine and lactate (Table 3) were also in the physiological range and they did not significantly change after 90 min of incubation. Moreover, glycogen, estimated by histochemistry using the periodic acid/Schiff reaction [29] on cryosections from bovine rectus abdominis muscle, showed no difference between fresh and incubated fibre strips (results not shown).

Table 3 Metabolite levels in control (quickly frozen) and incubated (60 or 90 min) muscle samples

ATP, phosphocreatine and lactate were assayed as described in the Experimental section. Results are expressed as μmol/g of wet tissue and are means ± S.E.M. *Significant difference ($P < 0.05$) between control and incubated sample.

Incubation time (min)	Number of observations	[ATP] (μmol/g)	[Phosphocreatine] (μmol/g)	[Lactate] (μmol/g)
0	5	5.73 ± 0.43	14.07 ± 2.88	4.71 ± 0.96
60	4	4.65 ± 0.61	15.55 ± 4.35	4.00 ± 0.88
90	4	4.23 ± 0.42*	15.87 ± 1.50	5.59 ± 1.31

Measurement of the rate of glucose transport

The basal rate of 2-dGlc transport was not significantly different in bovine muscle fibre strips (394 ± 17 nmol of 2-dGlc/20 min per g; $n = 6$) from that in rat muscle (316 ± 63 nmol of 2-dGlc/20 min per g; $n = 3$). Insulin stimulated glucose transport in both rat and bovine muscle ($P < 0.05$). However, the maximum insulin response was significantly lower in bovine muscle fibre

strips (719 ± 22 nmol of 2-dGlc/20 min per g; $n = 6$) than in rat muscle (1130 ± 238 nmol of 2-dGlc/20 min per g; $n = 3$) ($P < 0.05$). Most of the intracellular 2-dGlc in bovine muscle was phosphorylated (92.4 ± 1.0 and 90.8 ± 3.6 % in the absence and presence of insulin respectively; $n = 6$), indicating that hexokinase activity was higher than the rate of glucose transport; therefore glucose transport must be the rate-limiting step in glucose metabolism in bovine muscle.

DISCUSSION

Presence of a GLUT4 protein in tissues from ruminants

In the present study, an *in vitro* approach was used to demonstrate that insulin stimulates glucose transport in bovine muscle (rectus abdominis) as previously shown for ovine adipose tissue [13]. This suggests that translocation of a GLUT4-like protein may operate after insulin stimulation in at least one bovine muscle. Furthermore, antibodies raised against rat GLUT4 detected a protein in ruminant tissues at a molecular mass of 42 kDa, similar to that found for rodent GLUT4 [15,25]. It was only detected in tissues in which glucose uptake is sensitive to insulin, namely muscles (especially rectus abdominis) and adipose tissue, and not in tissues that are not sensitive to insulin (liver, brain, small intestine and erythrocytes). Taken together, these results strongly suggest that the protein we detected indeed corresponds to the insulin-responsive glucose transporter GLUT4. A GLUT4-like protein has previously been demonstrated in goat adipose tissue [14], but, to our knowledge, its presence has never been reported in ruminant muscles. However, recent work using a human probe showed that GLUT4 mRNA was expressed in bovine skeletal muscle and adipose tissue but not in the duodenum [30], which is in agreement with our results at the protein level.

GLUT4 protein content is higher in glycolytic than in oxidative muscles from calves and goats

It has been clearly shown in rodents that GLUT4 content is higher in oxidative than in glycolytic muscles [15,27,28]. This higher expression is related to a high capacity for glucose uptake [31] and a high stimulation of glucose transport by insulin and/or exercise [27]. In humans, glucose uptake after submaximal or maximal insulin stimulation is positively correlated with percentage of slow-oxidative fibres and negatively correlated with percentage of fast-glycolytic fibres, suggesting that GLUT4 is expressed more abundantly in oxidative than glycolytic fibres [32]. The present study indicates the converse situation in ruminants, i.e. a higher expression of GLUT4 in glycolytic and oxidoglycolytic muscles than in oxidative muscles. However, it remains to be determined whether GLUT4 expression in various skeletal muscles is under pretranslational control as is the case in rat muscles [27].

Regulation of glucose utilization by ruminant muscle

This experiment shows that the rate of glucose transport can be measured in incubated fibre strips of bovine muscle as described for human muscle [17]. *In vitro* incubation of bovine muscle fibre strips has previously been described for measurement of palmitate esterification [33], but not for glucose-metabolism studies. Muscles or muscle fibre strips incubated *in vitro* provide a convenient preparation for glucose-transport studies and for demonstration of differences between groups of animals, but they do not necessarily reflect conditions *in vivo* [34]. The rate of glucose transport is difficult and expensive to measure *in vivo* in large species compared with rats, so this *in vitro* technique enabled us to demonstrate that, as in rat muscle, glucose transport is the rate-limiting step in glucose utilization in bovine muscle [9,10].

If blood flow and blood glucose level are taken into account, energy provided by glucose to bovine muscle (as reflected *in vivo* by the hind limb) represents 3900 kJ/h, but caloric uptake by muscle from glucose is only 130 kJ/h [35]. In contrast, supply and uptake for acetate are 230 and 90 kJ/h respectively because of large differences in the extraction ratios of the two nutrients [35]. Indeed, before and after insulin infusion, bovine muscle extracts, *in vivo*, only 3 and 11 % respectively of the glucose passing through in blood, but 36 % of the circulating acetate [35]. This suggests a strong rate-limiting step for glucose but not for acetate uptake. Our results indicate that this rate-limiting process is likely to be glucose transport which is regulated by insulin. This conclusion emphasizes the physiological importance of glucose transport, which limits the rates of glucose use and metabolism in muscle from ruminant as well as monogastric animals [10].

Physiological consequences of a limited glucose-transport capacity in ruminant oxidative muscles

In fed rats, oxidative muscles make a large contribution to overall glucose utilization even in the resting state [31]. In ruminants, glucose availability in the fed state is low because of fermentation of food in the rumen, the bulk of the glucose arising from the gluconeogenic process [2]. In contrast, blood concentrations of oxidative energy-yielding substrates, especially short-chain fatty acids (acetate, ketone bodies), are two to seven times higher in ruminants than in monogastrics [1]. As glucose transport is a rate-limiting step in glucose utilization, a low GLUT4 concentration in ruminant oxidative muscles should limit glucose uptake and utilization by these muscles and contribute to sparing of glucose for the benefit of tissues, such as glycolytic muscles, that are obligatory glucose utilizers.

However, other mechanisms could be involved in glucose sparing: in the sheep hind limb [36] and rat heart [37], an infusion of acetate changed glucose uptake very little but almost completely inhibited glucose oxidation. Indeed, lactate output increased 11-fold in sheep when arterial acetate concentration increased to 3 mM [36], and the amount of labelled glucose incorporated into glycogen increased 6-fold in rat when the acetate level increased to 10 mM [37]. The mechanism for inhibition of glucose oxidation induced by short-chain fatty acids may be a decrease in the percentage of active pyruvate dehydrogenase [38] or a decrease in glycolytic flux [37]. This interaction between glucose and short-chain fatty acids may be important because blood ketone bodies and acetate levels are high in ruminants [1], and these energy-yielding substrates are utilized by muscles at a rate that is directly proportional to their concentrations in blood [39].

In humans and rats at least, the amount of GLUT4 in peripheral tissues correlates with insulin-stimulated glucose transport or disposal [27,28,40]. A comparison of *in vitro* metabolic studies revealed a lower insulin responsiveness of glucose transport in ovine adipocytes than in rat adipocytes [13]. A comparison of euglycaemic clamp studies in human [41], rat [42] and growing or adult ruminants [3–6] also revealed lower insulin responsiveness of peripheral tissues in ruminants: when results were corrected for blood glucose level (which tends to be lower in ruminants) and expressed as clearance rates in ml/min per kg, rates of insulin-stimulated glucose utilization for sheep, goat and cattle were 50–75 % and 15–25 % of those for humans

and rats respectively. Our results are in agreement, since the insulin-stimulated increase in glucose transport in fibre strips from a bovine oxidoglycolytic muscle, rectus abdominis (+325 nmol/20 min per g of wet tissue), is lower than in rat oxidative or glycolytic muscle (+626–1530 nmol/20 min per g) [16] or human rectus abdominis muscle [17]. However, euglycaemic clamps and *in vitro* metabolic studies described by various laboratories cannot be compared exactly because they have been performed under somewhat different experimental conditions and with subjects of various ages or nutritional status. Nevertheless, it can be concluded that peripheral tissues might be less sensitive to insulin in ruminants than in non-ruminants. Consequently, it can be hypothesized from our data that the overall decreased responsiveness of glucose metabolism to insulin in ruminants could be explained by the low content of the insulin-responsive glucose transporter GLUT4 in oxidative muscles and oxidative fibres from different muscles.

In summary, our results from *in vitro* experiments indicate that glucose transport is a rate-limiting step in glucose utilization by muscles, both in ruminants and monogastrics, agreeing with data from *in vivo* metabolic studies by others. The low amount of GLUT4 in oxidative muscles from ruminants might be one of the molecular mechanisms involved in control of glucose utilization and catabolism: oxidative muscles from ruminants may use lower amounts of glucose than other muscles even when blood insulin concentration increases, in contrast with the situation in rodents.

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