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Differential regulation of the HepG2 and adipocyte/muscle glucose transporters in 3T3L1 adipocytes

Effect of chronic glucose deprivation

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Glucose transport in 3T3L1 adipocytes is mediated by two facilitated diffusion transport systems. We examined the effect of chronic glucose deprivation on transport activity and on the expression of the HepG2 (GLUT 1) and adipocyte/muscle (GLUT 4) glucose transporter gene products in this insulin-sensitive cell line. Glucose deprivation resulted in a maximal increase in 2-deoxyglucose uptake of 3.6-fold by 24 h. Transport activity declined thereafter but was still 2.4-fold greater than the control by 72 h. GLUT 1 mRNA and protein increased progressively during starvation to values respectively 2.4- and 7.0-fold greater than the control by 72 h. Much of the increase in total immunoreactive GLUT 1 protein observed later in starvation was the result of the accumulation of a non-functional or mistargeted 38 kDa polypeptide. Immunofluorescence microscopy indicated that increases in GLUT 1 protein occurred in presumptive plasma membrane (PM) and Golgi-like compartments during prolonged starvation. The steady-state level of GLUT 4 protein did not change during 72 h of glucose deprivation despite a > 10-fold decrease in the mRNA. Subcellular fractionation experiments indicated that the increased transport activity observed after 24 h of starvation was principally the result of an increase in the 45–50 kDa GLUT 1 transporter protein in the PM. The level of the GLUT 1 transporter in the PM and low-density microsomes (LDM) was increased by 3.9- and 1.4-fold respectively, and the GLUT 4 transporter content of the PM and LDM was 1.7- and 0.6-fold respectively greater than that of the control after 24 h of glucose deprivation. These data indicate that newly synthesized GLUT 1 transporters are selectively shuttled to the PM and that GLUT 4 transporters undergo translocation from an intracellular compartment to the PM during 24 h of glucose starvation. Thus glucose starvation results in an increase in glucose transport in 3T3L1 adipocytes via a complex series of events involving increased biosynthesis, decreased turnover and subcellular redistribution of transporter proteins.

INTRODUCTION

Glucose transport in insulin-sensitive tissues is a control point for the regulation of blood glucose levels, and a possible target for the derangement of glucose homeostasis in certain disease states such as type II diabetes [1]. Insulin-sensitive tissues (fat and muscle) express at least two distinct glucose transporter species [2,3]. The adipocyte/muscle-specific transporter [4–8] (GLUT 4 protein [9]) is present at much greater abundance than the HepG2 transporter [10,11] (GLUT 1 protein [12]) in muscle and fat, and is thus likely to account for most of the insulin-sensitive hexose uptake in these tissues. Glucose transport in muscle and fat is acutely augmented by insulin, which causes a redistribution of intracellular transporters to the plasma membrane [13–15]. Both the GLUT 1 and GLUT 4 transporters are primarily intracellular in the absence of insulin, and both are capable of insulin-induced translocation to the plasma membrane [3,16].

Glucose transporters in muscle and fat are also subject to chronic regulation, which may be important in mediating the insulin resistance observed *in vivo* under certain conditions. However, the actual factors responsible for the long-term regulation of glucose transport *in vivo* are poorly defined. For example, fasted or streptozotocin-treated hypoinsulinaemic rats exhibit decreased levels of GLUT 4 protein in fat [17–20]. Because blood glucose levels change in opposite directions under these two conditions, it was suggested that hypoinsulinaemia *per se* was responsible for the decreased expression. However, because of the complexity of changes, both known and unknown, that occur in the whole organism during the perturbation of glucose homoeostasis by various treatments, it is impossible to draw firm conclusions about the proximal stimulus for the regulation of transport in these studies *in vivo*. Studies *in vitro* indicate that the expression of the GLUT 1 protein, but not of the GLUT 4 protein, is directly responsive to insulin in cultured 3T3L1 adipocytes [21].

Here we describe the direct effect of altered glucose levels on the expression of the GLUT 1 and GLUT 4 genes in 3T3L1 adipocytes. We demonstrate that glucose deprivation affects expression of the two glucose transporter mRNAs in opposite directions, increasing GLUT 1 mRNA and decreasing GLUT 4 mRNA, whereas the same treatment appears to stabilize both proteins in the plasma membrane. The increase in transport activity observed during starvation in these cells appears to be due primarily to an increased level of the GLUT 1 transporter and to the translocation of both the GLUT 1 and GLUT 4 proteins from an intracellular compartment to the plasma membrane.

Abbreviations used: GLUT 4 protein, glucose transporter cloned from adipose/muscle tissue; GLUT 1 protein, glucose transporter cloned from HepG2 cells/rat brain; DMEM, Dulbecco's modified Eagle's medium; PM fraction, plasma membrane fraction; LDM fraction, low-density microsomal fraction; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate

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EXPERIMENTAL

Cell culture

3T3L1 preadipocytes were maintained and differentiated as described previously [21]. The conversion of the fibroblast-like to adipocyte-like cells was greater than 95%, as judged by the morphological appearance of the cells by phase-contrast microscopy. Glucose deprivation experiments were initiated 9–11 days after starting differentiation by changing the medium to Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) dialysed fetal calf serum, 2 mM-glutamine, 50 units of penicillin/ml, 50 μg of streptomycin/ml, and the concentrations of glucose and other carbon compounds as indicated in the Figure legends. During glucose deprivation the medium was changed every 24 h. Adipocytes were incubated for 1 h in the same medium lacking serum prior to subcellular fractionation. This removes insulin-growth factor I, which has an insulin-omimetic action in promoting translocation of intracellular transporters to the plasma membrane. Hormone-treated cells were incubated in medium containing 100 nM-insulin for 20 min prior to subcellular fractionation or measurement of 2-deoxyglucose uptake.

2-Deoxyglucose uptake, immunoblot and Northern blot analyses

These methods were conducted exactly as described previously [21]. The specificity of the cDNA and antibody probes has been described [4,21]. 3-*O*-Methylglucose transport assays at 30 s time points were conducted periodically to confirm that the 2-deoxyglucose uptake measurements reflected true transport rates (results not shown).

Blots were quantified either by excision of bands and counting of radioactivity in a γ-radiation counter or liquid scintillation spectrometer, or by scanning autoradiograms using an LKB laser scanner. Each of the quantification methods was conducted within the linear response range.

Subcellular fractionation

Plasma membrane (PM) and low-density microsomal (LDM) subcellular fractions were isolated from cultured adipocytes as described by Simpson *et al.* [22]. Yields of PM and LDM were approx. 4 and 15 pg of protein per cell for control and approx. 3 and 12 pg of protein per cell for 24 h-glucose-starved adipocytes respectively. Preliminary experiments were conducted to determine the distribution of a PM marker in the subcellular fractions. Cells were incubated for 5 min on ice with ^{125}I-labelled wheat-germ agglutinin, washed extensively and then subjected to the fractionation procedure. Control and 24 h-glucose-starved cells exhibited 12.4- and 13.7-fold enrichment respectively of the marker in the PM fraction relative to the total homogenate, indicating that the procedure was effective for enriching the PM and that starvation for 24 h did not affect the fractionation characteristics of the cells. Assuming that all of the ^{125}I label represented PM protein, the recovery of total plasma membrane in the PM fraction was approx. 25%. Approx. 25% of the total label was recovered in the LDM fraction. Because the LDM fraction contains approx. 4-fold more protein than the PM fraction, this represents a 6–7% contamination of LDM with PM. The extent of contamination of the PM fraction with membranes derived from the intracellular glucose-transporter-containing pool(s) is impossible to determine, because of the lack of a specific marker for this organelle(s) other than the transporter itself.

Immunofluorescence

Preadipocytes were grown and then differentiated on glass coverslips before reaching confluence. Differentiated adipocytes were fixed in serum-free DMEM containing 4% paraformaldehyde for 20 min. Cells were then washed three times in phosphate-buffered saline (PBS; 150 mM-NaCl/10 mM-sodium phosphate, pH 7.2) and excess fixative was quenched by incubation in PBS containing 100 mM-glycine for 15 min. Cells were permeabilized with 0.1% Triton X-100 in PBS for 15 min, washed three times with PBS and then incubated in PBS containing 2% horse serum for 20 min. After washing three times with PBS, coverslips were incubated with 5 μg of R493 IgG/ml [4] in 0.2% horse serum at 4 °C overnight. Cells were then washed three times in PBS and incubated for 50 min in fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit antibody (5 μg/ml; Cappel, West Chester, PA, U.S.A.). FITC-conjugated antibody was purified by Sephadex G-50 chromatography to remove residual free FITC. After incubation with secondary antibody, the cells were washed for 15 min in PBS and mounted in 90% glycerol containing 1% n-propylgallate.

Samples were imaged with a Zeiss Axioplan microscope equipped with a Bio-Rad MRC-500 laser confocal imaging system. Images were digitally enhanced and scaled with the Bio-Rad MRC-500 system software. Specificity was demonstrated by lack of staining in the absence of primary antibody.

RESULTS

3T3L1 adipocytes deprived of glucose for 24 h appeared to be minimally affected with respect to cellular size and general morphology. Starvation for longer periods of time, however, resulted in a progressive diminution in the size of the cells, which was reflected in decreased protein and RNA contents per cell (Figs. 1*a* and 1*b*). Control cells also exhibited some decrease in total protein and RNA, which was caused by the switch from non-dialysed to dialysed fetal calf serum. Glucose transport activity, as measured by uptake of 2-[^{3}H]deoxyglucose, increased progressively with starvation by a maximum of $\sim$ 3.6-fold at 24 h, and declined thereafter (Fig. 2*a*). The decline in activity after 24 h was steeper when the data were normalized on a per cell basis, because of the decrease in total adipocyte protein observed at later time points. The induction of transport activity was a function of glucose concentration in the medium. The induction effect at 48 h was maximal at between 0.1 and 1 mM-glucose (Fig. 2*b*). This may be due to aberrant glycosylation of the transporter during total glucose deprivation (see below). The specificity of the repressive effect of high glucose concentrations was demonstrated by substituting fructose for glucose in the medium. Fructose at a concentration of up to 20 mM was incapable of preventing the increase in transport activity observed in the absence of glucose. Rather, fructose augmented the increase in transport activity caused by total glucose deprivation (Fig. 2*b*).

The GLUT 1 and GLUT 4 transporters are expressed at similar abundance in 3T3L1 adipocytes (approx. 3:1 molar ratio; D. M. Calderhead & G. E. Lienhard, personal communication). Thus changes in the expression of either protein could conceivably influence the rate of glucose uptake. *C*-Terminal-peptide-specific antibodies were used to quantify relative changes in the levels of both transporters during the course of glucose deprivation (Figs. 3*a* and 3*b*). As previously described [21], the two transporters in control adipocytes migrated as diffuse bands of between 45 and 50 kDa, with the GLUT 4 transporter exhibiting slightly lower mobility. Glucose deprivation for up to 72 h had no apparent effect on the relative mobility or abundance of the GLUT 4 transporter. The 45–50 kDa GLUT 1 transporter polypeptide increased in relative abundance up to 72 h of starvation. Additionally, a $\sim$ 38 kDa polypeptide species that reacted with the GLUT 1 antibody had appeared by 12 h of starvation. This species also progressively increased in abundance up to the 72 h time point. The 38 kDa polypeptide co-migrated

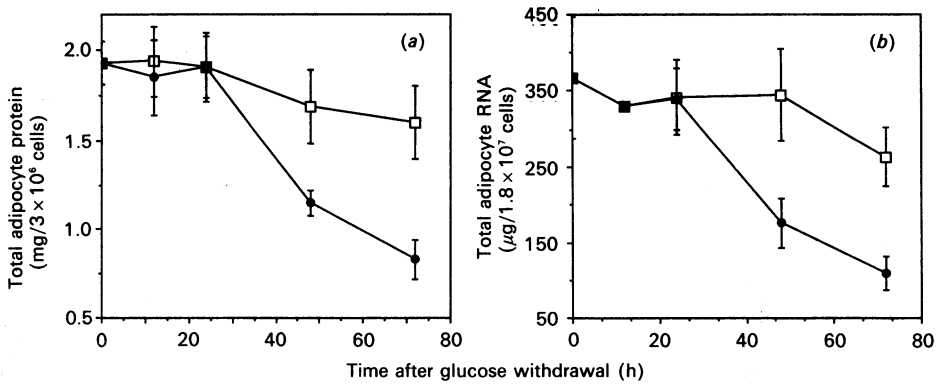

Fig. 1. Changes in total adipocyte protein and RNA during 72 h of glucose deprivation

Adipocytes were incubated in medium containing 20 mM-glucose (□) or no glucose (●) for up to 72 h. At various time points total cellular protein (*a*) and RNA (*b*) were determined. Protein assays were conducted using the BCA Reagent Kit (Pierce Biochemicals). RNA values represent the yield of total RNA after guanidinium thiocyanate/CsCl purification of total cellular RNA. The decreases in total protein and RNA per cell in the control adipocytes were caused by the change from non-dialysed to dialysed fetal calf serum. Values represent means ± S.E.M. for four to seven independent experiments.

with an *N*-glycanase-treated 45–50 kDa polypeptide (results not shown), and thus most likely represents a non-glycosylated or aberrantly glycosylated form of the transporter. A similar observation has been reported for the HepG2/brain-type transporter in glucose-starved mouse 3T3-C2 fibroblasts [23]. Occasionally the 45–50 kDa band for either transporter species resolved into a doublet. The doublets probably correspond to polypeptide conformers [2]. Note that a poor correlation was observed between transport activity during prolonged starvation and the level of either total GLUT 1 protein or the 45–50 kDa band. Activity decreased between 24 and 72 h, whereas the protein levels increased.

Glucose refeeding for 24 h after 48 h of starvation reversed the increase in the 45–50 kDa GLUT 1 species and dramatically reduced the level of the 38 kDa polypeptide. Interestingly, the presence of 20 mM-fructose during 48 h of glucose deprivation completely suppressed the appearance of the 38 kDa polypeptide and augmented the increase in the 45–50 kDa species. This is consistent with the increased transport activity observed in the presence of fructose. However, 20 mM-pyruvate present during the same time interval had little effect on the abundance of either polypeptide.

We next investigated the subcellular distribution of glucose transporters during starvation. We examined the levels of both transporter species in LDM- and PM-enriched subcellular fractions at 24 h after the commencement of glucose deprivation, at which time the maximal increase in transport activity was observed. The LDM fraction contains the intracellular pool of transporters that translocate to the plasma membrane in response to insulin [13,14,22]. A representative immunoblot is shown in Fig. 4(*a*) and a quantitative analysis of several independent experiments is presented in Fig. 4(*b*). Control cells maintained in standard medium containing 20 mM-glucose contained 29 ± 4% and 10 ± 5% of the total GLUT 1 and GLUT 4 transporter protein respectively in the PM fraction (means ± S.D., four experiments). Thus a greater proportion of total GLUT 1 than of total GLUT 4 transporter protein appeared to be in the plasma membrane under these basal conditions. Glucose deprivation for 24 h resulted in 3.9- and 1.5-fold increases in the content of GLUT 1 transporters in the PM and LDM respectively.

The PM and LDM contents of GLUT 4 transporters were 1.7 and 0.6 times control values respectively. Thus glucose deprivation resulted in the translocation of intracellular GLUT 4 transporters to the plasma membrane and the selective shuttling

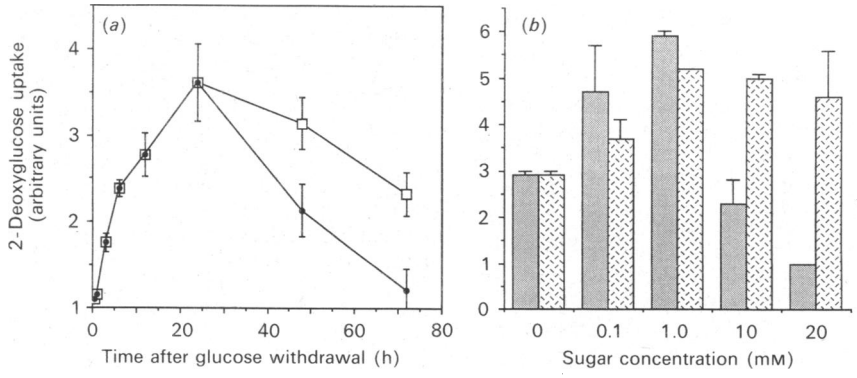

Fig. 2. Glucose transport activity as a function of time after glucose withdrawal and sugar concentration

Adipocytes were incubated in medium containing (*a*) no glucose or (*b*) the indicated concentration of glucose (▨) or fructose (▨) for up to 72 h (*a*) or for 48 h (*b*). The rate of 2-deoxyglucose uptake was then determined as described [21]. Values represent means ± S.E.M. or means ± range for two to nine independent experiments. Within experiments uptake measurements were conducted in triplicate. In (*a*), uptake per total cellular protein is indicated by □ and uptake per cell is indicated by ●. Values in (*b*) are normalized to total cellular protein. In both cases, control values are equal to 1 unit.

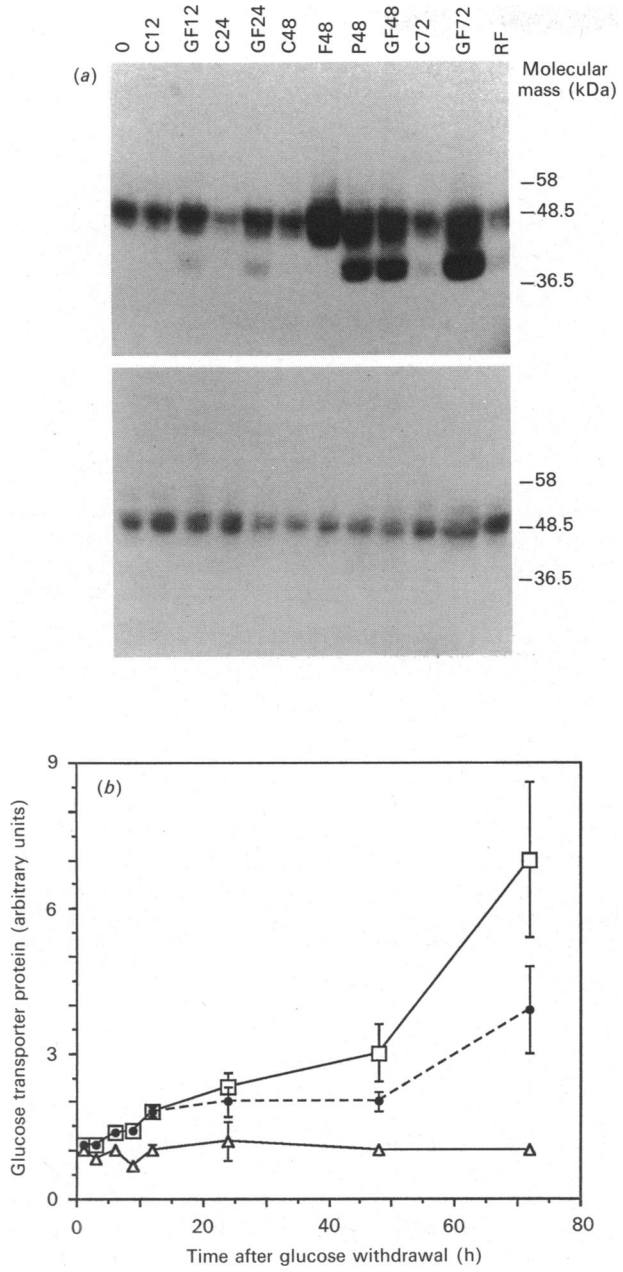

Fig. 3. Levels of GLUT 1 and GLUT 4 transporter proteins during glucose deprivation

(*a*) Representative immunoblots. Adipocytes were incubated in medium containing dialysed fetal calf serum and no glucose for 12 (GF12), 24 (GF24), 48 (GF48) or 72 h (GF72), or in medium containing no glucose but 20 mM-fructose (F48) or 20 mM-pyruvate (P48) for 48 h. Controls were incubated in medium containing dialysed fetal calf serum and 20 mM-glucose for 12 (C12), 24 (C24), 48 (C48) or 72 h(C72). 0, time zero control; RF, adipocytes were re-fed with 20 mM-glucose for 24 h after starvation for 72 h. Total protein was extracted from adipocytes and 100 μg portions were subjected to SDS/PAGE using a 10% resolving gel as described [21]. The gels were then subjected to immunoblot analysis using R493 antiserum specific for the GLUT 1 transporter (upper panel) or R820 antiserum specific for the GLUT 4 transporter (lower panel). (*b*) Quantification of changes in total GLUT 1 (□), 45–50 kDa GLUT 1 (●), or GLUT 4 (△) protein during glucose deprivation. Values represent means ± S.E.M. or means ± range of two to six independent experiments, except for the 3 and 9 h time points which are from single experiments. Missing error bars for other time points indicate that the error was too small to be shown for that point. The control value = 1 unit.

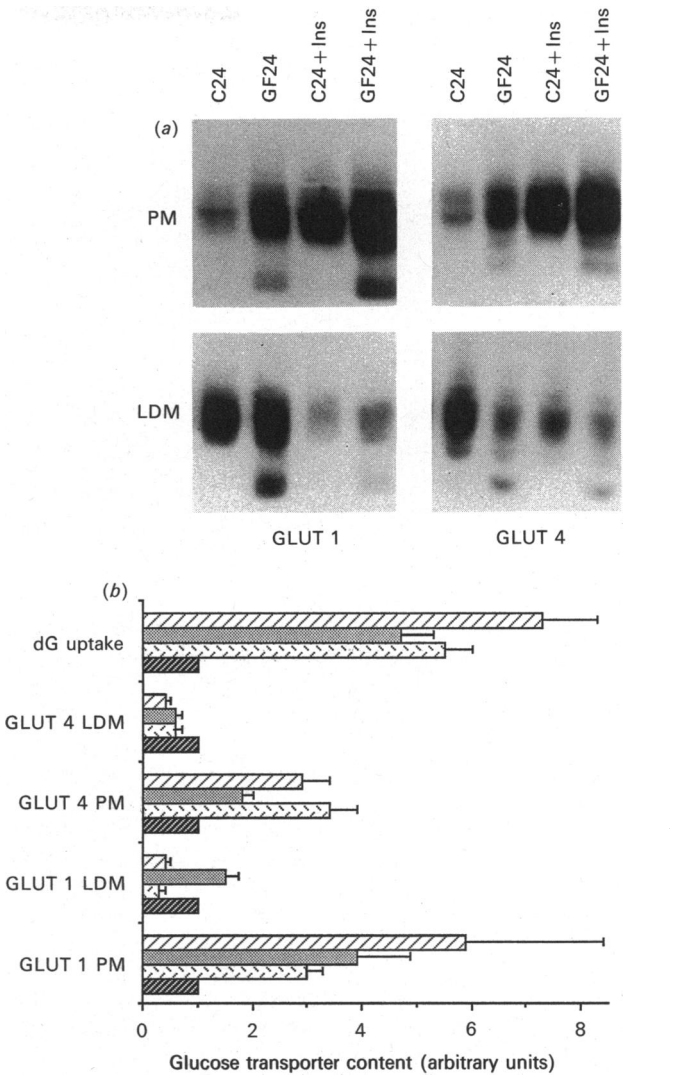

Fig. 4. Levels of GLUT 1 and GLUT 4 transporter proteins in PM and LDM subcellular fractions from glucose-starved adipocytes

Starved adipocytes were maintained in glucose-free medium containing 10% dialysed fetal calf serum for 23 h and then changed to the same medium lacking serum for 1 h. Control cells were treated identically, except that the media contained 20 mM-glucose. Cells treated with 100 nM-insulin (Ins) were exposed to the hormone for the final 20 min of the 1 h incubation period. (*a*) Representative immunoblot. Portions of each sample (50 μg) were analysed by SDS/PAGE and immunoblotting as described in the legend to Fig. 3. The blot represented in the lower right panel (GLUT 4 LDM) was exposed to film for one-third of the time of the other blots in order to allow accurate quantification. Abbreviations are the same as in Fig. 3. (*b*) Quantitative representation of the results of independent subcellular fractionation experiments. ▨, Control cells; ▩, cells incubated in glucose-free medium; ⊠, control cells treated acutely with 100 nM-insulin; ▨, cells incubated in glucose-free medium and then treated acutely with 100 nM-insulin. dG uptake, 2-deoxyglucose uptake; other abbreviations are as in the legend to Fig. 3. Values represent means ± S.E.M. or means ± range of two to four independent experiments. The control value = 1 unit.

of newly synthesized GLUT 1 transporters and/or the translocation of pre-existing GLUT 1 transporters to the plasma membrane. Acute insulin stimulation of starved cells resulted in further increases in the PM content of the GLUT 1 and GLUT 4 transporters (5.8 and 3.0 times control respectively), indicating that the translocatable pools of both transporters were not

Table 1. Correlation between glucose transport activity and plasma membrane content of transporter proteins

3T3L1 adipocytes were maintained for 23 h in DMEM containing 10% dialysed fetal calf serum and either no added glucose (Starved, Starved + insulin) or 20 mM-glucose (Basal, Insulin). The adipocytes were then incubated in the same medium lacking serum for 1 h prior to subcellular fractionation or measurement of 2-deoxyglucose uptake (Activity). Insulin-treated cells were exposed to 100 nM-insulin for the final 20 min of the serum-free incubation period. The data are taken from Fig. 4. All values have been normalized to the plasma membrane content of total GLUT 1 + GLUT 4 glucose transporters (= 1.0) or the rate of 2-deoxyglucose uptake (= 1.0) measured in basal cells. A 3:1 molar ratio of total cellular GLUT 1/GLUT 4 was assumed. The 38 kDa GLUT 1 polypeptide comprised 30% of the total plasma membrane GLUT 1 immunoreactive protein in starved cells.

Treatment	GLUT 1		GLUT 4	GLUT 1 + GLUT 4	45–50 kDa + GLUT 4	Activity
	Total	45–50 kDa				
Basal	0.90	0.90	0.10	1.0	1.0	1.0
Starved	3.51	2.45	0.17	3.68	2.62	4.7
Insulin	2.70	2.70	0.34	3.04	3.04	5.5
Starved + insulin	5.22	3.65	0.30	5.52	3.95	7.3

totally depleted after 24 h of glucose starvation. The changes in the PM content of transporter proteins was reflected by qualitatively similar changes in transport activity under these conditions (see Table 1 and the Discussion section). The percentage of total GLUT 1 transporter represented by the 38 kDa polypeptide in starved cells was the same in both the PM and LDM fractions (30% and 28% respectively) and did not change significantly in either fraction upon insulin stimulation (33% and 30% respectively). This suggests that the glycosylation state of the transporter did not influence its subcellular targeting or its capacity to translocate to the PM in response to insulin after 24 h of glucose deprivation. Unfortunately, quantification of transporters in subcellular fractions from cells deprived of glucose for more than 24 h was unreliable, because of changes in the fractionation characteristics of the chronically starved adipocytes.

The increase in total GLUT 1 transporter protein shown by the immunoblot analyses was consistent with the results of immunofluorescence studies using a GLUT 1 *C*-terminal-peptide-specific antibody (Fig. 5). A gradual increase in fluorescence intensity was observed in adipocytes immunostained after between 0 and 72 h of glucose deprivation. Adipocytes exhibited perimeter staining suggestive of PM reactivity. This apparent PM staining increased in intensity throughout the starvation period. Adipocytes starved for 24 h showed perinuclear staining, probably corresponding to a distinct intracellular organelle,

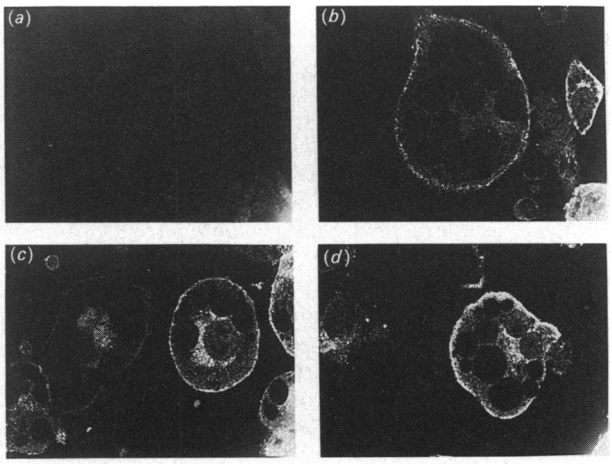

Fig. 5. Immunofluorescence microscopy of adipocytes during glucose starvation

Adipocytes grown on coverslips were incubated in glucose-free medium for 0 (*a*), 24 (*b*), 48 (*c*) or 72 h (*d*) and then processed for immunofluorescence microscopy as described in the Experimental section. The primary antibody used (R493) was specific for the GLUT 1 transporter.

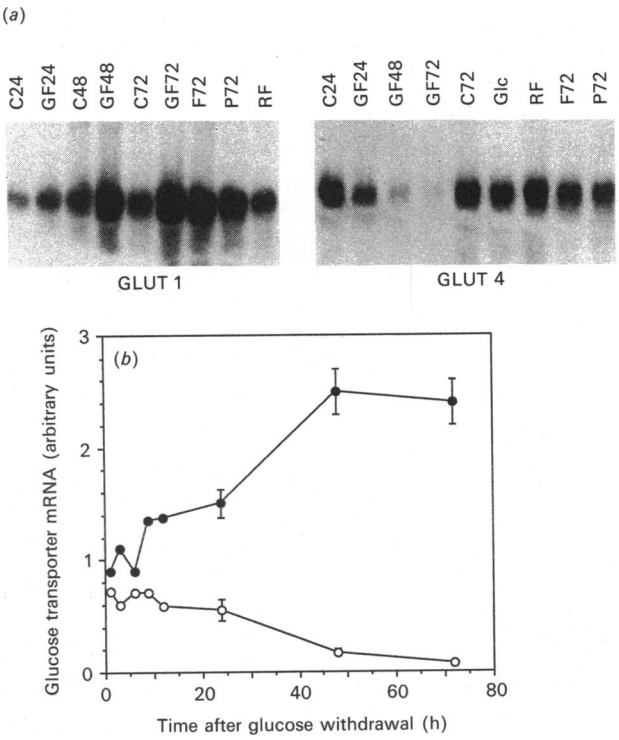

Fig. 6. Levels of GLUT 1 and GLUT 4 mRNAs during glucose deprivation

Adipocytes were maintained in medium containing dialysed fetal calf serum and either no glucose or 20 mM-glucose (control) for up to 72 h. Total RNA was isolated from the cells and 10 μg of each sample was subjected to Northern blot analysis as described [21] using cDNA probes specific for the GLUT 1 or GLUT 4 mRNAs. (*a*) Representative Northern blots. Glc, adipocytes were maintained for 72 h in medium containing 1 mM-glucose; other abbreviations are as described in the legend to Fig. 3. Note that an increase in GLUT 1 mRNA occurred in control cells between 24 and 48 h due to the change from non-dialysed to dialysed calf serum at time zero. (*b*) Quantitative presentation of the results of independent Northern blot experiments; ●, GLUT 1; ○, GLUT 4. Values represent means ± S.E.M. or means ± range of two to eight independent experiments.

possibly the Golgi. The perinuclear staining increased considerably between 24 and 72 h of glucose deprivation. No differences were observed among the immunofluorescent images of starved or control adipocytes stained with the GLUT 4 *C*-terminal-peptide-specific antibody (results not shown).

Northern blots were performed to determine whether changes in the level of the GLUT 1 transporter protein were due to increased levels of mRNA (Fig. 6). Glucose deprivation resulted in a progressive increase in the relative level of GLUT 1 transporter mRNA to a level 2.5-fold greater than the control value (20 mM-glucose) by 48 h of starvation. However, the actual GLUT 1 transporter mRNA content by 72 h of starvation may have been lower than in the control cells due to a 3.6-fold decrease in total RNA per cell. The relative increase in mRNA level did not quantitatively parallel the relative increase in total GLUT 1 transporter protein during starvation. An ~ 7-fold increase in protein was observed after 72 h of deprivation, whereas the mRNA had only increased by ~ 2.5-fold. Surprisingly, the steady-state level of the GLUT 4 transporter mRNA declined steadily during glucose deprivation, to a level < 8% of that of control adipocytes by 72 h, despite there being no change in the steady-state level of the corresponding protein relative to total cellular protein. These data suggest that both transporter proteins were selectively spared the enhanced turnover of total cellular protein that occurred in the starved adipocytes.

Glucose refeeding for 24 h after 72 h of glucose deprivation largely reversed the increase in GLUT 1 transporter mRNA and the decrease in GLUT 4 transporter mRNA, returning their levels to 1.2 and 0.8 times that of the control (average of two experiments). The presence of 20 mM-fructose or -pyruvate during glucose deprivation minimally suppressed the increase in GLUT 1 transporter mRNA, resulting in levels 2.1- and 1.7-fold greater than the control at 72 h. Fructose and pyruvate largely prevented the decrease in GLUT 4 transporter mRNA during starvation, resulting in levels 0.7 and 0.6 times those of the control at 72 h.

DISCUSSION

The purpose of undertaking these experiments was to determine the direct effect of glucose deprivation on the expression of the GLUT 1 and GLUT 4 gene products within the same insulin-sensitive cell line, and to correlate changes in transport activity with changes in the levels of the two transporter proteins. A striking observation was the lack of correlation between transport activity and total transporter protein which was present at later time points during starvation. Total GLUT 1 immunoreactive protein continued to accumulate between 24 and 72 h, despite a decline in transport activity. Part of the explanation may lie in the increasing proportion of the 38 kDa polypeptide comprising total GLUT 1 protein. This presumptive non-glycosylated or aberrantly glycosylated form of the transporter may be non-functional or improperly targeted during its biosynthesis. This possibility has been proposed to explain a similar discrepancy between transporter protein and activity levels during starvation of murine 3T3-C2 fibroblasts [23]. This is unlikely to be the only explanation, however, because the 45–50 kDa protein also increased at times when transport activity was decreasing. It is possible that some of the protein migrating in the 45–50 kDa region is also improperly modified and therefore non-functional or mistargeted. Although technical difficulties prevented quantification of transporter protein in the plasma membrane by subcellular fractionation at later time points, qualitative impressions from the immunofluorescence experiments suggest that the increase in GLUT 1 protein is greater in the cytoplasmic compartment (perinuclear staining) than in the plasma membrane (perimeter staining) between 24 and 72 h of starvation. This suggests that an increasing proportion of transporter protein fails to reach the plasma membrane during protracted starvation. Additionally, it is possible that the 38 kDa polypeptide and the properly modified 45–50 kDa species are both mistargeted during prolonged starvation due to a general disruption in the biosynthetic machinery. This view is supported by the subcellular fractionation experiments conducted on adipocytes starved for 24 h, which suggest that the 38 kDa polypeptide is not inherently excluded from the plasma membrane at earlier times in starvation.

The subcellular fractionation data can be used to evaluate whether a correlation exists between the amount of total transporter protein in the plasma membrane and transport activity at the 24 h time point. The data compiled in Table 1 indicate that transport activity is best correlated with the amount of 45–50 kDa + GLUT 4 or 45–50 kDa protein in the plasma membrane (r^2 = 0.996, 0.995, 0.924, 0.872 and 0.704 for transport activity as a function of 45–50 kDa + GLUT 4, 45–50 kDa, GLUT 1 + GLUT 4, GLUT 1 and GLUT 4 respectively). In this case the ratio of transporter protein/activity is strikingly similar for all three treatment conditions (0.54–0.55). This analysis suggests that the 38 kDa polypeptide may be non-functional or not exposed to the extracellular milieu, despite its presence in the PM fraction. The transporter protein activity ratio may reflect the presence of contaminating LDM in the PM fraction, or an increase in the intrinsic activity of one or both transporters during starvation and insulin administration. Cross-contamination between the subcellular fractions is unavoidable, and considering that there is approx. 4-fold more protein in the LDM than in the PM fraction, a relatively small amount of total LDM contaminating the PM would result in a significant underestimation of the extent of transporter redistribution.

The increase in transporter activity during glucose deprivation is thus most probably due to an increase in the plasma membrane content of both transporter species, with the GLUT 1 protein making a greater quantitative contribution. The increased steady-state level of the GLUT 1 transporter appears to be the result of an increase in its biosynthetic rate relative to total protein, due to an increase in the mRNA, and of a decreased turnover of the protein. Increased stability of the protein is suggested by the large discrepancy between the change in mRNA and protein at later time points. Previous studies in which transport activity was measured in the presence of cycloheximide are also consistent with both increased biosynthesis and decreased turnover contributing to the glucose deprivation effect [24]. It should be emphasized, however, that the correlation analysis does not necessarily indicate a cause and effect relationship, and that the only firm conclusion that can be drawn from our data is that no simple relationship exists between levels of either transporter isoform and transport activity during glucose deprivation.

The changes in GLUT 1 and GLUT 4 mRNA levels that occur during glucose deprivation are consistent with the proposed physiological roles for these two transporters *in vivo* [1]. The GLUT 1 transporter is responsible for supplying many cells with their basal glucose requirement for energy metabolism and for anabolic reactions requiring sugars. It is thus reasonable that expression of the GLUT 1 gene should be induced when glucose is in short supply. The GLUT 4 transporter, on the other hand, appears to be primarily involved in decreasing the level of glucose in the blood by facilitating its uptake and storage in muscle and fat. This activity would be counterproductive when blood glucose is low. The decrease in GLUT 4 mRNA during starvation of 3T3L1 cells is consistent with changes that occur in the level of the mRNA in the adipose tissue of fasted rats [18–20].

However, the GLUT 4 protein level also declines in the adipocytes of fasted rats. Unlike our observations in 3T3L1 adipocytes, expression of the GLUT 1 gene is unaffected in adipose or muscle tissue of fasted rats [17–20]. However, the GLUT 1 mRNA and protein are both induced in cultured neurons [25] and L6 myocytes [26] which are starved of glucose.

The differences in the regulation of these two transporter species *in vitro* and *in vivo* are difficult to evaluate. They may simply reflect secondary and tertiary changes that occur *in vivo* during disruption of glucose homeostasis, species differences, or possibly genetic changes that occur in the cell line during adaptation to growth *in vitro*. Further studies are clearly required to elucidate the mechanisms of GLUT 1 and GLUT 4 gene regulation during fluctuations in glucose levels. However, the studies reported herein clearly demonstrate that both genes are capable of responding directly to a lack of glucose with alterations in expression.

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