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Fructose transport and metabolism in adipose tissue of Zucker rats: Diminished GLUT5 activity during obesity and insulin resistance

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Abstract

Fructose is a major dietary sugar, which is elevated in the serum of diabetic humans, and is associated with metabolic syndromes important in the pathogenesis of diabetic complications. The facilitative fructose transporter, GLUT5, is expressed in insulin-sensitive tissues (skeletal muscle and adipocytes) of humans and rodents, where it mediates the uptake of substantial quantities of dietary fructose, but little is known about its regulation. We found that GLUT5 abundance and activity were compromised severely during obesity and insulin resistance in Zucker rat adipocytes. Adipocytes from young obese (*fa/fa*), highly insulin-responsive Zucker rats contained considerably more plasma membrane GLUT5 than those from their lean counterparts (1.8-fold per microgram membrane protein), and consequently exhibited higher fructose transport (fivefold) and metabolism (threefold) rates. Lactate production was the preferred route for fructose metabolism in these cells. As the rats aged and become more obese and insulin-resistant, adipocyte GLUT5 surface density (12-fold) and fructose transport (10-fold) and utilisation rates (threefold) fell markedly. The GLUT5 loss was more dramatic in adipocytes from obese animals, which developed a more marked insulin resistance than lean counterparts. The decline of GLUT5 levels in adipocytes from older, obese animals was not a generalised effect, and was not observed in kidney, nor was this expression pattern shared by the $\alpha 1$ subunit of the Na^+/K^+ ATPase. Our findings suggest that plasma membrane GLUT5 levels and thus fructose utilisation rates in adipocytes are dependent upon cellular insulin sensitivity, inferring a possible role for GLUT5 in the elevated circulating fructose observed during diabetes, and associated pathological complications. (Mol Cell Biochem **xxx**: 1–11, 2004)

Key words: GLUT4, glucose, membrane, muscle, diabetes

Introduction

Fructose is an important dietary sugar, but one which is associated with adverse conditions, such as glucose intolerance and hyperlipidaemia [1], and non-enzymatic fructosylation of proteins [2]—all factors in the pathogenesis of diabetic complications. Despite these associations, it has until recently been thought that fructose levels were the same between normal and diabetic subjects. In a recent technological advance, however, Kawasaki *et al*. [3] have been able to measure fructose levels free from the interfering effects of glucose and have shown that serum levels of fructose are 50% higher in diabetic than in non-diabetic or healthy human subjects. This finding strengthens the proposal that circulating fructose levels are a factor in the progression of diabetic complications.

Dietary fructose is absorbed into tissues mostly via the specific fructose transporter GLUT5, first isolated from small intestine [4]. GLUT2 has been shown also to have a role in fructose transport across the intestinal basolateral membrane [5], although this transporter has a broad substrate specificity

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for hexoses [6]. We and others have shown that aside from the jejunum, insulin-sensitive tissues of humans and rats also express the GLUT5 fructose transporter isoform [7–10]. It is thought that adipocytes are responsible for the uptake of a significant proportion of dietary fructose [11], where it is thought to enter metabolic pathways via hexokinase activity [12]. Indeed, at least in rats, it has been suggested that fat is the secondary site of fructose uptake after the liver [11].

In the small intestine and kidney, GLUT5 expression is regulated by substrate availability [13, 14]. In the colon carcinoma cell line Caco-2, GLUT5 expression is modulated by adenylate kinase activity, in addition to carbohydrate availability [15] and thyroid hormone [16]. Glucose is also a regulator of GLUT5 expression in BeWo human choriocarcinoma cells [17]. However, little is known about how GLUT5 expression in insulin-sensitive tissues is regulated, except that there appears to be no substrate effect in rat skeletal muscle [8]. In this study, we used Zucker rats to investigate whether GLUT5 expression in adipocytes is modulated during ageing obesity and insulin resistance. We also examined the modulation of fructose transport in this system and explored possible routes for fructose metabolism.

Materials and methods

Animals

Male obese Zucker rats (*fa/fa*) and their lean littermates were purchased from Harlan and Olac (Bicester, Oxon, UK). Rats were anaesthetised by administration of sodium pentobarbitone (May and Baker, Dagenham, UK) at 60 mg $\cdot$ kg^{-1} body weight, before removal of tissue samples, then killed by cervical dislocation. All animal procedures were performed in accordance with UK. Home Office regulations regarding the care and use of laboratory animals.

Crude kidney membrane preparations

Kidneys were ground under liquid nitrogen using a pestle and mortar, then homogenised by 10 strokes in a Dounce homogeniser in TES buffer (0.25 M sucrose, 10 mM Tris–HCl pH 7.5, 1 mM EDTA, 0.1 mM PMSF). The crude homogenate was centrifuged at 2500 $\times$ *g*, for 15 min at 4°C. The resulting supernatant fluid was centrifuged at 24000 $\times$ *g* for 20 min at 4°C to pellet the membranes. Crude membranes were resuspended in a small volume of TES buffer.

Subcellular fractionation of isolated fat tissue

Rat epididymal fat was minced and subjected to collagenase (1 mg/ml) treatment in Krebs–Ringer–HEPES buffer (KRH) (pH 7.5) at 37°C for 40 min. Adipocytes were washed in TES buffer and filtered through 200 μm nylon mesh (Cadisch Precision Meshes Ltd., London, UK), prior to homogenisation and subfractionation as described previously [18] to isolate fractions enriched in plasma membranes (PM), low density microsomes (LDM) and high density microsomes (HDM). The protein content of membrane fractions was determined by the method of Bradford [19].

Assays of hexose transport

Uptake of [^{3}H]-2-deoxyglucose and [^{14}C]-fructose (radiolabelled sugars from NEN, Boston, MA, USA) was measured as described previously [7]. Briefly, 400 μl of adipocyte suspension in KRH buffer were incubated with combinations of 100 nM insulin for 30 min and 10 μM cytochalasin B (to preclude GLUT-mediated glucose transport and non-GLUT5-mediated fructose transport) for 40 min. Assays were started by the addition of 50 μM [^{3}H]-2-deoxyglucose (26.2 Ci/mmol) or 100 μM [^{14}C]-fructose (235 mCi/mmol). Assays were terminated after 1 min (2-deoxyglucose) or 40 min (fructose) by centrifugation of the adipocyte suspension through a 100 μl di-isononyl phthalate oil cushion (Fluka, Gillingham, UK). Corrections for non-specific cell-associated radioactivity were made by including [^{14}C]- or [^{3}H]-inulin (NEN) in 2-deoxyglucose or fructose uptake assays, respectively.

Measurement of lipid synthesis

Assays of lipid synthesis were performed essentially as described previously [20]. Briefly, adipocytes were incubated in KRH buffer containing 1 mM glucose or fructose (including 1.5 mCi/ml [^{14}C]-glucose or [^{14}C]-fructose as tracer) and/or insulin at 100 nM, and cytochalasin B at 10 μM, for 1 h at 37°C in a 5% CO_2/95% air (v/v) atmosphere. Addition of 3.3 volumes of isopropanol/heptane (4:1) and 1 volume of H_2O (37°C) was followed by agitation at room temperature for 1 h. Heptane was added (0.4 volumes) and samples were agitated for 2 h. Phases were cleared by centrifugation and upper phase was removed, dried and radioactivity assessed by liquid scintillation counting.

Assay of lactate production

Lactate production was measured as described previously [20]. In brief, adipocytes were isolated and incubated in KRH buffer containing 5 mM glucose or fructose and/or 100 nM insulin and 10 μM cytochalasin B (to eliminate GLUT-mediated transport of glucose or non-GLUT5-

mediated transport of fructose) for 4 h at 37°C in a 5% CO_2/95% air (v/v) atmosphere. To each 1 ml adipocyte sample was added 200 μl of 10% (w/v) trichloroacetic acid to precipitate proteins, which were sedimented in a microfuge at 13,000 × *g* for 10 min at 4°C. To 200 μl aliquots of supernatant fluid in spectrophotometer cuvettes were added 800 μl buffer A (1 M glycine, 10 mM hydrazine, 1 mg/ml NAD, pH 9.0), and $A_{340\,nm}$ was measured. Lactate dehydrogenase was added (Sigma, Dorset, UK) to cuvettes and mixed. Cuvettes were incubated for 45 min at room temperature before the measurement of $A_{340\,nm}$ and the quantification of lactate production using the Beer–Lambert law.

SDS-PAGE and immunoblotting

Samples were subjected to SDS-PAGE on 10% (w/v) Schagger-type polyacrylamide gels [21] or regular 10% (w/v) polyacrylamide gels [22]. Resolved proteins were transferred to ECL-nitrocellulose membranes (Amersham) and membranes probed with primary antibodies (α-GLUT5, kind gift from Dr. Yoshitoma Oka, University of Tokyo, Japan; 1F8 α-GLUT4, Genzyme Ltd, Suffolk; or MCK1 anti-α1 subunit of Na^+/K^+ ATPase kind gift from Dr K. Sweadner, Massachusetts General Hospital, MA, USA) at 4°C. Protein A–horseradish peroxidase conjugate (1/8000; ICN Biomedicals, OH, USA) or anti mouse IgG–horseradish peroxidase conjugate (SAPU, Lanarkshire, UK) were incubated with blots for 1 h at room temperature and used subsequently to expose X-ray film (Konica, Hohenbrunn, Germany) after incubation with ECL reagents (Pierce & Warriner, Chester, UK).

Statistical analyses

Statistical analyses of experimental data were performed using an unpaired two-tailed Student's *t* test. Means were considered significantly different at *p* values less than 0.05.

Results

Fructose transport in young Zucker rats

Table 1 shows the measured blood glucose and insulin levels of Zucker rats used in this study. The blood insulin levels of young (5 week) obese animals was 2.6-fold greater than that of their lean counterparts, while blood glucose from animals on fast was not significantly different. Figure 1 illustrates the fructose transport characteristics of epididymal adipocytes from 5-week old Zucker rats. Experimental conditions were designed to assess an initial, linear rate of uptake, as we have determined previously [7]. At 5 weeks of age, the basal fructose transport rate of adipocytes from obese animals was approximately four times that of their lean counterparts (324.6 ± 18.2 versus 76.5 ± 4.8 fmol/40 min/1000 cells). Adipocytes from lean animals did not exhibit any significant increase in fructose transport upon acute stimulation with insulin. A marginal increase in fructose transport was induced by the hormone in adipocytes from obese rats, as has been reported previously for Sprague-Dawley rats [7]. However, this was not statistically significant and this effect was inhibited by prior treatment with the fungal metabolite cytochalasin B, indicating the involvement of transport via GLUT1 and GLUT4 [7].

Table 1. Blood glucose and insulin values from lean and obese Zucker rats

Animals	Blood glucose (mg/dl)	Blood insulin (ng/ml)
5 weeks		
Lean	101.3 ± 3.3 (6)	1.95 ± 0.53 (6)
Obese	107.0 ± 6.0 (6)	5.18 ± 0.94 (6)*
10 weeks		
Lean	128.7 ± 7.2 (3)	2.90 ± 0.23 (3)
Obese	192.3 ± 21.3 (3)	11.25 ± 0.14 (3)*
15 weeks		
Lean	124.5 ± 8.6 (3)	6.34 ± 1.63 (3)
Obese	238.9 ± 30.9 (3)	39.67 ± 1.74 (3)*

Values represent means ± S.E.M. of the indicated number of rats. *Statistically significant differences compared with values from age-matched lean rats ($p < 0.05$ Student's *t* test).

For purposes of comparison, the inset box in Fig. 1 shows glucose transport rates into 5-week old Zucker rat adipocytes. The figure illustrates previously reported insulin-responsive glucose transport characteristics in both lean and obese animals, including high fold increases in response to insulin, and elevated rates of transport in adipocytes from genetically obese (*fa/fa*) animals [23].

Fructose transport in mature Zucker rat adipocytes

Figure 2 illustrates the fructose transport characteristics of epididymal adipocytes from 10-week and 15-week old rats. At 10 weeks of age, adipocytes from lean animals exhibited similar fructose transport rates compared to fat cells from younger animals (Figs. 2A and 1). In the case of adipocytes from obese rats, fructose transport rates were approximately 2.7-fold those for lean animals, a reduced differential compared to that observed at 5 weeks. There was no significant elevation of fructose transport upon insulin stimulation of adipocytes from 10-week old rats. Inhibition of GLUT isoforms other than GLUT5, by incubation with cytochalasin B, resulted in a 25% decrease in fructose transport in both lean and obese animals, indicating that 75% of fructose uptake occurred through GLUT5 in these cells.

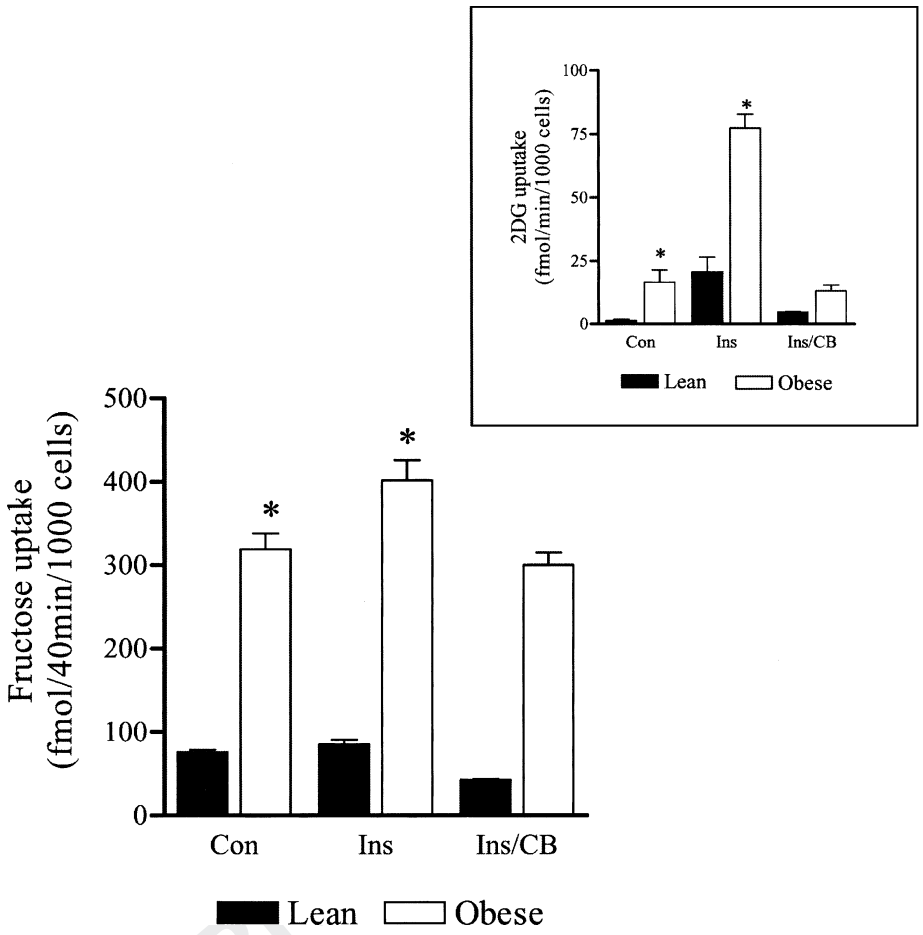

Fig. 1. Sugar uptake into adipocytes from young Zucker rats. Suspensions of isolated adipocytes were taken from Zucker rats of 5 weeks of age. Adipocytes were pre-incubated with 100 nM insulin for 20 min or with 10 μM cytochalasin B 10 min prior to the addition of hormone, or with vehicle alone. Assays of fructose (main figure) or 2-deoxyglucose (boxed inset) were performed as described. Data are means ± S.E. of 3–6 observations made in duplicate. ∗: Values significantly different from lean controls.

We were unable to measure transport into adipocytes from obese animals of 15 weeks of age because of the large size and consequent fragility of the cells. This problem has been noted previously [24]. Adipocytes from 15-week old lean rats exhibited reduced fructose transport rates compared to 5-week old animals (32.7 ± 2.3 versus 76.5 ± 4.8 fmol/40 min/1000 cells) (Figs. 2B and 1). Furthermore, pre-incubation of 15 week lean adipocytes with cytochalasin B reduced the fructose transport rate to 14.7 ± 1.3 fmol/40 min/1000 cells, indicating that in these cells only around 45% of fructose uptake is mediated by GLUT5. There was no insulin stimulation of fructose transport noted.

For comparative purposes, glucose transport data are included for mature Zucker adipocytes (inset box in Fig. 2). In adipocytes extracted from lean rats aged 15 weeks, we observed only a modest (2.5-fold basal values) increase in glucose transport after insulin stimulation, compared to those exhibited by fat cells from younger animals (Fig. 2B inset). These data are consistent with the observed decline in GLUT4 with age (Fig. 3C), and with the view that these adipocytes were relatively resistant to insulin compared to those from animals at 10 weeks, which exhibited 16-fold glucose transport stimulation with insulin (Fig. 2A inset).

GLUT5 abundance in adipocytes from lean and obese Zucker rats

Figure 3A shows the pattern of GLUT5 abundance in the PM of Zucker rat adipocytes. Previous work has shown that the PM is the major site of GLUT5 localisation in adipocytes [7]. The GLUT5 content of PMs from young obese animals, which are hyperinsulinaemic but not hyperglycaemic, was elevated 1.8-fold compared to lean controls (densitometric analysis in Fig. 3B), correlating with the fourfold greater rate

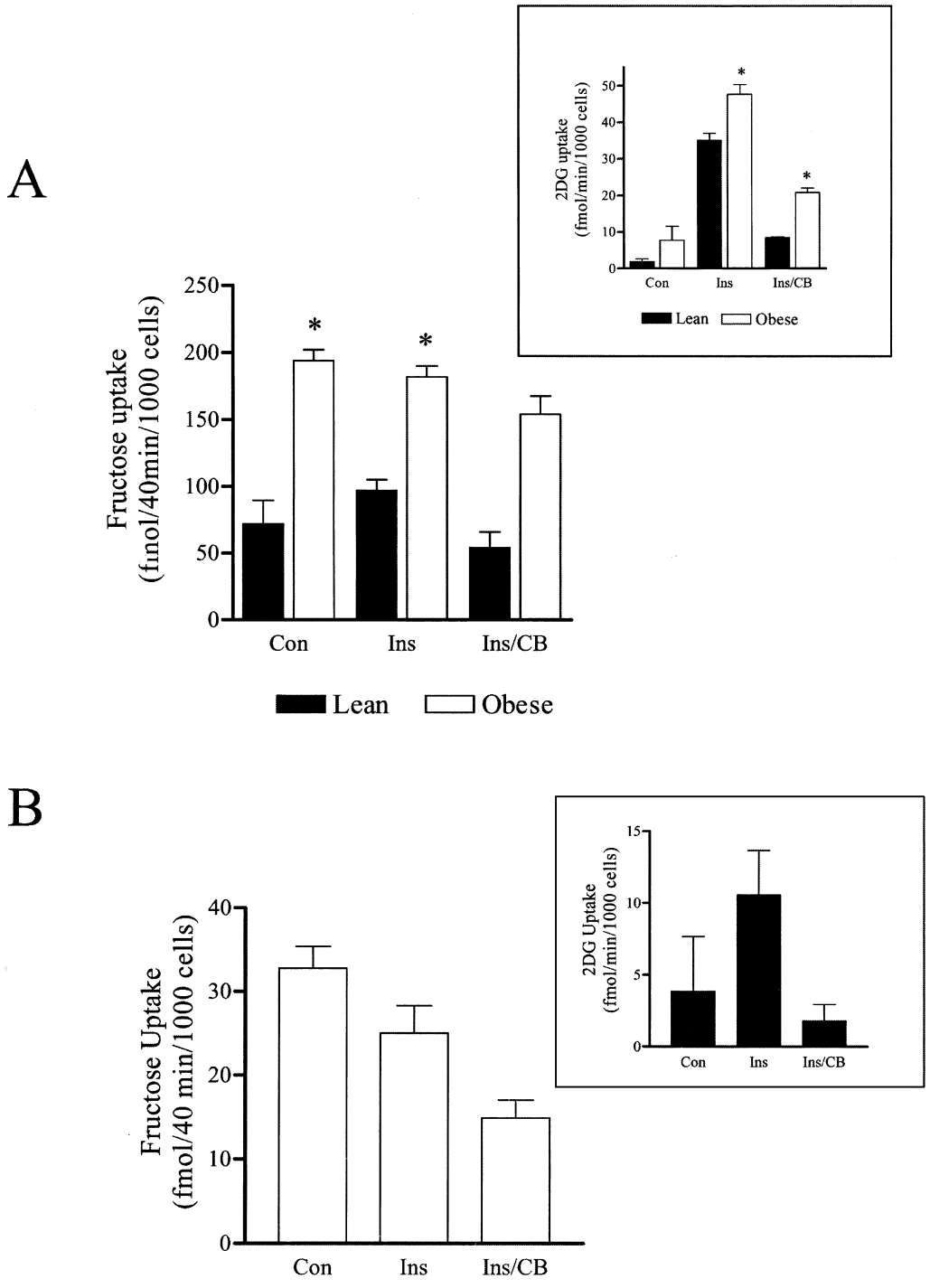

Fig. 2. Uptake of hexoses into adipocytes from mature Zucker rats. Suspensions of isolated adipocytes were taken from Zucker rats of (A) 10 weeks or (B) 15 weeks of age. Adipocytes were pre-incubated with 100 nM insulin for 20 min or with 10 μM cytochalasin B 10 min prior to the addition of hormone, or with vehicle alone. Assays of fructose (main figures) or 2-deoxy glucose (boxed insets) uptake were performed as described. Data shown are means $\pm$ S.E. of 3–6 observations made in duplicate. $*$: A statistically significant change from corresponding lean controls.

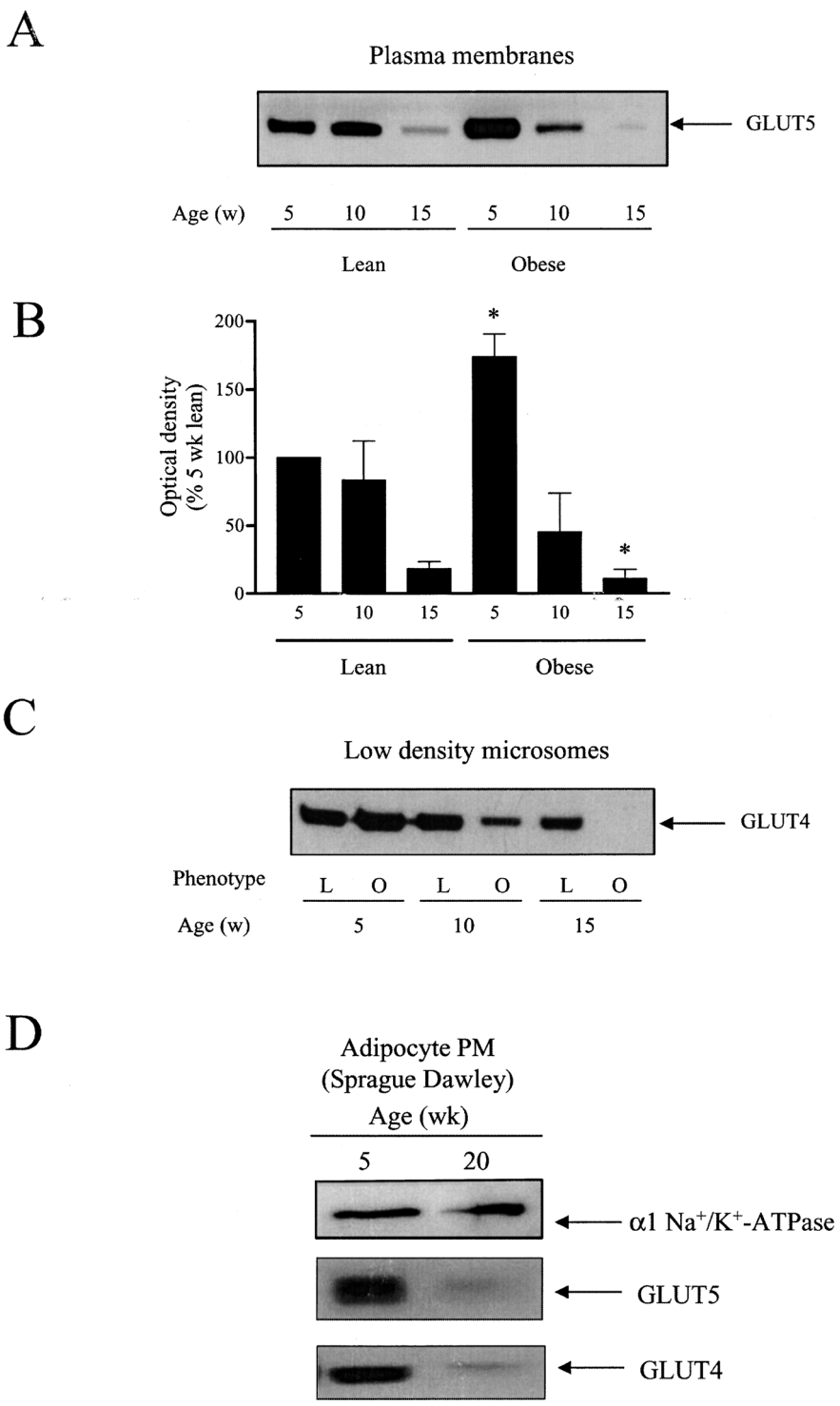

Fig. 3. GLUT5 transporter abundance in Zucker rat adipocytes. Adipocytes from epididymal fat pads of Zucker rats of the ages and phenotypes indicated were isolated and subcellular fractionation was performed as described. Protein (20 μg) from membrane fractions was separated by SDS-PAGE and immunoblotted. (A) Plasma membrane (PM) protein was blotted and probed for GLUT5 content. (B) PM GLUT5 abundance was quantified by densitometry. ∗: Statistically
Q3 significant change from values obtained from 5-week old lean animals. (C) Low-density microsome proteins were blotted and probed for GLUT4 content. (D) PMs were isolated from epididymal adipocytes from 5-week or 20-week old "Sprague-Dawley" rats, blotted and probed for membrane transporter content. All blots shown are representative of three or more experiments.

of fructose transport in these adipocytes compared to lean controls (Fig. 1).

Obese Zucker rats at 10 weeks (Table 1) exhibited increased blood insulin levels (3.9-fold control values) and were substantially hyperglycaemic (blood glucose 150% of lean controls). At 10 weeks of age, the GLUT5 content per microgram of adipocyte PM from obese animals was reduced by approximately 50% compared to lean controls and approximately fourfold lower than values obtained from 5-week old obese rats. There was no significant loss of GLUT5 from adipocyte PM of lean animals, compared to 5-week old controls.

At 15 weeks of age (Table 1), the obese Zucker rats exhibited extremely high circulating insulin (6.5-fold control values) and were also increasingly hyperglycaemic (blood glucose almost twofold lean controls). At this stage of maturity, even the lean animals had markedly elevated blood insulin levels (over three times those observed in corresponding 5-week old animals), but exhibited relatively normal blood glucose concentrations. At 15 weeks of age, there was a striking reduction in GLUT5 content per microgram of adipocyte PM from both lean (fourfold) and obese (12-fold) Zucker rats, compared to 5-week old controls.

For comparison, Fig. 3C shows the level of the insulin-regulated glucose transporter GLUT4 in adipocytes from Zucker rats, in LDM membranes. It was evident that the abundance of GLUT4 in this membrane compartment was also diminished markedly as a function of age, particularly in the more insulin-resistant cells from obese rats. This relationship has been studied previously in Zucker rat adipocytes, where GLUT4 regulation is understood to be regulated as a function of age, obesity and insulin sensitivity [23–25].

Figure 3D shows that the age-related decrease in adipocyte GLUT5 was not merely a strain-specific feature of Zucker rats. PMs were isolated from epididymal adipocytes of "Sprague-Dawley" rats at 5 and 20 weeks of age, and analysed for membrane transporter abundance. We observed a substantial decrease in the abundance of GLUT5 as well as GLUT4 in these membranes. However, no change was observed in the abundance of the PM marker, the α1 subunit of the Na^+/K^+ ATPase [26], indicating that the decrease in GLUT5 abundance does not reflect a generalised expression pattern.

Figure 4A further illustrates the modulation of GLUT5 density in relation to age and phenotype in Zucker rat adipocytes. The decline of PM GLUT5 in older, obese animals was in stark contrast to the density of the α1 subunit of the Na^+/K^+ ATPase. Immunoblotting for this transporter showed a dramatic increase in adipocyte PM density in 15-week old, genetically obese Zucker rats.

To examine whether the obesity and insulin resistance-associated changes in GLUT5 abundance that we observed

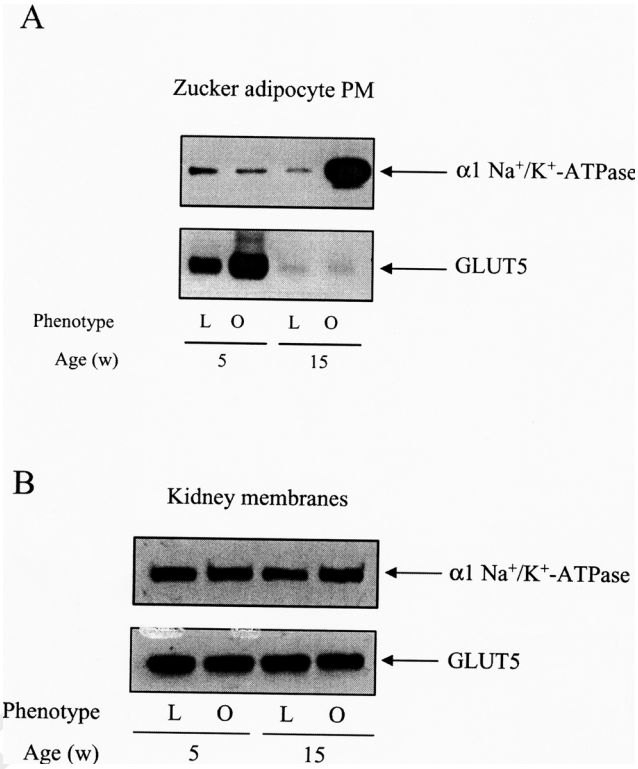

Fig. 4. GLUT5 loss during obesity and insulin resistance is tissue-specific. (A) Plasma membranes (PM) from young (5-week old) and mature (15-week old) Zucker rat adipocytes were blotted and GLUT5 abundance was compared with that of the PM marker α1 Na^+/K^+ ATPase. (B) Crude membranes from young (5-week old) and mature (15-week old) Zucker rat kidneys were blotted and GLUT5 abundance was compared with that of α1 Na^+/K^+ ATPase. All blots shown are representative of three or more experiments.

in Zucker rats were specific to adipocytes, we examined membranes prepared from the kidneys of these animals. No such regulation of GLUT5 was observed in kidney membrane preparations (Fig. 3B), where regulation has been shown to be exerted by substrate availability [13, 14]. This indicates that the regulation of GLUT5 exhibited by Zucker rat adipocytes is a tissue-specific phenomenon. Furthermore, the marked increase in Na^+/K^+ ATPase abundance that we observed in adipocytes from 15-week old obese animals was not a feature of kidney membranes, in which expression of this protein remained stable.

Fructose and glucose utilisation in Zucker rat adipocytes

Figure 5A shows the rate of lipid synthesis from radiolabelled fructose in Zucker rat adipocytes, with comparative rates for incorporation of radiolabelled glucose. In young rats, adipocytes from obese animals synthesised lipid from glucose at approximately 13 times the rate of fat cells from

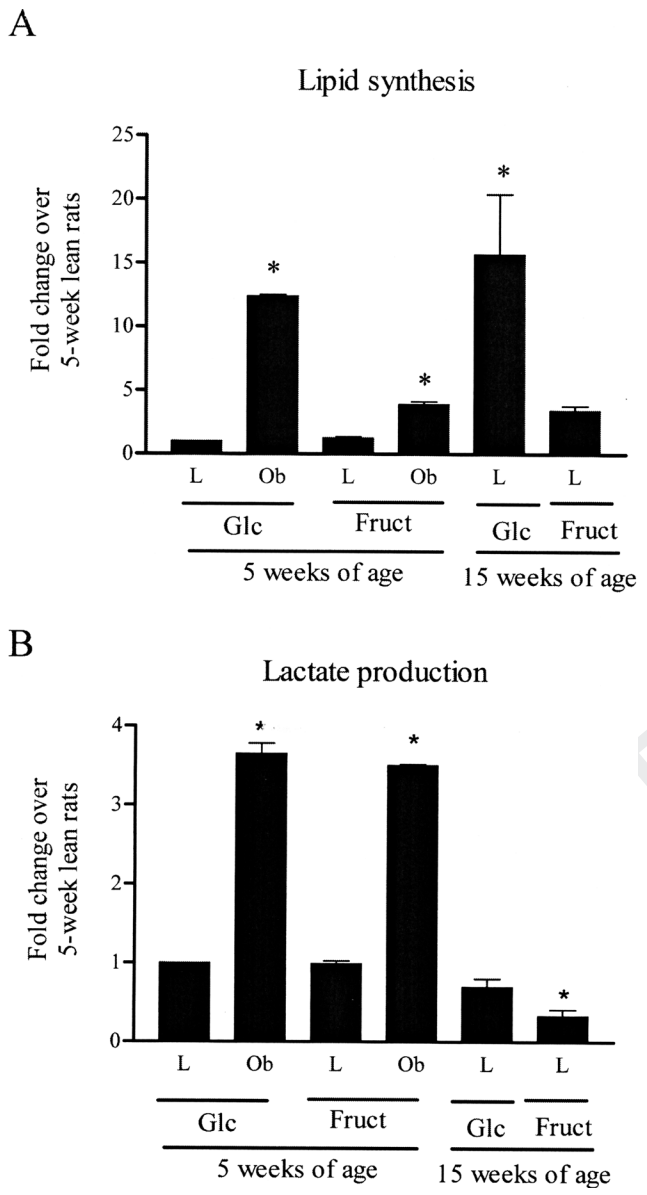

Fig. 5. Metabolic fate of fructose or glucose in adipocytes from Zucker rats. Suspensions of isolated adipocytes were taken from lean (L) or obese (Ob) Zucker rats at 5 or 15 weeks of age. Adipocytes were incubated with radiolabelled fructose (Fruct) or glucose (Glc), and assays of lipid synthesis (A) were performed as described. Alternatively, lactate production (B) was assayed as described. Data are means ± S.E. of 3–6 observations made in duplicate. ∗: A statistically significant change from values obtained from 5-week old lean animals.

Q4

lean animals. When fructose was used as the carbon source, adipocytes from obese animals again synthesised lipid at an elevated rate compared to lean controls, but with this sugar the increase was only threefold the rate of lean rats. When adipocytes from lean rats of 15 weeks were compared to their young counterparts, the rate of lipid synthesis from both fructose and glucose was increased by 2.5- and 16-fold, respectively.

Figure 5B illustrates the rates of lactate production from glucose or fructose by Zucker rat adipocytes. The data show that adipocytes from young, lean animals utilised either glucose or fructose for lactate production at the same rate. Adipocytes from young obese rats exhibited greater rates of lactate production (approximately 3.5-fold lean values), but again there was no apparent preference for glucose over fructose. At 15 weeks of age, the capacity for lactate production from fructose by adipocytes from lean Zucker rats was reduced significantly (30% of 5-week old control), and in these cells fructose was incorporated into lactate less readily than glucose.

Discussion

The recent finding that serum fructose levels are elevated in diabetic subjects [3] suggests a ready explanation for the involvement of this sugar in diabetic complications, such as the non-enzymatic fructosylation of proteins [2], and is consistent with previously suggested roles for serum fructose in adverse conditions such as glucose intolerance and hyperlipidaemia [1]. A plausible mechanism for increased serum fructose levels in diabetic subjects would be a reduction in the activity of the facilitative fructose transporter GLUT5 and consequently reduced fructose utilisation.

This report provides novel information on fructose uptake and GLUT5 abundance in adipocytes from Zucker rats and shows that GLUT5 surface density declines markedly during age, obesity and insulin resistance in adipose tissue. The data clearly show that fructose transport and metabolism, and plasma density of GLUT5, are up-regulated in adipocytes from young obese Zucker rats, which are highly responsive to insulin [23], as is illustrated by the glucose uptake data. Furthermore, we show that GLUT5 density and fructose uptake and metabolism rates are decreased in adipocytes from older rats, which exhibit insulin resistance. The increasing blood insulin levels observed in ageing Zucker rats indicate that, as is broadly accepted for both rats and humans, insulin sensitivity is reduced with age. Indeed, the glucose transport rates and elevated blood insulin levels in 15-week old Zucker rat adipocytes indicate that even lean animals are relatively insulin resistant at this age, since only a very small stimulation of transport is observed upon addition of insulin. The marked loss of GLUT5 with age is mirrored in adipocytes from male "Sprague-Dawley" rats, and is, therefore, not specific to the Zucker strain. We have observed routinely that adipocytes from 20-week old "Sprague-Dawley" rats respond very poorly to insulin in terms of glucose transport, compared to fat cells from young animals (unpublished data).

We have presented GLUT5 abundance in membranes of Zucker rat adipocytes in terms of equal protein loading, to illustrate differences in GLUT5 density at the PM. However, it is well established from several previous reports that adipocytes from obese Zucker rats have approximately fivefold the volume of adipocytes from age-matched lean animals [23–25, 27], and would therefore have almost three times the surface area, assuming spherical cell shape. This feature complicates the relationship between surface density of GLUT5, overall expression levels and fructose transport rates. However, our data would suggest that the expression levels of some genes encoding PM proteins, such as the $\alpha 1$ subunit of the Na^+/K^+ ATPase, are enhanced in larger fat cells, presumably to maintain normal activity levels. This is clearly not the case for GLUT5, and the data are consistent with a correlation between GLUT5 expression and cellular insulin sensitivity, and thus with the suggestion that insulin could be a controlling factor in GLUT5 expression and thus fructose uptake and utilisation.

The apparent discrepancy between GLUT5 density and fructose transport rates in adipocytes from 10-week old Zucker rats (obese cells exhibit 50% lean GLUT5 density but twofold higher fructose transport) can be explained readily by the increased size of adipocytes from obese, compared to lean animals [24]. The increased size of adipocytes in obese Zucker rats of 5 weeks of age suggests that GLUT5 expression may be increased by a factor more than the surface density indicates, since three times the level of GLUT5 expression may be required just to maintain surface density equal to lean controls, assuming that the PM is the major site of GLUT5 localisation in adipocytes [7]. Whatever the actual GLUT5 expression levels, in 10-week old obese animals, where the surface density of adipocyte GLUT5 is fourfold lower than in 5-week old animals, it is clear that the diminished GLUT5 abundance at the PM of these cells results in a diminution of fructose transport activity.

Although GLUT4 levels also decline in low-density adipocyte membranes from aged, obese Zucker rats, this is not a generalised phenomenon, since under the same circumstances, a dramatic increase in expression of the αl subunit of the Na^+/K^+ ATPase is observed. The reason for such a marked increase in the expression of this protein is unclear, but is likely to be in some way a reflection of its requirement by the cell for the maintenance of ion gradients and membrane potential. The burden of this task is likely to be much increased in the enlarged adipocytes of mature, obese Zucker rats. Also, in "Sprague-Dawley" rats, the abundance of the αl subunit of the Na^+/K^+ ATPase remains unchanged, despite marked decreases in GLUT5 and GLUT4 levels.

One previous report has described GLUT5 expression in human adipose tissue [9], in which no difference in GLUT5 content of adipocytes was observed between normal and obese/diabetic subjects. However, the wide range of subject age (29–64 years) of each group may preclude valid comparisons with our present findings. Furthermore, the obese/diabetic group in this study was made up of three obese, one very obese and one controlled type 2 diabetic patient. The degree of obesity and insulin resistance in this group may have been insufficient to bring about the clear loss of GLUT5 abundance seen in the present work.

We have previously investigated the effect of acute, streptozotocin-induced diabetes on adipocyte GLUT5 expression [7] in "Sprague-Dawley" rats. We found that induction of diabetes caused a profound reduction in GLUT5 abundance. Furthermore, this effect was only slightly relieved through blood glucose correction using vanadate treatment [7]. These data are consistent with the present work, suggesting that GLUT5 expression in rat adipocytes may be modulated as a function of insulin sensitivity, rather than by glycaemia. The elevated GLUT5 abundance in fat cells from obese compared to lean 5-week old Zucker rats indicates that insulin is likely to be the controlling factor, since the twofold increase in GLUT5 accompanies a similar increase in blood insulin, in the absence of any significant variation in blood glucose levels. In addition, we have performed studies with insulin-sensitive L6 myotubes and have observed no correlation between ambient glucose concentrations and GLUT5 expression. Interestingly, however, we do note a clear stimulation of GLUT5 expression at both mRNA and protein levels in L6 myotubes by increasing concentrations of insulin. Furthermore, sequence analysis of the GLUT5 promoter reveals the presence of a number of putative insulin-response elements (IREs), confirming the potential for regulation of GLUT5 expression by insulin. Promoter deletion studies utilising luciferase reporter constructs in this system support a possible role for insulin in regulating the activity of the GLUT5 promoter via these putative IREs (Hajduch, Litherland, Turban, Brot-Laroche & Hundal, in press). Q5

The present findings in Zucker rat adipose tissue suggest that insulin may provide a tonic stimulus for GLUT5 expression, in common with many other adipocyte genes, for instance glycerol 3-phosphate dehydrogenase [28], leptin [29] and hexokinase II [30]. We have reported previously that GLUT5 expression in rat skeletal muscle is not controlled by substrate availability (which is a controlling factor for intestinal and kidney GLUT5 expression [13, 14]), since the expression of this transporter in muscle cells was not altered by fructose feeding [8]. A recent report has shown that there is no difference in the expression of GLUT5 in the small intestine of obese, compared to lean Zucker rats [31], although the thiazolidinedione insulin sensitiser [32], troglitazone, appeared to cause a post-translational reduction in intestinal GLUT5 levels in obese rats. However, there is a precedent for the hormonal modulation of GLUT5 expression, which

appears to be stimulated by thyroid hormone in Caco-2 cells [16].

Our data concerning the utilisation rates of fructose to lipid synthesis or lactate production respectively indicate that, while glucose is likely to be the major source of carbohydrate for the glycerol backbone of storage triglyceride, especially in the post-prandial state where serum insulin levels are elevated, adipocytes may well utilise fructose as an oxidative fuel to utilise glucose for lipid synthesis more efficiently. This is consistent with the idea of GLUT5 expression being regulated by prevailing insulin levels.

The recent findings of Kawasaki *et al*. [3] that serum fructose levels are elevated in diabetic patients bring particular pertinence to the present work with regard to diabetic complications. It is possible that diminished adipocyte GLUT5 expression plays a significant role in reduced fructose utilisation in diabetes, resulting in elevated serum fructose. In turn, elevated serum fructose could be an important factor in the progression of diabetic complications [3]. Given our findings that GLUT5 expression in rat L6 muscle cells is regulated positively by insulin (Hajduch, Litherland, Turban, Brot-Laroche & Hundal, in press), skeletal muscle and adipose tissue may both play a role in controlling serum fructose levels.

In conclusion, we present evidence that surface density of the GLUT5 fructose transporter is regulated during age, obesity and insulin resistance in Zucker rat adipocytes. Our data also suggest that the uptake and metabolism of dietary fructose by adipocytes may be impaired during insulin resistance. Clearly, further work is required to establish whether there is a direct effect of insulin on the level of GLUT5 expression in adipocytes, as we have recently established in L6 skeletal muscle cells (Hajduch, Litherland, Brot-Laroche & Hundal, in press). Furthermore, it would be of value to ascertain whether improvement of insulin sensitivity using drugs such as thiazolidinediones [32] could restore the depressed GLUT5 abundance observed during insulin resistance. Such studies may shed light on the precise role of fructose metabolism in adipose tissue physiology, and tell us whether the disruption of fructose utilisation during insulin resistance has a significant role to play in the progression of diabetic complications.

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Queries

Q1. Au: Kindly check whether the edited sentence conveys the intended meaning.
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