Stimulation of glucose transport by thyroid hormone in 3T3-L1 adipocytes: increased abundance of GLUT1 and GLUT4 glucose transporter proteins

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Abstract

In 3T3-L1 adipocytes we have examined the effect of tri-iodothyronine (T_3) on glucose transport, total protein content and subcellular distribution of GLUT1 and GLUT4 glucose transporters. Cells incubated in T_3-depleted serum were used as controls. Cells treated with T_3 (50 nM) for three days had a 3·6-fold increase in glucose uptake ($P<0·05$), and also presented a higher insulin sensitivity, without changes in insulin binding. The two glucose carriers, GLUT1 and GLUT4, increased by 87% ($P<0·05$) and 90% ($P<0·05$), respectively, in cells treated with T_3. Under non-insulin-stimulated conditions, plasma membrane fractions obtained from cells exposed to T_3 were enriched with both GLUT1 ($3·29 \pm 0·69$ vs $1·20 \pm 0·29$ arbitrary units (A.U.)/5 μg protein, $P<0·05$) and GLUT4 ($3·50 \pm 1·16$ vs $0·82 \pm 0·28$ A.U./5 μg protein, $P<0·03$). The incubation of cells with insulin produced the translocation of both glucose transporters to plasma membranes, and again cells treated with T_3 presented a higher amount of GLUT1 and GLUT4 in the plasma membrane fractions ($P<0·05$ and $P<0·03$ respectively). These data indicate that T_3 has a direct stimulatory effect on glucose transport in 3T3-L1 adipocytes due to an increase in GLUT1 and GLUT4, and by favouring their partitioning to plasma membranes. The effect of T_3 on glucose uptake induced by insulin can also be explained by the high expression of both glucose transporters.

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Introduction

The enhancement of the metabolic rate by thyroid hormone (Barker & Klittgard 1952) implies an increase in the requirement of cellular glucose. Thyroid hormone has been shown to increase glucose disposal in humans and in animals (Hardeveld & Kassenaar 1977, Sandler *et al.* 1983, Foss *et al.* 1990, Casla *et al.* 1993) and to stimulate glucose transport in cultured cells (Segal & Gordon 1977, Weinstein *et al.* 1990). The cellular glucose uptake by facilitated diffusion is mediated by a family of homologous glucose transporter proteins, of which the insulin-responsive glucose transporter (GLUT4) is expressed primarily in adipose tissue and skeletal and cardiac muscle, and the GLUT1 glucose transporter is expressed in several tissues as well as in cultured cell lines. Insulin stimulates the rapid translocation of glucose transporters from an intracellular location to the plasma membrane in adipocytes and muscle cells; this process seems to be the major mechanism for the acute stimulation of glucose transport by insulin in these cells (for review see Gould & Holman 1993).

The stimulatory effect of thyroid hormone on glucose uptake has been related to an increase in glucose transporter proteins (Casla *et al.* 1993). The excess of thyroid hormone affects the two glucose carrier isoforms, GLUT1 and GLUT4, in a different way depending on specific tissues. It has been shown that in experimental hyperthyroidism GLUT4 is selectively increased in skeletal muscle (Weinstein *et al.* 1991), whereas both GLUT1 and GLUT4 are increased in adipocytes (Matthaei *et al.* 1995). However, it has also been reported that there is a direct inhibitory effect of tri-iodothyronine (T_3) on glucose transport in isolated rat adipocytes (Goto *et al.* 1997).

In this study, we aimed to investigate the direct effect of thyroid hormone on the cell content of the glucose transporters GLUT1 and GLUT4 as well as on their subcellular distribution, in order to have a better understanding of the reported stimulatory action of thyroid hormone on cell glucose uptake. For this purpose, we have used 3T3-L1 adipocytes exposed to T_3. The mouse 3T3-L1 cell line can be differentiated from fibroblasts to adipocytes. This process is accompanied by the acquisition of the insulin-dependent glucose transport and the

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expression of GLUT4 (de Herreros & Birnbaum 1989). Mature 3T3-L1 adipocytes express both GLUT4 and GLUT1 transporter proteins in similar abundance (Calderhead *et al.* 1990), thus providing a useful model system for studying these glucose carriers within the same cellular environment.

Materials and Methods

Materials

Murine-derived 3T3-L1 fibroblasts were obtained from the American Type Culture Collection (Rockville, MD, USA). Tissue culture flasks and culture dishes were obtained from Nunc (Roskilde, Denmark). Dulbecco's modified Eagle's medium (DMEM) was purchased from Bio-Whittaker (Verviers, Belgium). Fetal bovine serum (FBS) was from JRH Biosciences (Sussex, UK). Human insulin (Actrapid) was from Novo Nordisk (Bagsvaerd, Denmark). 2-Deoxy-D-[^{3}H]glucose ([^{3}H]2-DG) was obtained from New England Nuclear Corp. (Boston, MA, USA). Porcine monocomponent insulin (Novo Research Institute, Copenhagen, Denmark) was used for binding studies and iodination by the Chloramine T method (Greenwood *et al.* 1963) at a specific activity of 185 μCi/μg. Bovine serum albumin radioimmunoassay-grade Fraction V, N-2-hydroxyethylpiperazine-N′-2-ethane sulphonic acid (HEPES), vanadate, phenylmethylsulphonyl fluoride (PMSF), leupeptin, pepstatin, bacitracin, sodium fluoride, ethylenediaminetetracetic acid (EDTA), pyrophosphate, 3,3′,5-tri-iodo-L-thyronine, 3-isobutyl-1-methyl-xanthine (IBMX) and dexamethasone were obtained from Sigma (St Louis, MO, USA). [2-^{3}H] Adenosine 5′-monophosphate ammonium salt and adenosine 5′-monophosphate were from Amersham (Chicago, IL, USA). Cytochrome C, NADPH tetrasodium salt and aprotinin were obtained from Boehringer Mannheim (Mannheim, Germany). Reagents for polyacrylamide gel electrophoresis, Triton X-100 and AG 1-X8 anion exchange resin (200–400 mesh chloride form) were obtained from Bio-Rad Laboratories (Richmond, CA, USA). Protein determination was performed by the Bradford dye method (Bio-Rad Laboratories GmbH, Munich, Germany). Polyclonal antisera were raised in rabbits specific for the 12 amino acid peptide based on the deduced carboxyl-terminal sequence of GLUT1 and GLUT4 (Mueckler *et al.* 1985, James *et al.* 1989). Immunoglobulin G (IgG) fractions of both antisera were prepared using protein A-linked sepharose (Bio-Rad Laboratories, USA). The validity of GLUT4 antisera has been reported previously (Suárez *et al.* 1995) and the specificity of GLUT1 antisera was also assayed (data not shown).

Methods

Cell culture Murine-derived 3T3-L1 fibroblasts were cultured in DMEM supplemented with 10% FBS. Adipocyte differentiation was initiated by treating confluent cells with 0·5 mM IBMX, 5 μM dexamethasone and 10 μg/ml insulin. Two days later this medium was replaced with a medium supplemented only with insulin (10 μg/ml) for three additional days. On the fifth day, when cells were differentiated (more than 95% of cells contained lipid droplets), adipocytes were incubated for three days in the absence (untreated cells) or presence of 50 nM T_3 (T_3-treated cells). Fetal bovine serum used for this last incubation period was depleted from T_3 by treatment with AG 1X-8 anion exchange resin as described by Samuels *et al.* (1979). This treatment reduced serum thyroxine to less than 1 μg/100 ml and T_3 to less than 20 ng/100 ml, measured by radioimmunoassay. All the assays were performed on the ninth day.

Glucose transport The rate of glucose transport was determined by measuring the uptake of [^{3}H]2-DG as described by de Herreros and Birnbaum (1989), with few modifications. Cells grown in 35 mm dishes, incubated in the absence or the presence of T_3 were maintained in serum-free DMEM for 2 h. Then, cell monolayers were washed and incubated for 15 min at 37 °C in KRP-HEPES (131·2 mM NaCl, 4·71 mM KCl, 2·47 mM $CaCl_2$, 1·24 mM $MgSO_4$, 2·48 mM sodium phosphate, 10 mM HEPES, pH 7·45) containing 0·5% BSA. The buffer was replaced by the same buffer without or with insulin (10^{-11}–10^{-7} M) for 20 min. Glucose uptake was initiated by the addition of 0·5 μCi/ml [^{3}H]2-DG and 2-deoxyglucose (2-DG) (0·1 mM final concentration). After 6 min the reaction was stopped by aspirating the medium and washing the dishes three times with cold PBS (20 mM sodium phosphate buffer, pH 7·4, 140 mM NaCl). Cells were solubilised with 1 ml 0·1 M NaOH and radioactivity was determined by liquid scintillation spectrometry. Nonspecific uptake, determined in the presence of 100 mM D-glucose, was subtracted from all values. Uptake was always measured in triplicate.

Insulin binding Cells (5×10^5), grown in 6-well plates, were washed twice with binding buffer (100 mM HEPES, 120 mM NaCl, 2·5 mM KCl, 1·2 mM $MgSO_4$, 1 mM EDTA, 15 mM CH_3COONa, 10 mM glucose, 2% BSA and 1 mg/ml bacitracine, pH 7·8) and incubated in the same medium for 15 min at 37 °C. Then, cells were incubated with 0·1 ng/ml ^{125}I-insulin in the absence or presence of 10^{-10}–10^{-7} M non-radioactive insulin for 16 h at 4 °C. The reaction was finished by aspirating the medium and washing the wells three times with cold PBS. The cells were solubilised with 0·1 M NaOH, and the radioactivity was measured in a gamma-counter. The nonspecific binding, determined in the presence of 10^{-6} M non-radioactive insulin was subtracted from all values. Assays were performed in triplicate.

Preparation of total cellular extracts 3T3-L1 adipocytes from a 25-cm^2 flask were solubilised with 1 ml 50 mM HEPES buffer (pH 7·4) containing 50 mM NaCl, 1% Triton X-100, 1 mM PMSF, 1 M pepstatin, 8 mM EDTA, 1000 U/ml aprotinin and 1 mg/ml bacitracin. Then, the solubilised extract was microfuged for 60 min at 4 °C. The resulting supernatant was kept at −80 °C for the immunodetection of GLUT1 and GLUT4 proteins.

Subcellular membrane fraction 3T3-L1 adipocytes (30×10^6) treated in the absence or presence of T_3 were maintained for 18 h in serum-free DMEM containing 1 g/l glucose. Then, the cells were washed with KRB buffer containing 121·1 mM NaCl, 4·8 mM KCl, 1·2 mM KH_2PO_4, 1·2 mM $MgSO_4$-$7H_2O$, 25 mM $NaHCO_3$, 10 mM HEPES, 5 mM glucose and 2% BSA, pH 7·4, and maintained in the same buffer for 70 min at 37 °C. Later, the cells were incubated in the absence or presence of 10^{-7} M insulin for 15 min, followed by three washes with HES buffer (20 mM HEPES, 1 mM EDTA, 255 mM sucrose, pH 7·4). Cells were scraped vigorously with a rubber policeman and homogenised in 18 ml HES buffer by passing 10 times through an homogeniser equipped with a teflon pestle of 1200 r.p.m (Glas-Col, Terre Haute, IN, USA). All steps subsequent to the incubation of the cells with or without insulin were performed at 0–4 °C.

The homogenate was subjected to subcellular fraction using a procedure similar to that developed by Simpson *et al.* (1983), as modified by Piper *et al.* (1991). Briefly, the homogenate was centrifuged at 19 000 × ***g*** for 20 min. The resulting supernatant was centrifuged at 41 000 × ***g*** for 20 min, yielding a pellet of high-density microsomes (HDM). The supernatant from this spin was centrifuged at 180 000 × ***g*** for 75 min, yielding a pellet of low-density microsomes (LDM). The pellet obtained from the initial spin was layered onto 1·12 M sucrose in HES buffer and centrifuged at 100 000 × ***g*** in a Beckman SW-50 rotor for 60 min. This yielded a white fluffy band at the interface corresponding to the plasma membrane (PM) fraction. The PM fraction was resuspended in 15 ml HES buffer and pelleted at 40 000 × ***g*** for 20 min. All fractions were resuspended in HES buffer to a final protein concentration of 1–5 mg/ml and stored at −80 °C. Protein concentrations in the subcellular fractions and in the total cellular extract were determined by the Bradford dye method.

Membrane marker enzyme assays 5′-Nucleotidase activity was selected as a specific marker for plasma membranes. The enzyme activity was assayed as described by Avruch & Hoelzl-Wallach (1971) in the presence of 0·05% Triton X-100. In order to inhibit nonspecific phosphatase activity, 2,3′-AMP (5 mM) was also included in the assay. Rotenone-insensitive NADH-cytochrome C reductase activity was determined (Dallner *et al.* 1966) as a specific marker for microsome membranes.

SDS-PAGE and immunodetection of GLUT1 and GLUT4 glucose transporters For the immunological identification of GLUT1 and GLUT4 glucose transporters, aliquots of the total cellular protein extract (15 μg) or subcellular membranes (5 μg protein) were solubilised in Laemmli sample buffer (Laemmli 1970) and subjected to SDS polyacrylamide gel electrophoresis (PAGE) on 8% resolving gel. Proteins were blotted onto nitrocellulose membranes by electrotransfer. The nitrocellulose was blocked for 2 h with 5% non-fat dry milk (Carnation) in Tris-buffered saline (TBS) and treated overnight with GLUT1 and GLUT4 antisera respectively. After incubation, the membrane was washed alternately in TBS and 0·05% TBS-TWEEN. Then, the blot was exposed to a peroxidase-labelled anti-rabbit antibody (1:2000). An enhanced chemiluminescence Western blotting system was used for detection. Bands were scanned using the Image Quant densitometer (Molecular Dynamics, Sunnyvale, CA, USA).

Statistical analysis

Statistical significance was assessed by Students's two-tailed *t*-test, and a *P* value of $<0·05$ was taken to be significant.

Results

Effect of T_3 treatment on basal and insulin-stimulated [^{3}H]2-DG uptake

Figure 1 shows the data on glucose transport in 3T3-L1 adipocytes cultured in the absence and in the presence of T_3. The basal glucose uptake was increased 3·6-fold by T_3 treatment ($0·47 \pm 0·12$ vs $0·13 \pm 0·047$ nmol/5×10^5 cells × 6 min; $P<0·05$). Insulin induced a significant increase in the glucose uptake by 3T3-L1 adipocytes at each of the concentrations tested (10^{-11}–10^{-7} M). Insulin-stimulated glucose transport was significantly increased ($P<0·05$) in cells treated with T_3; however, the increment over the basal value induced by insulin did not show an additive effect on that produced by the thyroid hormone in the basal situation. The insulin response curve for glucose uptake was displaced to the left and the insulin concentration necessary to achieve the half-maximal glucose transport (ED_{50}) was significantly lower in T_3-treated 3T3-L1 adipocytes, indicating a higher insulin sensitivity ($8·8 \pm 0·5 \times 10^{-10}$ M vs $32 \pm 10 \times 10^{-10}$ M, $P<0·01$).

Insulin binding

The specific ^{125}I-insulin binding to 3T3-L1 cells cultured in the absence and in the presence of T_3 was similar at each of the insulin concentrations tested. The concentration of unlabelled insulin needed to produce 50% inhibition

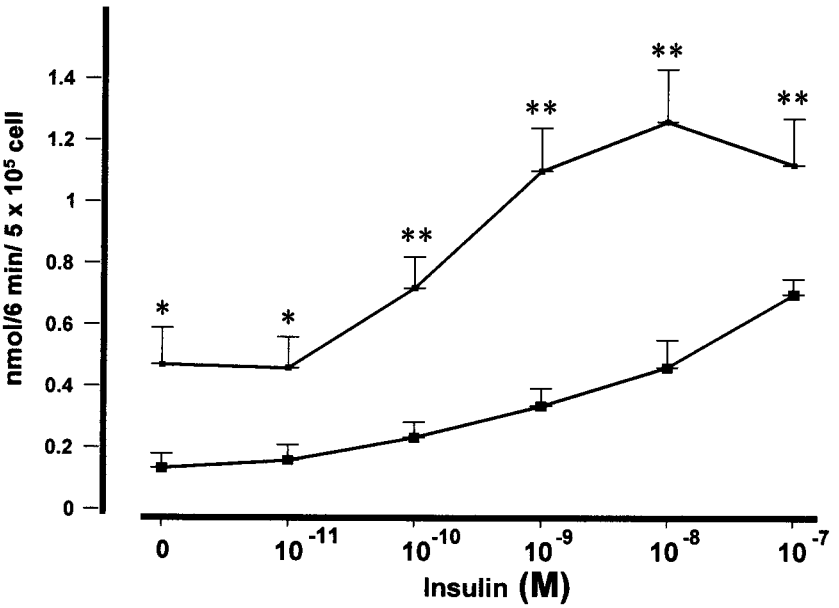

Figure 1 Glucose transport in 3T3-L1 adipocytes cultured in the presence (—■—) or absence (—■—) of 50 nM T_3. The results shown are specific 2-deoxyglucose uptake in the absence and presence of insulin as described in Materials and Methods. The results are expressed as means ± S.E.M. from 10 experiments in each group performed in triplicate. *P<0·05, **P<0·01 vs absence of T_3.

of ^{125}I-insulin binding was also similar in the two groups of cells. The Scatchard analysis of the binding data showed that the binding capacity of insulin receptors of high and low affinity were not significantly altered by T_3 treatment (Fig. 2).

Effect of T_3 treatment on GLUT1 and GLUT4 protein content in total cellular membrane fractions

Homogenates were analysed by immunoblotting, with the use of antibodies against GLUT1 and GLUT4 proteins respectively. These antisera recognized a major protein band of around 50 kDa, corresponding to GLUT1 and GLUT4 proteins (Fig. 3). GLUT1 protein content was increased by 87% in cells treated with T_3 (7·61 ± 1·06 vs 4·06 ± 1·08 arbitrary units (A.U.)/15 μg protein, P<0·05). T_3 treatment also increased GLUT4 protein content by 90% (11·94 ± 1·65 vs 6·26 ± 1·40 A.U./15 μg protein, P<0·05). Figure 3 shows a representative autoradiogram of immunoblots with GLUT1 and GLUT4 antisera obtained in total membrane extracts from 3T3-L1 adipocytes cultured in the absence and in the presence of T_3, illustrating the higher expression of both proteins induced by the thyroid hormone.

Characterization of subcellular fractions

The protein recovery in subcellular fractions was similar in all the experimental cell groups (Table 1). The three subcellular fractions obtained from 3T3-L1 adipocytes cultured in the absence or in the presence of T_3, either in basal or in insulin-stimulated conditions, showed a similar

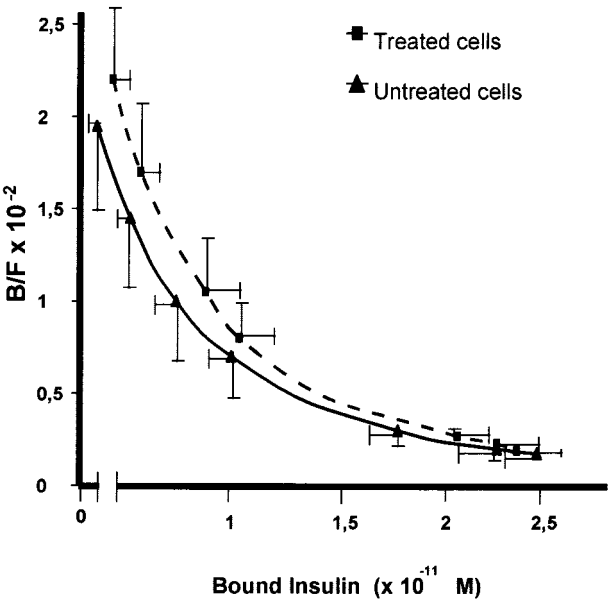

Figure 2 Scatchard plots of the specific ^{125}I-insulin binding to 3T3-L1 adipocytes incubated for three days in the absence —▲— and presence —■— of T_3. Values are means ± S.E.M. of 10 experiments performed in triplicate.

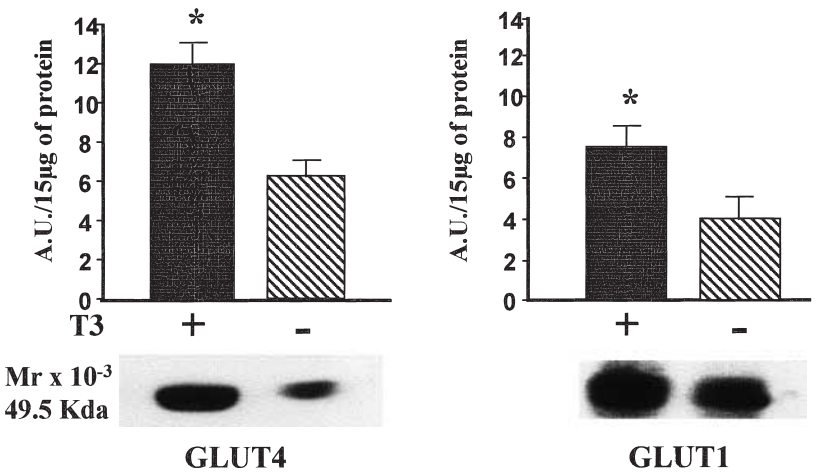

Figure 3 Quantitation of the glucose transporter proteins GLUT1 and GLUT4 in 3T3-L1 adipocytes incubated in the absence (hatched bars, $n=5$) and presence (solid bars, $n=5$) of T_3. Fifteen micrograms protein were subjected to SDS-PAGE in 8% acrylamide resolving gel under reducing conditions. Proteins were transferred to immobilon membrane and immunoblotted with polyclonal antisera raised in rabbits against the carboxyl-terminal peptide of GLUT1 and GLUT4 as described in Materials and Methods. The 49·5 kDa band from the autoradiogram was scanned and the results are expressed as arbitrary units/15 µg proteins (means ± S.E.M.). *$P<0·05$ compared with untreated group. Representative autoradiograms of GLUT1 and GLUT4 immunoblots are also shown.

degree of purity as assessed by the measurement of specific enzyme markers. 5′-Nucleotidase activity was 10-fold and 3-fold higher in PM fractions compared with LDM and HDM fractions respectively, indicating the enrichment of the plasma membrane fraction with plasma membrane (Table 1). Rotenone-insensitive NADH-cytochrome C reductase activity, a marker enzyme characteristic of membranes of the endoplasmic reticulum, was 5-fold higher in HDM and 2-fold higher in LDM fractions compared with PM fractions (Table 1).

Subcellular distribution of GLUT1 and GLUT4

Effect of thyroid hormone on the distribution of GLUT1 glucose transporter in subcellular membrane fractions To examine whether thyroid hormone had an effect on the subcellular distribution of GLUT1 glucose transporter, HDM, LDM and PM were obtained in four separate experiments from basal and maximal insulin-stimulated adipocytes cultured in the absence and in the presence of T_3. Immunoblots of the three fractions using anti-GLUT1 antiserum detected a major band of around 50 kDa, as is shown in the autoradiogram displayed in Fig. 4. Under basal conditions, 47% of GLUT1 was distributed in PM and the remainder was distributed in microsomal fractions in cells cultured in the presence of T_3, while 37% of GLUT1 was distributed in PM in cells cultured in the absence of T_3. Exposure of both groups of cells to insulin resulted in an increase in GLUT1 in PM fractions, associated with a depletion of GLUT1 from the intracellular LDM and HDM fractions (Fig. 4). For quantitative immunoblotting, cells treated with T_3 presented a higher content of GLUT1 in PM fractions both in non-insulin-stimulated conditions ($3·29 \pm 0·69$ vs $1·20 \pm 0·29$ A.U./5 µg protein; $P<0·05$) and after insulin exposure ($6·33 \pm 0·73$ vs $3·52 \pm 0·85$ A.U./5 µg protein; $P<0·05$). No differences in the amount of GLUT1 in the other two fractions were observed between the two groups of cells (Fig. 5).

Effect of thyroid hormone on the distribution of GLUT4 glucose transporter in subcellular membrane fractions Subcellular membrane proteins obtained in four separate experiments were subjected to SDS-PAGE, blotted onto nitrocellulose and GLUT4 was identified by immunodetection using a specific GLUT4 antibody. In non-insulin-stimulated conditions, adipocytes cultured in the absence and in the presence of T_3 had a preferential GLUT4 distribution within intracellular membrane fractions. Exposure of cells to insulin resulted in an increase in GLUT4 glucose transporter in the PM fraction, accompanied by a depletion in the LDM fraction (Fig. 4).

The densitometry of the bands revealed that in the absence of insulin, cells treated with T_3 presented a higher content of GLUT4 in PM fractions compared with untreated cells ($3·50 \pm 1·16$ vs $0·82 \pm 0·28$ A.U./5 µg protein; $P<0·03$). The abundance of GLUT4 in LDM and

Table 1 Specific enzyme activities and protein recovery of PM, LDM and HDM from 3T3-L1 adipocytes treated or untreated with T_3 in basal and insulin-stimulated conditions. Results are means ± S.E.M., $n=4$ for each experimental condition

	T_3-treated cells		Untreated cells	
	Basal	Insulin	Basal	Insulin
5′-Nucleotidase (nmol/h . mg protein)				
PM	149 ± 23	215 ± 39	218 ± 61	182 ± 40
LDM	15 ± 1	14 ± 2	17 ± 2	17 ± 3
HDM	52 ± 4	48 ± 7	43 ± 3	49 ± 13
NADPH-cytochrome *c* reductase (nmol/min . mg protein)				
PM	15 ± 5	24 ± 5	22 ± 7	23 ± 7
LDM	50 ± 5	52 ± 5	55 ± 2	51 ± 5
HDM	130 ± 28	130 ± 32	100 ± 14	110 ± 16
Protein recovery (μg)				
PM	56 ± 12	40 ± 14	61 ± 25	36 ± 11
LDM	409 ± 78	434 ± 76	244 ± 141	363 ± 89
HDM	260 ± 34	320 ± 81	328 ± 143	232 ± 78

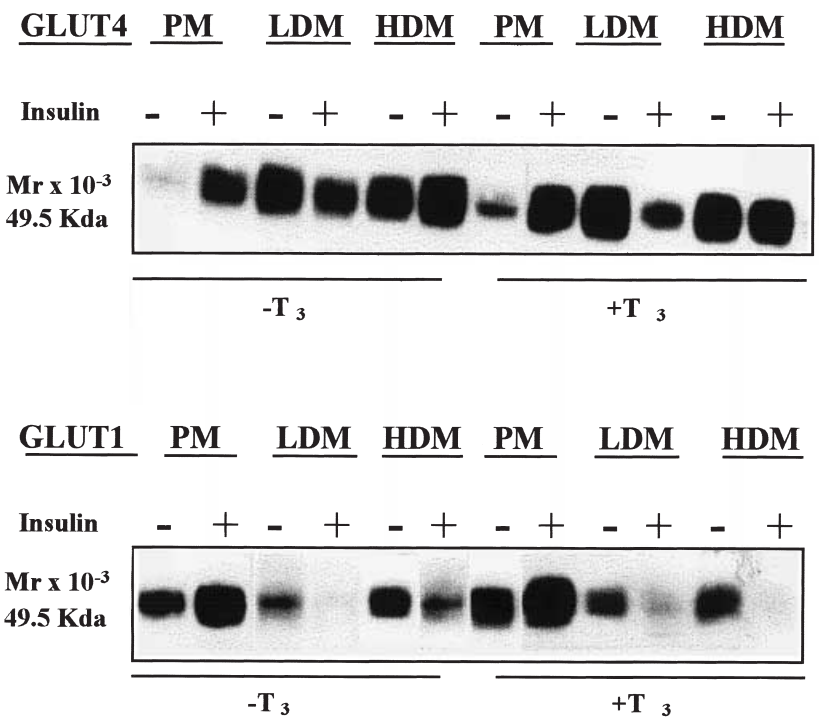

Figure 4 Representative autoradiogram of the Western blot analysis of GLUT1 and GLUT4 in subcellular membrane fractions of 3T3-L1 adipocytes cultured in the absence (-T_3) and presence (+T_3) of T_3 for 3 days. Cells were incubated in the absence (-) or presence (+) of insulin (10^{-7} mol/l) for 15 min, homogenised and subjected to a differential centrifugation procedure. Total protein (5 μg) from plasma membranes (PM), low density microsomes (LDM) and high density microsomes (HDM) were immunoblotted using anti-GLUT1 and anti-GLUT4 polyclonal antisera. The immunolabelled bands obtained were revealed by horseradish peroxidase anti-rabbit IgG followed by a chemiluminescence reagent.

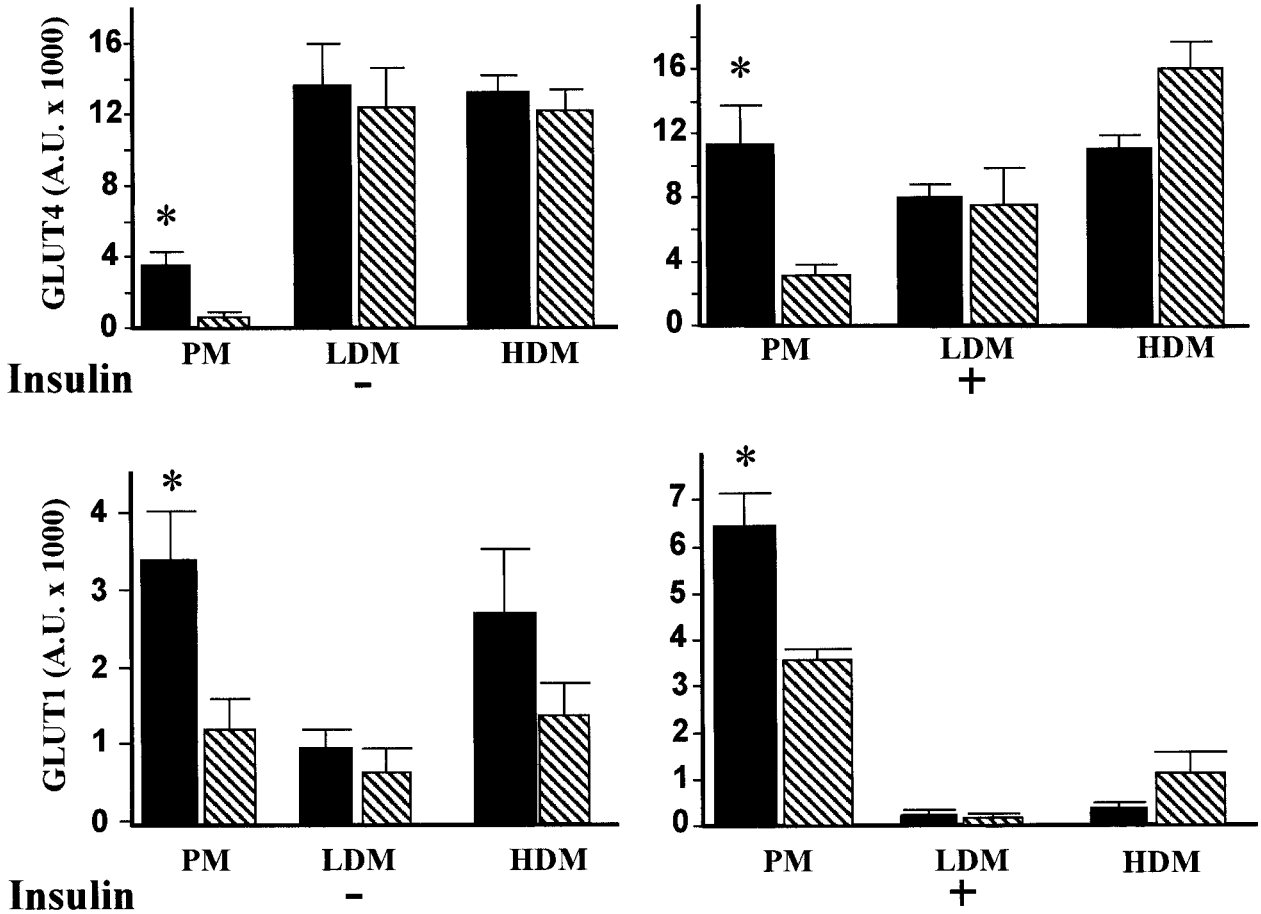

Figure 5 Quantitation of GLUT1 and GLUT4 in plasma membrane (PM), low density microsome (LDM) and high density microsome (HDM) fractions obtained from 3T3-L1 adipocytes treated for three days in the absence (hatched bars, $n=4$) and presence (solid bars, $n=4$) of T_3. Cells were incubated in the absence (-) and presence (+) of insulin, subjected to a differential centrifugation process, and the subcellular fractions were immunoblotted as described in Fig. 4. Results are expressed as arbitrary units/5 μg protein (means ± S.E.M.). *$P<0{\cdot}05$ between groups.

HDM fractions was similar in both group of cells in the insulin-unstimulated conditions. After exposure to insulin, cells treated with T_3 also had a higher content of GLUT4 in PM fractions ($11{\cdot}29 \pm 3{\cdot}12$ vs $3{\cdot}10 \pm 1{\cdot}13$ A.U./5 μg protein; $P<0{\cdot}03$). No differences were found in the amount of GLUT4 in the other two fractions between the two groups of cells (Fig. 5).

Discussion

The results of the present study indicate that the excess of T_3 has a direct stimulatory effect on glucose transport in 3T3-L1 adipocytes, supporting previous results obtained in adipocytes and skeletal muscle from hyperthyroid rats, in which an indirect action of T_3 through metabolic/hormonal changes could not have been ruled out. Specifically, hyperinsulinaemia secondary to hyperthyroidism is of great concern due to its recognized influence on glucose transport in peripheral tissues. Our results differ from those recently published using isolated adipocytes obtained from euthyroid rats, in which glucose transport was acutely inhibited by T_3 at concentrations higher than 10 μM (Goto *et al.* 1997). The different results may be explained by the distinct methodologies used, i.e. cell type, time of exposure to T_3 and concentrations of thyroid hormone.

We found that the excess of thyroid hormone induced an increase in the glucose uptake in non-insulin-stimulated 3T3-L1 adipocytes. Since GLUT1 is thought to be the glucose transporter protein responsible for most of the basal glucose transport in 3T3-L1 adipocytes (Piper *et al.* 1991), we measured the total cellular content of GLUT1 and its subcellular distribution. Cells treated with T_3 had a twofold increase in the GLUT1 protein content, with a higher distribution in PM fractions (174% increase relative to untreated cells). Our data are in line with those

previously obtained in the rat liver-derived ARL 15 cell line and in clone 9 cells exposed to 100 nM T3 (Haber *et al.* 1990, Shetty *et al.* 1996). According to our results, the effect of T_3 on glucose uptake (3·6-fold increase relative to untreated cells) could be partially explained by the high expression of GLUT1 transporter protein due to exposure to thyroid hormone.

It is of interest to notice the cell-type-specific effect of T_3 on the expression of GLUT1 protein. Thus, it appears that the excess of T_3 increases GLUT1 protein in adipose cells (Matthaei *et al.* 1995), but not in other tissues, according to previous studies referring to a lack of increase of this glucose transporter in skeletal muscle and in brain (Weinstein *et al.* 1991), and even a decrease in cardiac muscle from hyperthyroid rats (Weinstein & Haber 1992).

In order to gain more insight into the mechanism of the effect of T_3 on basal glucose transport, we measured the cellular content of GLUT4 transporter protein and its distribution in subcellular fractions. Cells cultured in the absence of T_3 had a small percentage of total GLUT4 disposed in PM, which was similar to the results obtained in 3T3-L1 adipocytes cultured in non-T_3 depleted medium (data not shown). These results agree with those reported by Piper *et al.* (1991) in 3T3-L1 adipocytes. Excess of T_3 induced a twofold increase in the total GLUT4 protein cell content and produced a change in its subcellular distribution. T_3-treated cells presented a higher GLUT4 content in PM fractions compared with untreated cells. Therefore, it is possible that increases in both GLUT 1 and GLUT4 in the PM fractions may be responsible for the high rate of glucose uptake displayed by cells exposed to T_3. However, the possibility of an increase in the functional activity of glucose transporters cannot be ruled out.

The higher content of GLUT4 in 3T3-L1 adipocytes exposed to T_3 is in line with results obtained in skeletal muscle (Casla *et al.* 1990, Weinstein *et al.* 1991, Weinstein *et al.* 1994), cardiac muscle (Weinstein & Haber 1992) and in adipocytes (Matthaei *et al.* 1995) from hyperthyroid rats. The increase in the GLUT4 protein content is probably related to a stimulatory effect of T_3 on GLUT4 mRNA synthesis, as has been reported to have occurred in skeletal muscle of hyperthyroid rats (Weinstein *et al.* 1991, Torrance *et al.* 1997). Moreover, it has been reported that the GLUT4 gene promotor presents thyroid hormone response elements (Richardson & Pessin 1993), and it has also been shown that T_3 increases the transcription rate of GLUT4 mRNA in rat skeletal muscle (Torrance *et al.* 1997). The increase in the abundance of GLUT4 in the plasma membrane could suggest that the new transporter synthesised carriers directly to the plasma membrane or could be due to a reduction in the recycling. The stimulatory effect of T_3 of GLUT4 mRNA synthesis has also been reported in skeletal muscle and in adipocytes from hyperthyroid rats (Weinstein *et al.* 1991, Matthaei *et al.* 1995).

Previous results obtained in isolated adipocytes (Casla *et al.* 1993, Matthaei *et al.* 1995) and in isolated skeletal muscles (Weinstein *et al.* 1994) from hyperthyroid rats have reported that T_3 produced an increase in the insulin-stimulated glucose transport in 3T3-L1 adipocytes. In our study, insulin sensitivity was enhanced by T_3 treatment. This effect was not related to changes in the insulin binding characteristics displayed by 3T3-L1 adipocytes.

Although our results and those from others point to a beneficial effect of thyroid hormone on glucose entry into the cells, there is still controversial data on clinical hyperthyroidism. Normal and low insulin-induced glucose disposal in peripheral tissues have been described in hyperthyroid subjects (Shen *et al.* 1988, Gonzalo *et al.* 1996). The apparent discrepancies can be accounted for due to the complexity of the metabolic responses occurring in long-standing spontaneous hyperthyroidism. The beneficial effect of T_3 on insulin action has been described in two models of insulin resistance, the obese Zucker (fa/fa) rats (Torrance *et al.* 1997) and the hyperglycaemic KK mice (Shimokawa *et al.* 1997). In both cases, T_3 treatment induced an increase in GLUT4 mRNA content in skeletal muscle, which, in the case of obese Zucker rats, was associated with a decrease in peripheral insulin levels, and, in the case of diabetic KK mice, was associated with an amelioration of hyperglycaemia (Shimokawa *et al.* 1997, Torrance *et al.* 1997).

In conclusion, this study shows that T_3 directly stimulates glucose uptake in 3T3-L1 adipocytes. This effect is due to a high expression of the two glucose transporter proteins that are expressed in these cells, GLUT1 and GLUT4, increasing their partitioning to plasma membranes. The effect of T_3 on glucose uptake induced by insulin can also be explained by the increase in both glucose transporters.

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