Biochemical and functional characterization of the GLUT5 fructose transporter in rat skeletal muscle

Froogh DARAKHSHAN*, Eric HAJDUCH*, Søren KRISTIANSEN†, Erik A. RICHTER† and Harinder S. HUNDAL*[1]

*Department of Anatomy & Physiology, The University of Dundee, Dundee DD1 4HN, Scotland, U.K., and †Copenhagen Muscle Research Centre, University of Copenhagen, Universitetsparken 13, DK-2100 Copenhagen, Denmark

Previous work has demonstrated that human skeletal muscle and adipose tissue both express the GLUT5 fructose transporter, but to date the issue of whether this protein is also expressed in skeletal muscle and adipose tissue of rodents has remained unresolved. In the present study we have used a combination of biochemical and molecular approaches to ascertain whether rat skeletal muscle expresses GLUT5 protein and, if so, whether it possesses the capacity to transport fructose. An isoform-specific antibody against rat GLUT5 reacted positively with crude membranes prepared from rat skeletal muscle. A single immunoreactive band of approx. 50 kDa was visualized on immunoblots which was lost when using anti-(rat GLUT5) serum that had been pre-adsorbed with the antigenic peptide. Subcellular fractionation of skeletal muscle localized this immunoreactivity to a single membrane fraction that was enriched with sarcolemma. Plasma membranes, but not low-density microsomes, from rat adipose tissue also displayed a single protein band of equivalent molecular mass to that seen in muscle. Reverse transcription–PCR analyses, using rat-specific GLUT5 primers, of muscle and jejunal RNA revealed a single PCR fragment of the expected size in jejunum and in four different skeletal muscle types. Sarcolemmal vesicles from rat muscle displayed fructose and glucose uptake. Vesicular uptake of glucose was inhibited by nearly 90% in the presence of cytochalasin B, whereas that of fructose was unaffected. To determine whether fructose could regulate GLUT5 expression in skeletal muscle, rats were maintained on a fructose-enriched diet for 4 days. This procedure increased jejunal and renal GLUT5 protein expression by approx. 4- and 2-fold respectively, but had no detectable effects on the abundance of GLUT5 protein in skeletal muscle or on fructose uptake in rat adipocytes. The present results show that GLUT5 is expressed in the sarcolemma of rat skeletal muscle and that it is likely to mediate fructose uptake in this tissue. Furthermore, unlike the situation in absorptive and re-absorptive epithelia, GLUT5 expression in insulin-sensitive tissues is not regulated by increased substrate supply.

INTRODUCTION

The uptake of glucose across the sarcolemma of skeletal muscle is known to be mediated by two members of the facilitative glucose transporter family, GLUT1 and GLUT4 [1,2]. Biochemical and immunocytochemical studies have localized GLUT1 to the plasma membrane, where it is considered to participate in maintaining the basal rates of glucose transport needed to meet the requirements of resting muscle [3]. In contrast, GLUT4 is sequestered in specialized intracellular storage vesicles and, in response to increased muscular activity or binding of insulin to its receptor, is mobilized or 'translocated' to the muscle surface [4–8]. The resulting increase in cell-surface GLUT4 facilitates the increase in glucose transport that is necessary for helping to meet the increased energy demands of working muscle or assisting in the disposal of blood glucose in the post-absorptive state [9,10].

The other major dietary sugar is fructose; however, unlike glucose, the absorption of fructose from the intestinal lumen takes place via the GLUT5 facilitative transporter, and the circulating blood level of this sugar is considerably lower [11–13]. The low blood fructose (between 0.05 and 0.1 mM) is due primarily to the presence of a very active hepatic enzyme system for metabolizing fructose that enables the liver to extract between 55% and 70% of the portal supply of this hexose after tube feeding of fed and starved rats respectively [14]. Thus approx. 30–45% of the absorbed fructose is available for extraction and utilization by other tissues, such as the kidney, adipose tissue and skeletal muscle [15–17]. In humans, adipose tissue and skeletal muscle both express the GLUT5 fructose transporter [18,19], and we have recently shown that sarcolemmal vesicles prepared from human muscle biopsies take up fructose and glucose by distinct carrier mechanisms: uptake of glucose was acutely sensitive to inhibition by the fungal metabolite cytochalasin B (CB), whereas that of fructose was not [20]. Heterologous expression studies in *Xenopus* oocytes have revealed that a characteristic hallmark of hexose transport via GLUT5 is the relative insensitivity of this carrier towards CB compared with other members of the GLUT family [11,12,21], an observation that would strongly support our view that the observed uptake of fructose in human muscle vesicles is mediated by the resident GLUT5 protein [20].

Interestingly, whereas human skeletal muscle expresses GLUT5, RNA blotting studies have indicated that this carrier is not expressed in rat skeletal muscle [12]. The reasons for this apparent discrepancy are not clear, but, if true, this would imply that there is a major difference in how fructose is handled in the skeletal muscle of rats and humans. In the present study we have investigated this issue in greater detail by specifically addressing the following questions: (i) do isolated membranes prepared from rat skeletal muscle express GLUT5 protein?; (ii) does reverse transcription–PCR (RT-PCR) analysis of rat muscle RNA enable detection of GLUT5 expression in rat muscle?; (iii) do sarcolemmal vesicles prepared from rat skeletal muscle exhibit fructose transport with characteristics consistent with uptake via GLUT5?; and (iv) does increased dietary intake of fructose modify the expression of GLUT5 in rat skeletal muscle, as is known to happen in the intestine and kidney?

Abbreviations used: CB, cytochalasin B; RT-PCR, reverse transcription–PCR.

[1]To whom correspondence should be addressed (e-mail h.s.hundal@dundee.ac.uk).

MATERIALS AND METHODS

Animals and experimental procedures

Male Sprague–Dawley rats (150–250 g; Bantin and Kingman, Hull, U.K.) were used throughout, and were killed by cervical dislocation. Hind-limb skeletal muscle, jejunum, brain, kidneys, testes and epididymal fat pads were rapidly excised. Jejunum and adipose tissue were immediately processed to isolate crude jejunal membranes or subcellular adipocyte fractions enriched with plasma membranes and intracellular 'light' microsomes, as previously described [13,22]. Brain, kidneys and testes were frozen in liquid N_2 at the time of tissue harvesting, and post-nuclear membranes were prepared from these tissues at a later stage, as previously described [18]. Skeletal muscle was either snap-frozen in liquid N_2 until required for subcellular fractionation or, alternatively, used immediately for preparing giant sarcolemmal vesicles, as described below.

Subcellular fractionation of rat skeletal muscle

Skeletal muscle was homogenized and subjected to differential centrifugation for isolation of crude muscle membranes, which were subfractionated on a discontinuous sucrose density gradient (25 %, 30 % and 35 %, w/v), as described previously [8,23]. This procedure results in the separation of three distinct membrane bands: one on top of the 25 % sucrose cushion representing membranes enriched with plasma membrane markers, a second band on top of the 30 % sucrose layer containing intracellular membranes largely of endosomal origin [8], and a third band on top of the 35 % sucrose layer composed of membranes endowed with the insulin-sensitive pool of GLUT4 [24]. The protein content of each muscle membrane fraction and that of membranes from the other tissues harvested was determined using the Bradford method [25].

Preparation of giant sarcolemmal vesicles from rat skeletal muscle

Preparation of giant sarcolemmal vesicles from rat skeletal muscle was as previously reported [5,26,27]. Briefly, rat muscle was cut into small longitudinal pieces and incubated in KCl/Hepes buffer (140 mM KCl/10 mM Hepes, pH 7.4) containing 0.37 mg/ml PMSF, collagenase (100 units/ml; type VII; Sigma) and aprotinin (0.01 mg/ml; Sigma) for 45 min at 34 °C. Sarcolemmal vesicles (2–35 μm in diameter) form spontaneously during incubation. EDTA (10 mM) in KCl/Hepes buffer was added at the end of the incubation period to terminate the collagenase digestion. Percoll (final concentration 16 %, w/v) and aprotinin were added to the crude vesicle suspension, and a three-layered density gradient was set up consisting of the vesicle/Percoll mixture, 4 % Nycodenz (Nycomed, Oslo, Norway) in KCl/Hepes buffer, and KCl/Hepes buffer. The gradient was spun gently at 50 ***g*** for 45 min at room temperature, and vesicles were isolated from the KCl/Hepes buffer prior to pelleting at 800 ***g*** for 10 min at 20 °C. The vesicle pellet was resuspended in KCl/Hepes buffer. The membrane vesicles isolated using this procedure have been extensively characterized with respect to sarcolemmal recovery, orientation, purity and contamination, and have been shown ostensibly to be of sarcolemmal origin [5,26,27].

Hexose uptake in isolated sarcolemmal vesicles and rat adipocytes

Vesicle uptake was performed as described previously [5,20]. Briefly, fructose and glucose uptake were measured under zero-*trans* conditions at room temperature, and were initiated by the addition of uptake solution (10 μl), containing either 0.5 μCi of D-[^{14}C]fructose/1 mM unlabelled D-fructose (or a fructose concentration of up to 20 mM for analyses of transport kinetics) plus 0.1 μCi of [^{3}H]mannitol or 0.5 μCi of D-[^{3}H]glucose/1 mM D-glucose plus 0.1 μCi of [^{14}C]mannitol, to 30 μl of membrane vesicle suspension. For experiments assessing the kinetics of fructose uptake, the osmolarity of the uptake media containing the different concentrations of fructose was maintained iso-osmotic, by the addition of mannitol, with that of the uptake solution containing the highest concentration of unlabelled fructose. Uptake was allowed to proceed for periods of up to 30 min, and was terminated by the addition of 1 ml of ice-cold KCl/Hepes buffer containing $HgCl_2$ (2.5 mM). Sarcolemmal vesicles were pelleted by high-speed centrifugation (14000 ***g***; 1 min) for 1 min in Eppendorf tubes and recovered by cutting off the bottom of the tubes prior to assessing intravesicular radioactivity by liquid scintillation counting (Packard Instruments). In some experiments we assessed the sensitivity of vesicular fructose (5 mM) and glucose (5 mM) uptake to CB. Membrane vesicles were pre-incubated with CB (at concentrations between 1 and 500 μM) for 30 min at room temperature prior to the addition of uptake solution as described above.

For analyses of fructose uptake in rat adipose tissue, we isolated fat cells by digestion of epididymal pads in Krebs–Ringer/phosphate buffer, pH 7.4, supplemented with 2 % (w/v) BSA (fraction V; Sigma) containing 0.1 % collagenase (Sigma) by the method of Rodbell [28]. A 400 μl aliquot of washed adipocyte suspension was then incubated with 50 μM [^{14}C]fructose (du Pont; specific radioactivity 235 mCi/mmol) and [^{3}H]mannitol (specific radioactivity 27 Ci/mmol). Fructose uptake was terminated after 40 min by the rapid centrifugation (14000 ***g***) of 300 μl of the adipocyte suspension through a 100 μl di-isononylphthalate oil cushion (Fluka) for 20 s, and cell-associated radioactivity was assessed by liquid scintillation counting as described previously [29].

Fructose-feeding studies

Previous studies have shown that intestinal GLUT5 expression is elevated in rats fed a fructose-enriched diet [30,31]. To assess whether GLUT5 protein expression is also elevated in skeletal muscle, we divided male Sprague–Dawley rats (body wt. 250 g) into two groups. One group was maintained on a standard diet of food pellets, while the second group were given food pellets that had been supplemented with fructose (25 %, w/w). Both groups were maintained on their respective diets for 4 days with free access to water. At the end of this period animals were killed by cervical dislocation. With the exception of adipose tissue, which was processed immediately for isolation of fat cells, hind-limb muscles, jejunum and kidneys were removed rapidly and frozen in liquid nitrogen. Prior to freezing, the isolated jejunal segment was washed three times with ice-cold PBS (pH 7.0) to remove any undigested lumenal contents. Samples were stored at −80 °C and used at a later stage for isolation of plasma membranes from skeletal muscle or of post-nuclear membranes from jejunum and kidneys, as described above.

Gel electrophoresis and Western blot analyses

Isolated membrane fractions were subjected to SDS/PAGE, as described by Laemmli [32], on 9 % (w/v) polyacrylamide resolving gels. Separated proteins were electrophoretically transferred on to nitrocellulose sheets, blocked with 3 % (w/v) BSA in Tris buffered saline, and incubated with the following: antibodies

to GLUT1 (1:2000 dilution; a gift from Dr. S. A. Baldwin, Department of Biochemistry and Molecular Biology, University of Leeds, U.K.), antibodies to GLUT4 (1:2000; 1F8; Genzyme), antibodies to rat GLUT5 (1:2000) or an anti-(rat GLUT5) antibody that had been pre-adsorbed with 0.1 mg/ml of the antigenic peptide (rat GLUT5 serum and GLUT5 peptide were both generously provided by Dr. Y. Oka, Yamaguchi University School of Medicine, Yamaguchi, Japan [30]), or antibodies to the α_1-subunit of Na,K-ATPase (Mck1; 1:100 [33]; kindly provided by Dr. K. Sweadner, Harvard Medical School, Boston, MA, U.S.A.). Following primary antibody incubation, membranes were washed with 0.05% Tween 20 in Tris buffered saline prior to incubation with horseradish-peroxidase-conjugated anti-(sheep IgG) (1:1000; Scottish Antibody Production Unit, Carluke, Larnarkshire, Scotland, U.K.) for the detection of polyclonal antibodies or with horseradish-peroxidase-conjugated anti-(mouse IgG) (1:500; Scottish Antibody Production Unit) for the detection of monoclonal antibodies. Membranes were subsequently washed and air-dried, and autoradiography was performed by exposure to XAR-5 Kodak film. Autoradiographs were quantified using a Bio-Rad GS-670 imaging densitometer.

RNA extraction and RT-PCR

Total RNA was extracted from rat jejunum and different rat skeletal muscles (soleus, gastrocnemius, extensor digitorum longus and tensor fasiolus longus) using an RNA isolator kit (Genosys). RNA was quantified by its 260/280 nm UV absorbance, and its integrity was confirmed by visualization of ethidium bromide-stained rRNA under UV light. Reverse transcription (1 μg of total RNA/sample) and PCR were performed using the Promega Access RT-PCR Kit according to the manufacturer's instructions. cDNA was amplified using rat specific primers for the GLUT5 gene (GenBank accession no. D13871), as described by Oka and co-workers [30]. The primer pair 5′-GAGCTTTGGGGACGTCGGAATCTT-3′ and 5′-CGGGTAGCAGGTGGGAGGTCAT-3′ were synthesized by Oligosyn (Department of Biochemistry, University of Dundee), and generated a single band of 496 bp. The control RNA with carrier contained a 1.2 kb RNA (prepared by *in vitro* transcription) and *Escherichia coli* rRNA (as specified in the kit). The primers used for control experiments were either our GLUT5 specific primers or the upstream and downstream primers supplied in the kit (upstream sequence, 5′ GCCATTCTCACCGGATTCAGTCGTC 3′; downstream sequence, 5′ AGCCGCCGTCCCGTCACGTCAG 3′). PCR was performed using a Hybaid thermal cycler under the following conditions: initial denaturation was for 2 min at 94 °C for one cycle, followed by 40 cycles of denaturation for 30 s at 94 °C, annealing for 1 min at 59.4 °C and extension for 2 min at 68 °C. The products were separated and visualized by ethidium bromide staining on a 1% (w/v) agarose gel.

Statistical analyses

Data were assessed for statistical differences using Student's *t*-test and considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Immunodetection of GLUT5 in rat tissues

An antibody raised to the C-terminal domain of rat GLUT5 was used to probe membranes from rat jejunum, skeletal muscle, brain, kidney and testes, as well as subcellular membrane fractions of rat adipose tissue enriched with plasma membranes or low-density microsomes. Figure 1 shows that the antibody reacted positively with all rat tissues tested except testicular membranes, which have been shown previously not to express GLUT5 at the RNA level [12]. GLUT5 migrated as a slightly broader band in crude jejunal membranes ($\sim$ 55 kDa) compared with that seen in membranes from the other tissues. This slight variation in electrophoretic mobility is most probably attributable to the differential glycosylation of GLUT5, as has been reported previously for human GLUT5 [18]. However, of notable interest was the finding that a single band of $\sim$ 50 kDa was detected in membranes prepared from skeletal muscle and adipose tissue. The presence of GLUT5 in the adipocyte plasma membrane is in line with very recent work from our group showing that fat cells can take up fructose by a mechanism that is insensitive to both CB and insulin [29], properties normally associated with GLUT5-mediated transport [11,12,21]. Moreover, while our studies represent the first report showing expression of GLUT5 in rat adipose tissue, the observation is fully consistent with a number of earlier studies that also proposed the existence of a specific fructose carrier in fat cells, based on differences in the characteristics with which glucose and fructose are taken up by rat adipocytes [16,34,35].

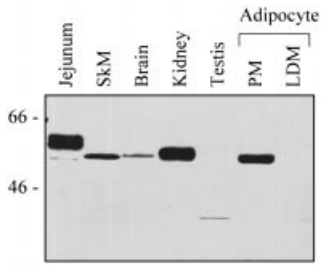

Figure 1 Tissue distribution of rat GLUT5

Post-nuclear membranes (50 μg of protein) from rat jejunum, skeletal muscle (SkM), brain, kidney and testis, and isolated plasma membranes (PM) and low-density microsomes (LDM) from rat adipocytes, were subjected to SDS/PAGE and immunoblotting using anti-(rat GLUT5) serum, as described in the Materials and methods section. Positions of molecular mass markers (kDa) are shown on the left.

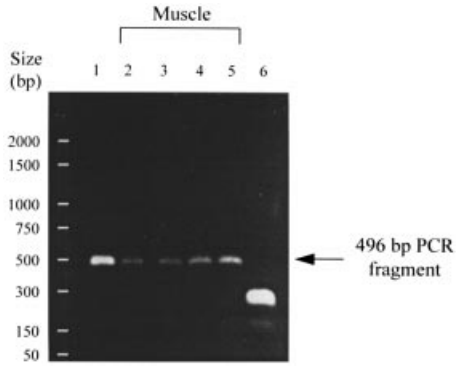

Figure 2 Expression of GLUT5 mRNA in different rat skeletal muscle types

cDNAs were prepared from total RNA isolated from rat jejunum (lane 1), soleus (lane 2), gastrocnemius (lane 3), extensor digitorum longus (lane 4) and tensor fasiolus longus (lane 5), and were used in RT-PCR with primers specific for rat GLUT5, as described in the Materials and methods section. The amplified PCR product of 494 bp was detected on a 1% agarose gel stained with ethidium bromide. No fragments of the expected size were observed when using control primers (lane 6) or control RNA.

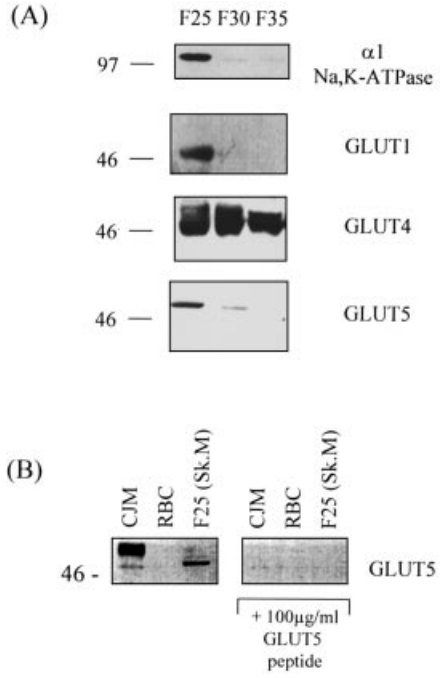

Figure 3 Subcellular distribution of the α_1-Na, K-ATPase subunit and GLUT transporters

(**A**) Representative immunoblots showing the subcellular distribution of the α_1 subunit of Na,K-ATPase and of the GLUT1, GLUT4 and GLUT5 transporters in isolated membrane fractions of rat skeletal muscle. The F25 fraction contains membranes predominantly of plasma membrane origin, fraction F30 is enriched with endosomal membranes, and fraction F35 contains membranes largely of intracellular origin [8]. Portions of 20 μg of membrane protein from each fraction were subjected to SDS/PAGE and immunoblotting using isoform-specific antibodies, as described in the Materials and methods section. (**B**) Crude jejunal membranes (CJM; 20 μg of protein), ghost membranes from red blood cells (RBC; 20 μg of protein) and rat sarcolemmal membranes (20 μg of protein) from the F25 fraction were immunoblotted using anti-GLUT5 serum or anti-GLUT5 serum that had been pre-adsorbed with 100 μg of the antigenic rat GLUT5 peptide/ml. Positions of molecular mass markers (kDa) are shown on the left.

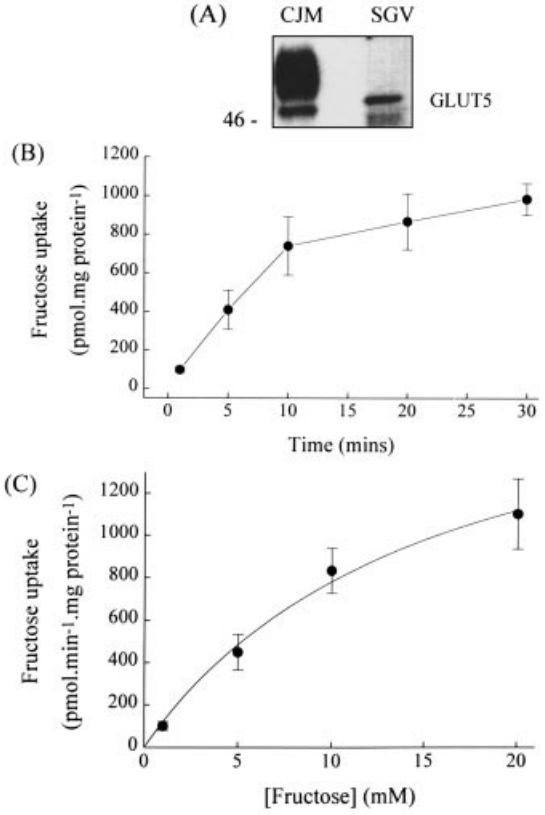

Figure 4 Fructose uptake in sarcolemmal giant vesicles

(**A**) Representative immunoblot showing the presence of GLUT5 in rat sarcolemmal giant vesicles (SGV). Crude jejunal membranes (CJM; 20 μg) and SGV (20 μg) were subjected to SDS/PAGE and immunoblotted using anti-(rat GLUT5) antibody, as described in the Materials and methods section. (**B**) Time course for fructose uptake in giant sarcolemmal vesicles prepared from rat skeletal muscle. The uptake of 1 mM D-[^{14}C]fructose was assayed over the times indicated, as described in the Materials and methods section (values represent means $\pm$ S.E.M. from four separate experiments). (**C**) Fructose uptake in sarcolemmal vesicles as a function of extravesicular fructose concentration. Uptake data presented are means $\pm$ S.E.M. for up to 11 individual experiments.

RT-PCR analyses of rat muscle RNA

Since previous work was unable to detect the presence of GLUT5 mRNA in rat skeletal muscle by Northern blotting [12], we elected to perform RT-PCR using rat specific GLUT5 primers. Skeletal muscle from younger rats (150 g) was used to isolate total RNA. Figure 2 shows that jejunum, soleus, gastrocnemius, extensor digitorum longus and tensor fasiolus longus all showed the expected 496 bp PCR fragment. In contrast, this fragment was not detected when using control RNA with GLUT5 specific primers (Figure 2) or when using primers that did not correspond to rat GLUT5 (results not shown) or RNA isolated from 3T3-L1 adipocytes, which do not express GLUT5 protein (results not shown). These observations would imply that rat skeletal muscle expresses GLUT5 mRNA, albeit at very low levels which may not be detected by conventional hybridization techniques utilizing total RNA.

Subcellular distribution of GLUT5 in rat skeletal muscle

We have reported previously that GLUT5 is principally expressed in the human sarcolemma [19]; interestingly, this surface localization also appears to be a feature in both rat and human adipocytes (Figure 1 and [18,29]). In an attempt to assess whether the immunoreactive GLUT5 signal that we observed in rat muscle originates from its presence in the sarcolemma, we fractionated skeletal muscle to isolate membrane fractions enriched with plasma membranes, endosomes and intracellular membranes [8]. Figure 3(A) shows representative immunoblots for the subcellular distribution of the α_1 subunit of Na,K-ATPase, GLUT1, GLUT4 and GLUT5. The α_1 Na,K-ATPase subunit and GLUT1 were both detected in the fraction denoted F25, which is enriched with sarcolemmal membranes. This finding is consistent with previous biochemical and immunocytochemical data showing that these two proteins are good sarcolemmal markers [3,36]. GLUT4 was detected in all three muscle fractions, but was most abundant in the F30 and F35 fractions, which contain membranes largely of endosomal and intracellular origin respectively [8]; the F35 fraction has previously been shown to contain the 'insulin-responsive' GLUT4 pool [8]. Of interest, however, was the finding that GLUT5 immunoreactivity was predominantly localized to the sarcolemmal (F25) fraction (Figure 3A), indicating that, as in human skeletal muscle [19], expression of rat GLUT5 appears to be largely restricted to the plasma membrane.

To show that the observed GLUT5 signal in the sarcolemmal fraction was not an experimental artefact, we immunoblotted membranes from the F25 fraction and crude jejunal membranes

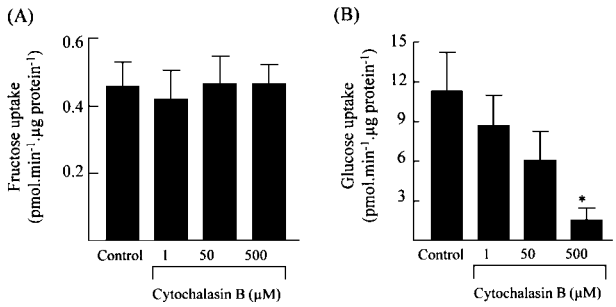

Figure 5 Effects of CB on uptake of (A) 5 mM D-fructose and (B) 5 mM D-glucose in rat sarcolemmal vesicles

Values represent means ± S.E.M. from four separate experiments. *Significant difference ($P < 0.05$) compared with the respective control value.

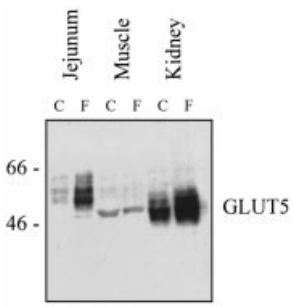

Figure 6 Effects of fructose feeding on GLUT5 protein expression in rat jejunum, skeletal muscle and kidney

Rats were placed on a control (C) or fructose-enriched (F) diet for 4 days, after which period post-nuclear membranes from jejunum (20 μg of protein), kidney (50 μg of protein) and sarcolemmal membranes (20 μg of protein) were subjected to SDS/PAGE and immunoblotted using anti-(rat GLUT5) serum. Positions of molecular mass markers (kDa) are shown on the left.

(run as a positive control) with anti-GLUT5 serum that had been pre-adsorbed with the antigenic peptide. Figure 3(B) shows that the GLUT5 immunoreactivity in membranes from both tissues was lost when using peptide-protected serum, suggesting that this signal was not attributable to a non-specific antibody reaction.

Fructose uptake in rat sarcolemmal vesicles

Based on the biochemical detection of GLUT5 in rat sarcolemma, we next attempted to demonstrate whether rat skeletal muscle has the capacity to take up fructose. For this purpose, giant sarcolemmal vesicles were prepared from rat hind-limb muscle, and vesicle fructose uptake was assayed. In line with the data shown in Figures 1 and 3(A), Western blot analysis of giant sarcolemmal vesicles confirmed the presence of rat GLUT5 (Figure 4A). Figure 4(B) shows that rat sarcolemmal vesicles took up fructose in a time-dependent manner. Uptake was linear over the first 5–10 min, but was assayed over 1 min in all subsequent experiments for convenience. Fructose uptake showed signs of saturation when measured as a function of extravesicular fructose concentration (Figure 4C). Eadie–Hofstee analyses of the data presented in Figure 4(C) indicated that sarcolemmal fructose uptake had a K_m of 13 ± 2 mM and a V_{max} of 1546 ± 379 pmol/min per mg of protein. It is noteworthy that previous studies investigating the kinetics of fructose uptake in jejunal membranes [21,37], human sarcolemmal vesicles [20] and *Xenopus* oocytes expressing rabbit GLUT5 [21] have all reported K_m values ranging from 8 to 18 mM. Thus the K_m value of 13 mM obtained in the present study falls within the range of values reported in the literature and would imply that, at normal physiological concentrations of blood fructose (up to 0.5 mM), uptake of the ketose across the sarcolemma is unlikely to be rate-limiting for any metabolic process that may utilize fructose.

Having established that sarcolemmal vesicles prepared from rat skeletal muscle could take up fructose, we subsequently investigated whether hexose uptake was sensitive to CB. Figure 5(A) shows that raising the extravesicular concentration of CB from 1 μM to 500 μM had no effect on fructose uptake. In contrast, when uptake of D-glucose was assayed in parallel experiments, we found that CB, at a concentration of 50 μM, suppressed glucose uptake by $\sim 50\%$; this increased to nearly 90% when the concentration of the inhibitor was raised 10-fold to 500 μM (Figure 5B). The inability of CB to inhibit fructose uptake is consistent with the idea that uptake of this sugar is mediated by GLUT5 which, when expressed heterologously in *Xenopus* oocytes, has been shown to transport fructose in a CB-insensitive fashion [11,12,21]. Furthermore, since a significant component of the sarcolemmal glucose uptake was CB-sensitive, our data support the idea that uptake of glucose and fructose in rat skeletal muscle is unlikely to be mediated by a common transporter.

Fructose feeding and GLUT5 expression in rat jejunum, kidney and skeletal muscle

It is well established that a marked increase in intestinal and renal GLUT5 protein expression takes place upon feeding rats a fructose-enriched diet [30,31]. This increase is thought to be primarily a post-transcriptional effect, given that GLUT5 mRNA levels undergo a disproportionately smaller increase than does GLUT5 protein. Interestingly, the stimulus that triggers an increase in GLUT5 expression does not require metabolism of fructose *per se*, as non-metabolizable fructose analogues (e.g. 3-*O*-methylfructose) can also stimulate intestinal fructose transport [38]. To assess whether the increased availability of fructose could also enhance GLUT5 expression in rat skeletal muscle, we isolated rat sarcolemmal membranes following a 4-day period of high fructose feeding and immunoblotted them using anti-GLUT5 antibodies.

Figure 6 shows that an increased dietary intake of fructose significantly enhanced intestinal and renal GLUT5 expression, by 3.9 ± 0.7- and 1.8 ± 0.1-fold respectively (values are means ± S.E.M. from three independent experiments; $P < 0.05$). The lower increase in renal GLUT5 is in line with the work of Burant and Saxena [31], and is most probably explained by the much higher levels of free fructose that exist in the intestinal lumen compared with the blood or kidney tubule. Whereas intestinal and renal GLUT5 expression were both elevated in animals fed a fructose-enriched diet, there was no detectable effect on the abundance of GLUT5 protein in skeletal muscle (Figure 6). Furthermore we also found that, when we assayed fructose uptake in rat adipocytes isolated from animals maintained on a normal or fructose-enriched diet, there was no significant difference in fructose uptake between the two groups (control, 58 ± 4 fmol/40 min per 1000 cells; fructose-fed, 55 ± 2 fmol/40 min per 1000 cells; values are means ± S.E.M. for between three and six separate experiments). This latter observation would indirectly suggest that expression of GLUT5 in

rat adipose tissue was also unlikely to have been altered in response to fructose feeding.

Taken together, these findings signify that there is a fundamental difference in the ability of fructose to regulate GLUT5 in the intestine and kidney compared with other tissues that also express this transporter. In our view, it is likely that the normal abundance of GLUT5 in the rat intestine and kidney becomes a limiting factor for the absorption and re-absorption respectively of fructose when there is a transition to a high-fructose diet. In order to avoid sustained intestinal or urinary loss of fructose, a rise in GLUT5 protein is instigated to facilitate increased absorption and re-absorption of the sugar in these tissues. In contrast, while glucose remains the major hexose substrate for skeletal muscle, this tissue can also utilize fructose for oxidation to lactate and incorporation into glycogen [39]. Based on the kinetics of the muscle fructose transporter, it is highly unlikely, even in the face of a substantial increase in blood fructose concentration, that the utilization of fructose by skeletal muscle will be limited by its transport across the sarcolemma. Consequently, unlike the intestine or kidney, there is no requirement to increase GLUT5 protein expression in this tissue. It should be stressed, however, that while 4 days of high-fructose feeding clearly enhanced intestinal and renal GLUT5 expression, we are unable to exclude the possibility that the duration of this feeding period may have been insufficient to elicit changes in muscle GLUT5 expression.

Conclusion

In summary, we have shown that the rat GLUT5 protein, like its human counterpart, is localized to the surface membranes of both skeletal muscle and adipose tissue, and that sarcolemmal vesicles prepared from rat muscle display fructose uptake that is not CB-inhibitable. These observations negate the possibility that expression of GLUT5 in human skeletal muscle and fat may be a species-related phenomenon. In addition, the present work has shown that, unlike in absorptive and re-absorptive epithelia, GLUT5 expression or fructose uptake in insulin-sensitive tissues is not regulated by an increased provision of fructose in the diet. This latter finding strongly suggests that the substrate-induced regulation of GLUT5 that occurs in the intestine and kidney may be a tissue-specific event.

We are extremely grateful to Dr. Yoshitomo Oka for providing us with antibodies to rat GLUT5 and with GLUT5 peptide. We thank Dr. Franck Rencurel, who participated in some of the studies. S.K. was supported by the Danish Natural Science Council (grant no. 9600422). This work was supported by grants from the Danish Natural Research Foundation (grant no. 504-14), the British Diabetic Association and The Wellcome Trust. H.S.H was the recipient of a Wellcome Trust University Award.

REFERENCES

1 Gould, G. W. and Holman, G. D. (1993) Biochem. J. **295**, 329–341
2 Stephens, J. M. and Pilch, P. F. (1995) Endocr. Rev. **16**, 529–546
3 Marette, A., Richardson, J. M., Ramlal, T., Balon, T. W., Vranic, M., Pessin, J. E. and Klip, A. (1992) Am. J. Physiol. **263**, C443–C452
4 Douen, A. G., Ramlal, T., Klip, A., Young, D. A., Cartee, G. D. and Holloszy, J. O. (1989) Endocrinology **124**, 449–454
5 Kristiansen, S., Hargreaves, M. and Richter, E. A. (1996) Am. J. Physiol. **270**, E197–E201
6 Kristiansen, S., Hargreaves, M. and Richter, E. A. (1997) Am. J. Physiol. **272**, E385–E389
7 Marette, A., Burdett, E., Douen, A., Vranic, M. and Klip, A. (1992) Diabetes **41**, 1562–1569
8 Aledo, J. C., Lavoie, L., Volchuk, A., Keller, S. R., Klip, A. and Hundal, H. S. (1997) Biochem. J. **325**, 727–732
9 Hayashi, T., Wojtaszewski, J. F. P. and Goodyear, L. J. (1997) Am. J. Physiol. **273**, E1039–E1051
10 DeFronzo, R. A., Gunnarsson, R., Bjorkman, O. and Wahren, J. (1985) J. Clin. Invest. **76**, 149–155
11 Burant, C. F., Takeda, J., Brot-Laroche, E., Bell, G. I. and Davidson, N. O. (1992) J. Biol. Chem. **267**, 14523–14526
12 Rand, E. B., Depaoli, A. M., Davidson, N. O., Bell, G. I. and Burant, C. F. (1993) Am. J. Physiol. **264**, G1169–G1176
13 Blakemore, S. J., Aledo, J. C., James, J., Campbell, F. C., Lucocq, J. M. and Hundal, H. S. (1995) Biochem. J. **309**, 7–12
14 Topping, D. L. and Mayes, P. A. (1971) Nutr. Metab. **113**, 331–338
15 Bjorkman, O. and Felig, P. (1982) Diabetes **31**, 516–520
16 Froesch, E. R. and Ginsberg, J. L. (1962) J. Biol. Chem. **237**, 3317–3324
17 Bergstrom, J. and Hultman, E. (1967) Acta Med. Scand. **182**, 93–107
18 Shepherd, P. R., Gibbs, E. M., Wesslau, C., Gould, G. W. and Kahn, B. B. (1992) Diabetes **41**, 1360–1365
19 Hundal, H. S., Ahmed, A., Guma, A., Mitsumoto, Y., Marette, A., Rennie, M. J. and Klip, A. (1992) Biochem. J. **286**, 348–353
20 Kristiansen, S., Darakhshan, F., Richter, E. A. and Hundal, H. S. (1997) Am. J. Physiol. **273**, E543–E548
21 Miyamoto, K., Tatsumi, S., Morimoto, A., Minami, H., Yamamoto, H., Sone, K., Taketani, Y., Nakabou, Y., Oka, T. and Takeda, E. (1994) Biochem. J. **303**, 877–883
22 Hajduch, E., Aledo, J. C., Watts, C. and Hundal, H. S. (1997) Biochem. J. **321**, 233–238
23 Klip, A., Ramlal, T., Young, D. A. and Holloszy, J. O. (1987) FEBS Lett. **224**, 224–230
24 Douen, A., Burdett, E., Ramlal, T., Rastogi, S., Vranic, M. and Klip, A. (1991) Endocrinology **128**, 611–616
25 Bradford, M. M. (1976) Anal. Biochem. **71**, 248–254
26 Pilegaard, H., Juel, C. and Wibrand, F. (1993) Am. J. Physiol. **254**, E156–E160
27 Ploug, T., Wojtaszewski, J., Kristiansen, S., Hespel, P., Galbo, H. and Richter, E. A. (1993) Am. J. Physiol. **264**, E270–E278
28 Rodbell, M. (1964) J. Biol. Chem. **239**, 375–380
29 Hajduch, E., Darakhshan, F. and Hundal, H. S. (1998) Diabetologia **41**, 821–828
30 Inukai, K., Asano, T., Katagiri, H., Ishihara, H., Anai, M., Fukushima, Y., Tsukuda, K., Kikuchi, M., Yazaki, Y. and Oka, Y. (1993) Endocrinology **133**, 2009–2014
31 Burant, C. F. and Saxena, M. (1994) Am. J. Physiol. **267**, G71–G79
32 Laemmli, U. K. (1970) Nature (London) **227**, 680–685
33 Felsenfeld, D. P. and Sweadner, K. J. (1988) J. Biol. Chem. **263**, 10932–10942
34 Schoenle, E., Zapf, J. and Froesch, E. R. (1979) Am. J. Physiol. **237**, E325–E330
35 Halperin, M. L. and Cheema-Dhaldi, S. (1982) Biochem. J. **202**, 717–721
36 Hundal, H. S., Marette, A., Mitsumoto, Y., Ramlal, T., Blostein, R. and Klip, A. (1992) J. Biol. Chem. **267**, 5040–5043
37 Schultz, S. G. and Strecker, C. K. (1970) Biochim. Biophys. Acta **211**, 586–588
38 Csaky, T. Z. and Fischer, E. (1984) Biochim. Biophys. Acta **772**, 259–263
39 Zierath, J. R., Nolte, L. A., Wahlstrom, E., Galuska, D., Shepherd, P. R., Kahn, B. B. and Wallberghenriksson, H. (1995) Biochem. J. **311**, 517–521

Received 29 May 1998/10 September 1998; accepted 28 September 1998