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Fructose uptake in rat adipocytes: GLUT5 expression and the effects of streptozotocin-induced diabetes

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Summary Previous studies have shown that rat adipocytes possess the capacity to take up fructose by a mechanism that is distinct from that involved in the transport of glucose. In this investigation we report that rat adipocytes express the GLUT5 fructose transporter and that it is responsible for mediating a substantial component (~ 80 %) of the total cellular fructose uptake. This proposition is based on the finding that only 21 % of the total fructose uptake was cytochalasin B (CB) sensitive which most likely reflects transport via GLUT1 and/or GLUT4. Consistent with this suggestion we found (i) that insulin caused a small, but significant stimulation in fructose uptake (~ 35 %) which was abolished in the presence of CB and (ii) that 3-O-methyl glucose inhibited fructose uptake to a level comparable with that observed in the presence of CB. GLUT5 was found to be localised only in the adipocyte plasma membrane and, unlike GLUT4 or GLUT1, its cell surface abundance was not modulated by insulin. GLUT5 expression fell substantially (by ~ 75 %) in adipocytes of streptozotocin-diabetic rats and was accompanied by a reduction in fructose uptake by approximately 50 %. Treatment of streptozotocin-diabetic rats with sodium orthovanadate for a period of 3 days led to a significant reduction in blood glycaemia by approximately 40 % and a partial restoration in both GLUT5 expression and adipocyte fructose uptake. We suggest that fructose uptake in rat adipocytes is principally mediated by GLUT5 in an insulin- and CB-insensitive manner and that expression of GLUT5 in rat adipocytes may be regulated by changes in blood glycaemia. [Diabetologia (1998) 41: 821–828]

Keywords Insulin, GLUT4, GLUT1, membrane, glucose.

The uptake of sugars across the plasma membrane of most mammalian cells is mediated by a family of structurally related glucose transport proteins (GLUTs). To date, five major isoforms of this family have been cloned (termed GLUT1–5) [1–3] which appear to be expressed in a tissue-specific manner and which display specific modes of regulation [1]. By far the most studied is GLUT4 and much of our current understanding regarding its regulation has stemmed from studies carried out in adipocytes [4, 5]. GLUT4 is the insulin-regulated glucose transporter and in the presence of low blood insulin is sequestered largely in specialised intracellular storage vesicles [6–8]. An increase in blood insulin as occurs, for example, after a meal causes a rapid translocation in GLUT4 from its intracellular storage pool to the plasma membrane. The resulting increase in cell surface GLUT4 forms the primary mechanism by which insulin induces a substantial (up to 20-fold) stimulation in cellular glucose uptake [9–11].

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Abbreviations: GLUT, Glucose transporter; CB, cytochalasin B; STZ, streptozotocin; KRP, Krebs-ringer-phosphate; TES, Tris EDTA Sucrose; 2DG, 2-deoxyglucose; BSA, bovine serum albumin; 3-OMG, 3-O-methyl glucose; PM, plasma membrane; LDM, low density microsomes.

Adipocytes also express small amounts of GLUT1 but, unlike GLUT4, its distribution is influenced by insulin to a lesser extent [10] and a much greater proportion is constitutively present in the plasma membrane in unstimulated cells [9]. Consequently, GLUT4 is considered to represent the principal glucose carrier expressed in the fat cell [10]. Rat adipocytes, however, are also capable of taking up and metabolising fructose and a number of early studies proposed that this hexose was taken up by at least two separate transport mechanisms [12–14]. This suggestion was based on the finding that whilst fructose uptake was marginally stimulated by insulin a much larger proportion of its uptake was mediated by a carrier that was insensitive to both insulin and cytochalasin B (CB) [14]. These latter observations would tend to exclude both GLUT1 and GLUT4 which are known to mediate glucose uptake in a CB-sensitive manner [15–17]. Moreover, it has also been shown previously that GLUT1, when expressed in *Xenopus* oocytes, is a very poor mediator of fructose uptake [18]. Taken together these observations raise the strong possibility that rat adipocytes may express a third hexose transporter that specifically transports fructose.

The molecular identity of this putative third carrier has remained unknown, but the most likely candidate from the family of facilitative glucose transporters is GLUT5 [1]. This suggestion is based on its abundant expression in the small intestine, where it is considered to play a role in the absorption of dietary fructose[19–21], and upon substrate selectivity studies of the expressed GLUT5 protein in *Xenopus* oocytes [22, 23]. However, the question of whether rat adipocytes express GLUT5 has remained polemic as previous studies have shown that although GLUT5 mRNA is expressed in human adipose tissue it appears not to be expressed at a detectable level in rat adipocytes [23, 24]. In our study we try to define the mechanism by which fructose is taken up by rat adipocytes and show (i) in line with previous studies [12–14], the majority of the fructose uptake in rat adipocytes is mediated by a carrier that is not regulated by insulin or inhibited by CB, (ii) a specific rat-GLUT5 antibody detects a single immunoreactive band on Western blots of adipocyte membranes which is lost when using GLUT5 antiserum preadsorbed with the antigenic peptide, (iii) the subcellular distribution of GLUT5 in adipocytes is distinct from that of GLUT1 and GLUT4, (iv) GLUT5 expression and fructose uptake are significantly reduced in adipocytes from streptozotocin (STZ)-diabetic rats and (v) changes in fat cell GLUT5 expression during STZ-diabetes may be influenced in part by blood glycaemia. We propose that GLUT5 is expressed in rat adipocytes and is responsible for facilitating fructose uptake in this tissue.

Materials and methods

Animals and experimental design. All reagent grade chemicals were obtained from Sigma (Poole, Dorest, UK), unless specified otherwise. Male Sprague-Dawley rats (~ 200 g, Bantin and Kingman, Hull, UK) were used throughout. Rats were treated with a single subcutaneous injection of 65 mg/kg anhydrous streptozotocin (STZ) reconstituted in citrate-buffered saline (pH 4.5). Control animals were injected with vehicle alone. STZ-injected rats were provided with a 5 % (w/v) glucose drinking solution for the first 24 h to ensure survival through the hyperinsulinaemic phase brought about in response to the STZ-induced lysis of beta-cells. Blood and urine samples were taken daily to assess diabetic status using Glucostix strips for blood glucose and Ladstix strips for urine glucose (Bayer Diagnostics, Basingstoke, Hampshire, UK). Rats were maintained diabetic for a period of 4 days. In some experiments, 1 day post STZ injection, diabetic rats were provided with drinking water supplemented with 0.7 mg/ml sodium orthovanadate [25] for the remaining duration of the study.

Isolation of rat adipocytes. On day 4 of the study rats were killed by cervical dislocation and epididymal fat-pads removed. Samples of venous blood were also taken at this time for analysis of blood glucose using a Reflolux S glucose meter (Boehringer Manneheim, Lewes, East Sussex, UK). Adipocytes were isolated by digestion in Krebs-Ringer-Phosphate (KRP) buffer pH 7.4, supplemented with 2 % bovine serum albumin (BSA) (fraction V) containing 0.1 % collagenase by the method of Rodbell [26]. Collagenase digestion was performed at 37 °C for 45 min and digested tissue filtered through nylon mesh (pore size ~ 180 μm; John Staniar and Co., Manchester, UK). Isolated adipocytes were washed in KRP buffer and finally resuspended in an appropriate volume of this buffer.

Adipocyte fractionation. Plasma membranes and low density microsomes were prepared as described previously [27]. In brief, isolated adipocytes were washed in Tris EDTA sucrose (TES) buffer (20 mmol/l Tris/HCl, pH 7.4; 1 mmol/l EDTA; 250 mmol/l sucrose; 10 μmol/l trans-Epoxysuccinyl-l-Leucilamido(4-guanidino)-butane; 1 μmol/l pepstatin; 1 μmol/l leupeptin) and homogenized in icecold TES buffer using 10 strokes of a dounce type-A glass homogenizer. The homogenate was subjected to a 16 000 *g* centrifugation spin. The resulting supernatant (S1) was retained on ice and the pellet resuspended and centrifuged at 100 000 *g* on a 1.12 mol/l sucrose cushion. Plasma membranes from this spin were recovered from the top of the sucrose cushion and diluted 10-fold in TES buffer and pelleted by centrifugation at 45 000 *g*. The S1 supernatant was centrifuged at 45 000 *g* to pellet high density microsomes and the resulting supernatant centrifuged at 200 000 *g* to pellet low density microsomes. The total protein content of each subcellular membrane fraction was assessed using the Bradford method [28].

Hexose uptake in adipocytes. Uptake of [^{3}H]-2-deoxyglucose and [^{14}C]-D-fructose was assayed as described previously [14, 27]. In brief, 400 μl aliquots of the adipocyte suspension were preincubated in the absence or presence of 100 nmol/l insulin, 10 μmol/l cytochalasin B (CB), 30 mmol/l of either fructose or glucose (or its related hexose analogues, 2 deoxyglucose (2DG) or 3-O-methyl glucose (3OMG)). Incubations were started by the addition of 50 μmol/l [^{3}H]-2DG (NEN, Boston, Mass., USA, specific activity 26.2 Ci/mmol) or 50 μmol/l of [^{14}C]-fructose (NEN, specific activity 235 mCi/mmol). Uptake assays were terminated after 1 min for glucose uptake and after 40 min for fructose uptake by the rapid centrifugation of

300 μl of the adipocyte suspension through a 100 μl di-isononyl phthalate oil cushion (Fluka, Gillingham, Dorset, UK) for 20 s. Non-specific cell-associated radioactivity was corrected by the inclusion in the uptake medium of either [^{14}C]-mannitol (NEN, specific radioactivity 54.5 mCi/mmol) for 2DG uptake or [^{3}H]-mannitol (NEN, specific radioactivity 27 Ci/mmol) for fructose uptake.

Western-blot analyses. In some experiments, to assess the specificity of the rat GLUT5 antibody, we immunoblotted crude rat jejunal membranes as a positive control alongside rat adipocyte membranes. Membrane samples were subjected to SDS/PAGE on 10% polyacrylamide gels as described by Leammli [29]. Proteins were transferred onto nitrocellulose membranes (Amersham Life Science, Little Chalfont, Bucks, UK), blocked for 1 h at room temperature (3% BSA/50 mmol/l Tris-HCl, pH 7.2/150 mmol/l NaCl/0.05% Tween 20) and probed overnight at 4 °C with isoform-specific antibodies against GLUT1 (1:2000; a gift from Dr S. A. Baldwin, University of Leeds), GLUT4 (1:500; East Acres Biologicals, Southbridge, Mass., USA), rat GLUT5 (1:2000) or rat GLUT5 antibody which had been pre-adsorbed with 0.5 mg/ml of the antigenic peptide (rat GLUT5 serum and GLUT5 peptide were both generously provided by Dr Y. Oka, Yamaguchi, Japan, [30]). Membranes were also immunoblotted with a monoclonal antibody against the α1-subunit of the Na,K-ATPase (Mck1 (1:100), kindly provided by Dr K. Sweadner, Harvard University, [31]). Membranes were washed (3 × 15 min) in 50 mmol/l Tris-HCl, pH 7.2, 150 mmol/l NaCl and 0.05% Tween 20. Primary antibody detection was carried out using either [^{125}I]-labelled Protein A (0.1 mCi/ml, Dupont) for polyclonal antibodies or using a horseradish peroxidase-conjugated secondary antibody for the monoclonal antibody. After secondary antibody incubation, membranes were washed in Tris-saline-Tween buffer (3 × 15 min) and immunoreactive bands visualised either by enhanced chemiluminescence (Amersham) or by exposure to Kodak XOMAT film. Autoradiographs were quantified using a Bio-Rad 670 Imaging Densitometer (Bio-Rad, Hemel Hempstead, Herts, UK).

Statistical analyses. Statistical analysis were carried out using a two-tailed Student's test, and data were considered significant at p values less than 0.05.

Results

Fructose uptake in rat adipocytes. As previously reported [13], we found fructose uptake in rat adipocytes was linear over a 60 min period and was substantially suppressed in the presence of 30 mmol/l 2DG (Fig. 1 and see below). In all subsequent studies initial rates of fructose uptake were assayed over a 40 min period for convenience. In an attempt to further characterise fructose uptake in adipocytes, we investigated the effects of 100 nmol/l insulin, 10 μmol/l CB and the inhibitory potential of unlabelled glucose and the glucose analogues, 2DG and 3OMG on the uptake of 50 μmol/l [^{14}C]-fructose. Figure 2 shows that insulin caused a modest, but significant increase in fructose uptake by approximately 35%. This small stimulation in fructose uptake was abolished completely when cells were exposed simultaneously to both insulin and CB. This latter finding suggests that fructose uptake in the fat cell appears, in part, to be mediated by an insulin-responsive transporter (Fig. 2). When fat cells were incubated with 10 μmol/l CB alone, fructose uptake was reduced by 21% (Fig. 2). This concentration of CB was maximally effective as raising the concentration of this inhibitor by five-fold to 50 μmol/l did not result in any further inhibition in fructose uptake (data not shown). Our findings signify that a much larger component (~80%) of the total fructose uptake in adipocytes was CB insensitive (Fig. 2). In the presence of 30 mmol/l unlabelled fructose, D-glucose or 2DG

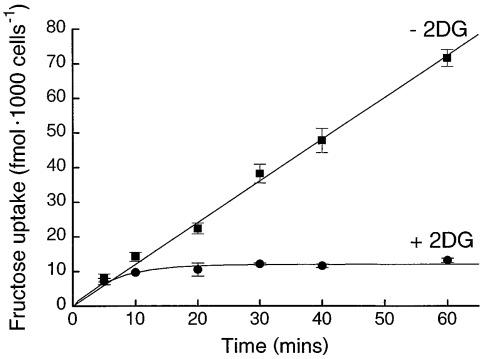

Fig. 1. Time course for fructose uptake in isolated rat adipocytes. Fructose uptake in rat adipocytes was assayed as described in the methods section in the absence or the presence of 2-deoxy-glucose (2-DG) (30 mmol/l) for the times indicated at 37 °C. Uptake values at each time point represent means ± SEM (from three separate experiments each carried out in triplicate)

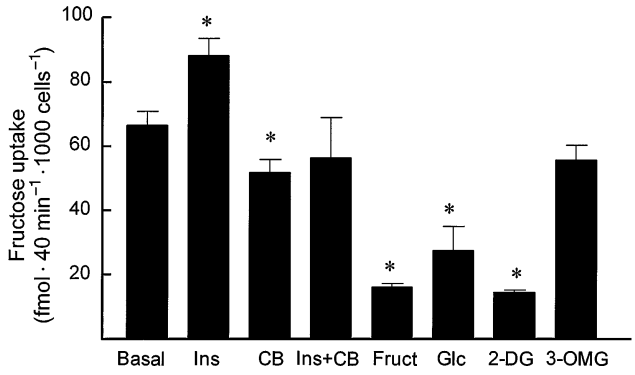

Fig. 2. Effect of insulin, cytochalasin B, fructose, glucose, 2-deoxy-glucose and 3-O-methyl-glucose on fructose uptake in rat adipocytes. Isolated rat adipocytes were incubated in the absence or the presence of insulin (100 nmol/l) for 30 min at 37 °C. Adipocytes were then used to assay fructose uptake as described in the methods section in the absence or the presence of 10 μmol/l cytochalasin B (CB), 30 mmol/l fructose (Fruct), 30 mmol/l glucose (Glc), 30 mmol/l 2-deoxy-glucose (2-DG) or 30 mmol/l 3-O-methyl-glucose (3-OMG). Uptake values represent means ± SEM (for up to 11 separate experiments each carried out in triplicate). * Statistically significant differences compared with basal fructose uptake ($p < 0.05$ Student's t test)

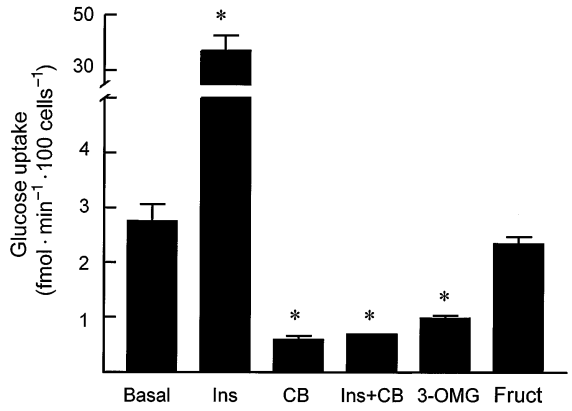

Fig. 3. Effect of insulin, cytochalasin B, 3-O-methyl-glucose and fructose on glucose uptake in rat adipocytes. Isolated rat adipocytes were incubated in the absence or the presence of insulin (100 nmol/l) for 30 min at 37 °C. Adipocytes were then used to assay 2-deoxy-D-glucose uptake as described in the methods section in the absence or the presence of 10 μmol/l cytochalasin B (CB), 30 mmol/l 3-O-methyl-glucose (3-OMG) or 30 mmol/l fructose (Fruct). Uptake values are means ± SEM (for up to 8 separate experiments each carried out in triplicate). * Statistically significant differences compared with basal glucose uptake ($p < 0.05$ Student's t test)

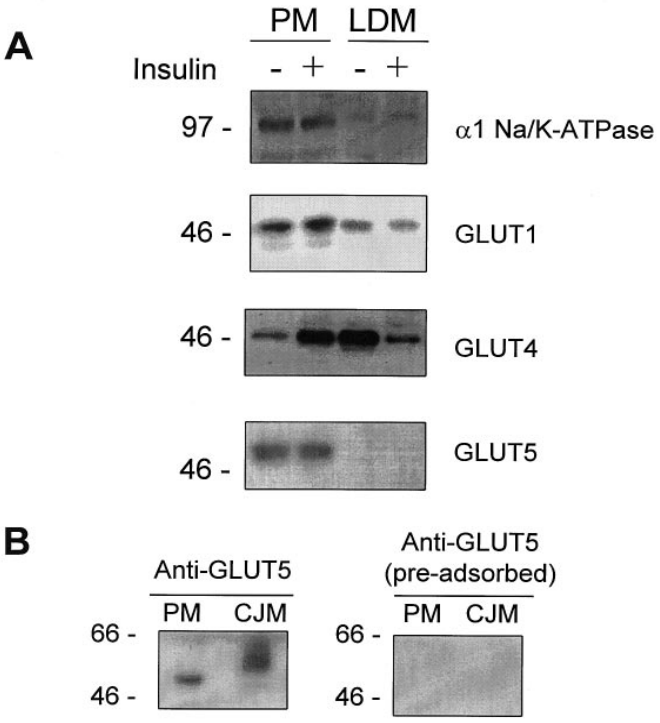

Fig. 4A, B. Representative Western Blots showing the abundance of α1 subunit of the Na, K-ATPase, GLUT1, GLUT4 and GLUT5 transporters in adipocyte membranes and an immunoblot showing the specificity of the GLUT5 immunoreactivity. **A** Isolated rat adipocytes were incubated in the absence or the presence of insulin (100 nmol/l) at 37 °C for 30 min before being subjected to subcellular fractionation. Plasma membrane (PM) and low density microsomes (LDM) (20 μg protein) were applied to SDS-PAGE gels and immunoblotted using isoform-specific antibodies to the α1 subunit of the Na,K-ATPase, GLUT1, GLUT4 and GLUT5 as described in the methods section. **B** Rat adipocyte plasma membranes (PM) and crude jejunal membranes (CJM) were subjected to SDS/PAGE and immunoblotting as described in the methods section with anti-GLUT5 antibody. Samples in the right panel were immunoblotted using an anti-GLUT5 antibody that had been pre-adsorbed with the antigenic peptide (0.5 mg/ml)

the uptake of [^{14}C]-fructose was suppressed substantially (Fig. 1, 2). In contrast, 3OMG only inhibited fructose uptake by approximately 16 %, an inhibition similar in magnitude to that seen in the presence of CB alone. In separate experiments we observed that the presence of 30 mmol/l sucrose had no inhibitory effect on adipocyte fructose uptake thus establishing that any changes in fructose uptake seen in the presence of different hexoses studied could not be attributed to osmotically induced-changes in cell volume (data not shown).

2-deoxyglucose (2DG) uptake. In parallel experiments we also assayed 2DG uptake. Figure 2 shows that 2DG uptake was stimulated by approximately 14-fold by insulin. In the presence of 10 μmol/l CB basal 2DG uptake was inhibited by over 80 % and the inhibitor completely blocked the stimulation in 2DG transport by insulin. Unlike fructose uptake, that of 2DG was inhibited in the presence of 3OMG by about 70 % (Fig. 3). In contrast, fructose had little inhibitory effect on the uptake of 2DG suggesting that it was a relatively poor substrate for the transport system mediating the uptake of glucose.

GLUT5 is expressed in rat adipocytes. To assess whether rat adipocytes express the fructose transporter GLUT5, we immunoblotted subcellular membrane fractions prepared from control and insulin-treated adipocytes. Figure 4a shows that in line with many previous studies GLUT4 was detected predominantly in intracellular membranes in unstimulated fat cells but following insulin treatment its abundance in the plasma membrane (PM) increased at the expense of GLUT4 in the low density microsomes (LDM) (Figure 4a). In contrast, there was significantly more GLUT1 in the PM fraction of unstimulated fat cells and its abundance was marginally increased following treatment of cells with insulin. We found that an antibody raised against the C-terminal domain of rat GLUT5 detected a single protein band migrating with a M*r* of approximately 50 kDa in the adipocyte PM fraction. No GLUT5 immunoreactivity was observed in the LDM (or high density microsomes (HDM) fraction, data not shown) indicating a pattern of distribution distinct from that seen for GLUT1 and GLUT4 (Figure 4a). Acute insulin treatment did not elicit any changes in GLUT5 abundance in the PM fraction consistent with the lack of any intracellular GLUT5. The subcellular distribution of GLUT5 was identical to that of the α1

subunit of the Na,K-ATPase which is a plasma membrane marker in these cells.

To confirm that the GLUT5 band detected in our adipocyte membranes did not represent an experimental artefact, we ran adipocyte PM alongside crude membranes prepared from rat jejunum, which are known to be enriched with GLUT5 [23]. Figure 4b shows that the rat GLUT5 antibody reacted positively against crude jejunal membranes; detecting a single protein migrating as a slightly broader band (~55 kDa). Importantly, the immunoreactivity in both the adipocyte and jejunal membranes was not observed when we used GLUT5 serum that had been pre-adsorbed with the antigenic peptide (Fig. 4b). The jejunal and adipocyte GLUT5 migrate with slightly different mobilities on SDS-gels and this most likely reflects differences in *N*-linked glycosylation of the protein in the two tissues as has been shown previously for GLUT5 in human fat and intestine [24].

GLUT5 expression and fructose uptake in adipocytes from diabetic rats. To assess whether expression of GLUT5 could be influenced under circumstances known to affect glucose uptake and GLUT4 expression, we investigated the effects of streptozotocin (STZ)-induced diabetes on GLUT5 expression and fructose uptake. Figure 5a shows a representative immunoblot of GLUT4 and GLUT5 in PM and LDM fractions prepared from adipocytes of control and diabetic rats. In line with previous reports we observed a marked reduction in both PM and LDM GLUT4 content 4 days post STZ-injection (Fig. 5a). We found that GLUT5 abundance in the PM fraction of diabetic adipocytes also fell substantially by approximately 75% (Fig. 5b). These findings imply that GLUT5 expression may be responsive to changes in blood insulin or glucose and it might therefore be expected that a marked reduction in GLUT5 expression may be accompanied by changes in fructose uptake. To test this proposition, we measured fructose uptake in adipocytes isolated from diabetic rats and found that it was reduced by approximately 50% (Fig. 6). To evaluate whether the reduction in GLUT5 was due to a lack of insulin or the hyperglycaemia our diabetic rats were experiencing, we tried to correct glycaemia in diabetic rats by supplementing their drinking water with vanadate. Blood glucose in diabetic animals was 20 ± 1.6 mmol/l (means ± SEM, $n = 6$) and was partially corrected by treatment with vanadate 12.4 ± 0.75 mmol/l (means ± SEM, $n = 5$). Animals treated with vanadate were still moderately hyperglycaemic since blood glucose in control animals was still 53% lower (5.8 ± 0.2 mmol/l, means ± SEM, $n = 6$). Analyses of fructose uptake in adipocytes isolated from vanadate treated rats showed GLUT5 expression and fructose uptake (Fig. 6b) were partially restored, although still lower than that

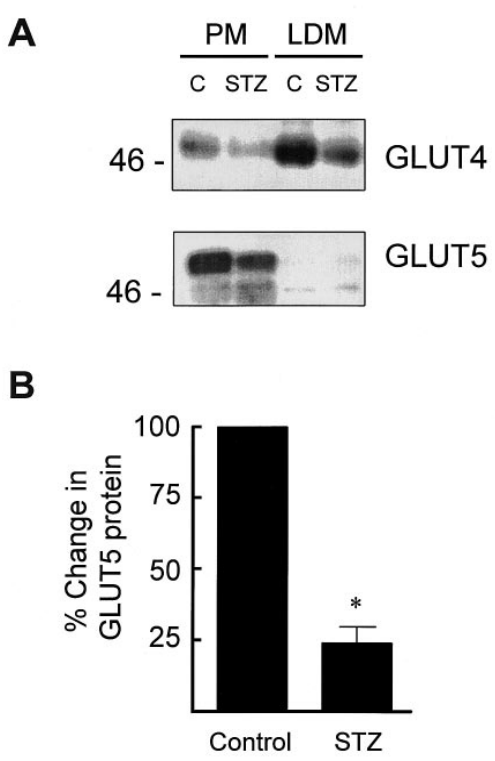

Fig. 5A,B. Effect of STZ-diabetes on GLUT4 and GLUT5 expression in rat adipocytes. Rats were rendered diabetic with streptozotocin. Adipocyte subcellular membranes were prepared and 20 μg membrane protein analysed by SDS/PAGE and Western blotting as described in the methods section. **A** Representative immunoblots showing GLUT4 and GLUT5 proteins in control (C) or diabetic (STZ) rats in plasma membrane (PM) and low density microsome (LDM) fractions. **B** Densitometric quantification of GLUT5 abundance in adipocyte plasma membranes from diabetic rats. GLUT5 signal density in adipocyte membranes from control animals were assigned a value of 100%. Values represent means ± SEM ($n = 5$). * Statistically significant change ($p < 0.05$ Student's t test)

observed in adipocytes from control rats (Fig. 6). Figure 6a also shows that unlike GLUT5, we did not observe any differences in the expression of the α1 subunit of the Na,K-ATPase in adipocyte membranes from diabetic and diabetic animals treated with vanadate compared with the control membranes. This latter finding helps dispel the possibility that the changes observed in GLUT5 expression may have partly arisen through differences in protein loading on SDS gels.

Discussion

It has long been established that rat adipocytes are capable of taking up fructose by a carrier whose properties deviate from that responsible for the uptake of glucose [12–14]. The molecular identity of this transporter has remained uresolved, but based on our work we suggest it is a member of the facilitative glu-

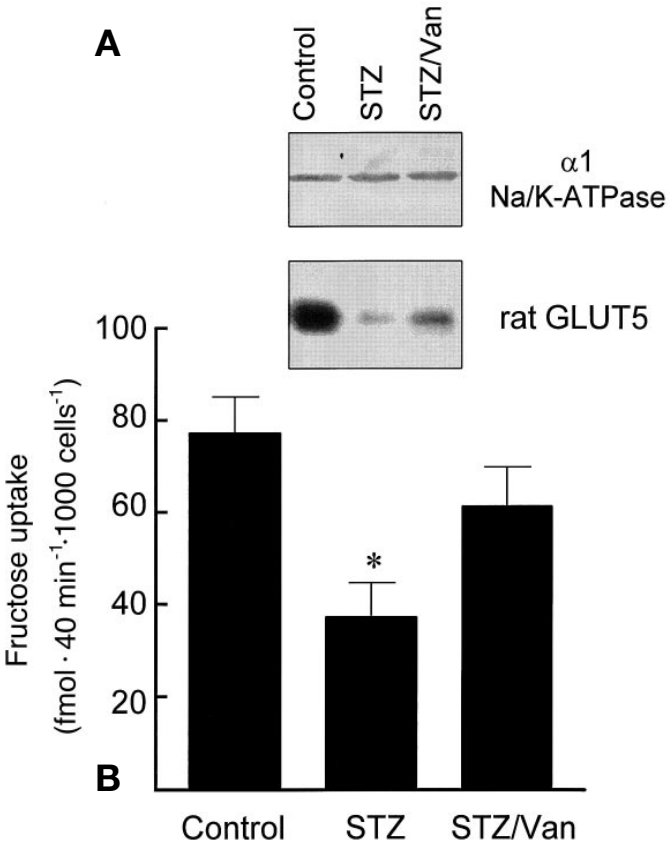

Fig. 6A,B. Effect of STZ diabetes and vanadate treatment on GLUT5 expression in rat adipocytes. Rats were rendered diabetic with streptozotocin and subsequently treated with sodium orthovanadate as described in the methods section. Adipocytes from control, diabetic and vanadate-treated diabetic rats were isolated and used either for isolating plasma membranes or for fructose uptake studies. **A** 20 μg of plasma membrane protein was subjected to SDS/PAGE and Western blotting using a GLUT5 antibody. **B** Fructose uptake was assayed as described in methods. Uptake values represent means ± SEM (data from 4 separate experiments each carried out in triplicate). *Statistically significant change compared with fructose uptake measured in adipocytes from control animals ($p < 0.05$ Student's t test)

cose transporter family, most likely GLUT5. This carrier operates as a specific fructose transporter in other tissues [17, 22, 23, 32] and we believe that it also functions in this capacity in rat adipocytes based on the following evidence. Firstly, heterologous expression of GLUT5 in *Xenopus* oocytes indicate that this transporter is insensitive to cytochalasin B (CB) [22, 23], whereas it is established that GLUT1 and GLUT4 mediate sugar uptake in a CB-sensitive fashion [15–17]. Thus the finding that about 80% of the fructose uptake was CB-insensitive is consistent with our suggestion that GLUT5 transports most of the fructose uptake in the adipocyte. Secondly, only a very small component of the fructose uptake in fat cells was insulin-responsive. We believe that since the hormone principally affects the subcellular distribution of GLUT4, but is also capable of inducing a modest increase in cell surface GLUT1, the small hormonal stimulation in fructose uptake is attributable to GLUT4 and/or GLUT1. This proposition is further strengthened by our finding that insulin fails to cause any stimulation in fructose uptake in the presence of CB which, as indicated earlier, inhibits only GLUT1 and GLUT4 mediated transport, but not that by GLUT5. The suggestion that some of the fructose uptake may be mediated by GLUT1 and GLUT4 is also supported by the finding that a very small fraction of the total fructose uptake was inhibited by 3OMG signifying a diminutive degree of competition between fructose and 3OMG for a common glucose carrier(s). Thirdly, the present work shows that the subcellular localisation of GLUT5 is distinct from that seen for GLUT4 and GLUT1. The absence of any intracellular GLUT5 also helps explain why insulin fails to promote an increase in plasma membrane GLUT5 in a manner similar to that seen for GLUT4. Fourthly, our data is consistent with the findings of Shepherd et al. [24] who reported that human adipocytes also express GLUT5, signifying that expression of GLUT5 in adipose tissue is a feature that has been preserved across at least two different species. These authors only reported data for the plasma membrane fraction but they too, did not observe any changes in GLUT5 content after treatment of human adipocytes with insulin [24]. Finally, based on biochemical and immunocytochemical evidence, the plasma membrane localisation of GLUT5 in rat adipocytes is strikingly similar to that in human skeletal muscle [32] which we, and others, have shown to take up and metabolise fructose in CB-independent manner [17, 33]. All these observations strongly support our view that GLUT5 operates as a specific fructose transporter in the rat adipocyte.

We observed a distinct pattern of inhibition by different hexose analogues on fructose uptake in our study. Both glucose and (2DG) inhibited fructose uptake by 60% and 75%, respectively, yet 3OMG caused only a modest inhibition in fructose uptake by 16%. These findings agree with earlier reports [12, 14] and the differences observed in the inhibitory potential of the different hexoses is probably attributable to the fact that both glucose and 2DG can be phosphorylated within the fat cell. It is likely that the presence of either 30 mmol/l glucose or 2DG during the fructose uptake assay results in the respective accumulation of glucose 6-phosphate and 2DG 6-phosphate which leads to an allosteric inhibition in hexokinase activity. In adipocytes, fructose is phosphorylated to fructose 6-phosphate by hexokinase in strong contrast to liver where the ketose is phosphorylated to fructose 1-phosphate by fructokinase [34, 35]. Thus, when hexokinase is inhibited the transmembrane fructose gradient required for 'driving' the uptake of the sugar into the fat cell is dramatically reduced. This phenomena is not restricted to adipo-

cytes as a positive relationship has been shown to exist between the down-regulation in hexose uptake and an increase in cellular 2-DG 6-phosphate content in rat L8 myotubes [36]. Since 3OMG is not phosphorylated, the inhibition observed in fructose uptake by this hexose simply reflects that proportion of the total fructose uptake which occurs via a glucose carrier for which 3OMG is a competing substrate. This route of fructose entry is likely to be CB-sensitive and indeed the inhibition in fructose uptake seen in the presence of CB (21 %) approximates very closely to that seen in the presence of 3OMG (16 %).

A number of studies have shown that the expression of the insulin-regulated glucose transporter, GLUT4, is reduced following induction of diabetes [37–41]. Our study shows that this is true also for GLUT5 in rat adipocytes given that its expression was reduced by about 75 % after just 4 days of STZ-diabetes. The lower GLUT5 expression in fat cells was associated with a reduction in fructose uptake providing further evidence of its role in the uptake of this sugar in rat adipocytes. The precise signal that triggers the down-regulation in GLUT5 in adipose tissue during STZ-diabetes is not known and although insulin does not exert any acute effects upon fructose uptake or GLUT5 abundance we cannot exclude the possibility that there may be a constitutive requirement for insulin action to help maintain its expression in the fat cell. Alternatively, given that blood glucose was elevated by up to four-fold in our diabetic rats, glycaemia could also be an important regulator of GLUT5 expression. Previous studies have shown that normalisation of blood glucose in diabetic rats can help restore adipocyte insulin-stimulated glucose transport in vitro and insulin-mediated glucose disposal in vivo [42]. To address this possibility we partially corrected the high blood glucose in our diabetic rats through treatment with vanadate. Vanadate acts like an insulin-mimetic in vivo and has been shown to modify carbohydrate metabolism in whole animals, isolated cells and tissues [43–45]. Although the 4 day period of our study may not have been sufficiently long to restore normal euglycaemia we were able to induce a significant reduction in blood glucose (by ~ 40 %) and as a result observed a slight sparing in GLUT5 expression accompanied by a modest restoration in fructose uptake in fat cells. The idea that glucose could be a regulator of GLUT5 expression is supported by the finding that it can modulate GLUT5 expression in the human colonic cell line, Caco-2 [46] and in the human choriocarcinoma (placental) cell line, BeWo (Shah and Hundal, unpublished data). How glucose exerts its effects on GLUT5 expression in primary rat adipocytes and established cell lines, such as Caco-2 and BeWo, is poorly understood. However, it should be stressed that whilst glycaemia could be an important regulator of GLUT5 expression, we are not able to exclude the possibility that the restoration in GLUT5 expression observed in adipose tissue of our diabetic rats could also, in part, be attributable to the insulin-like effects exerted by vanadate.

In summary, the present work shows that rat adipocytes express the GLUT5 transporter which is likely to participate in the uptake of fructose in these cells in a CB- and insulin-insensitive manner. Expression of GLUT5 in the fat cell was rapidly down-regulated during streptozotocin-induced diabetes and this reduction may, be regulated by changes in blood glucose.

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