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Research Article

Insulin action in cultured human skeletal muscle cells during differentiation: assessment of cell surface GLUT4 and GLUT1 content

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Abstract. In mature human skeletal muscle, insulin-stimulated glucose transport is mediated primarily via the GLUT4 glucose transporter. However, in contrast to mature skeletal muscle, cultured muscle expresses significant levels of the GLUT1 glucose transporter. To assess the relative contribution of these two glucose transporters, we used a novel photolabelling techniques to assess the cell surface abundance of GLUT1 and GLUT4 specifically in primary cultures of human skeletal muscle. We demonstrate that insulin-stimulated glucose transport in cultured human skeletal muscle is mediated by GLUT4, as no effect on GLUT1 appearance at the plasma membrane was noted. Furthermore, GLUT4 mRNA and protein increased twofold ($p < 0.05$), after differentiation, whereas GLUT1 mRNA and protein decreased 55% ($p < 0.005$). Incubation of differentiated human skeletal muscle cells with a non-peptide insulin mimetic significantly ($p < 0.05$) increased glucose uptake and glycogen synthesis. Thus, cultured myotubes are a useful tool to facilitate biological and molecular validation of novel pharmacological agents aimed to improve glucose metabolism in skeletal muscle.

Key words. Human skeletal muscle; cell culture; glucose transporter; insulin action; glucose transport; glycogen synthesis.

Insulin-stimulated skeletal muscle glucose disposal and metabolism are important factors in whole-body glucose homeostasis [1–3]. Importantly, skeletal muscle insulin resistance is a characteristic feature of non-insulin-dependent (type II) diabetes mellitus [4–6], and an early metabolic abnormality noted in pre-diabetics and in first-degree relatives of type II diabetic patients [7, 8]. Thus, understanding the mechanisms regulating insulin action in skeletal muscle is of paramount importance to delineate the molecular mechanisms underlying insulin action in healthy individuals and in people with type II diabetes mellitus.

Insulin target organs including skeletal muscle and adipose tissue express the insulin-sensitive glucose transporter, GLUT4 [9]. We investigated the expression of GLUT1 and GLUT4 during human skeletal muscle cell

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differentiation. Skeletal muscle myotubes in culture differ from adult skeletal muscle in that cultures express significant amounts of GLUT1 [10–12]. The GLUT1 contribution to insulin-mediated glucose transport in skeletal muscle cultures is not known [11]. Using a novel technique involving a biotinylated photoaffinity label, the appearance of specific glucose transporters on the cell surface can be assessed, and GLUT1- and GLUT4-mediated effects on glucose uptake differentiated. Thus, we hypothesised that this method may reveal insulin responsiveness at the level of GLUT4 in primary cultures of human muscle.

Primary cultures of human skeletal muscle provide a useful system to study insulin action because they display numerous features of mature skeletal muscle [11–18]. Recently, a small-molecule fungal metabolite with insulin-like activity (L-783,281) [19], as well as two synthetic analogues [20] have been described. We have utilised differentiated cultures of primary human skeletal muscle cells to determine the effect of these compounds on glucose metabolism in human cells. The use of differentiated human skeletal muscle cells should provide a relevant model for assessing biological effects of novel compounds.

Materials and methods

Materials

Dulbecco's minimum essential medium (DMEM), Ham's F-10 medium, fetal bovine serum (FBS), penicillin, streptomycin and fungizone were obtained from GibcoBRL (Life-Technologies, Stockholm, Sweden). Radiochemical, 2-[G-^{3}H]deoxy-D-glucose (6.0 Ci/mmol/l) and D-[U-^{14}C] glucose (310 mCi/mmol/l) were from Amersham (Life Science, Uppsala, Sweden).

Antibodies

The GLUT1 antibody has been described previously [21]. The GLUT4 antibody was from Dr. P. Pilch, (Boston, Mass.). Horseradish peroxidase-conjugated goat anti-rabbit and anti-mouse immunoglobulin G were from Bio-Rad (Richmond, Calif.). Reagents for enhanced chemiluminescence (ECL) were from Amersham (Arlington Heights, Ill.). All other reagents were analytical grade (Sigma, St. Louis, Mo.).

Subjects

Skeletal muscle biopsies were obtained with the informed consent of the donors during scheduled abdominal surgery. The characteristics of the study participants (four male and four female) are summarised in table 1. None of the subjects had known metabolic disease. The ethical committee at Karolinska Institutet approved protocols.

Table 1. Clinical characteristics of the study participants.

Gender (M/F)	4/4
Age (years)	55 ± 5
BMI (kg/m^2)	25.6 ± 1.5
Fasting plasma glucose (mM)	5.4 ± 0.3

Results are expressed as mean ± SE.

Cell culture

Satellite cells were isolated and cultures were established based on protocols for human fetal skeletal muscle [22]. Muscle biopsies (rectus abdominus, ~1–3 g) were collected in cold phosphate-buffered saline (PBS) supplemented with 1% PeSt (100 units/ml penicillin/100 µg/ml streptomycin). Skeletal muscle biopsies were dissected free from visible connective and fat tissues, minced finely, transferred to a digestion solution [0.015 g collagenase IV, 8% 10 × trypsin, 0.015 g bovine serum albumin (BSA), 1% PeSt, in Ham's F-10 medium], and incubated with gentle agitation at 37 °C for 15–20 min. Undigested tissue was allowed to settle and the supernatant containing liberated satellite cells was collected and mixed 1:1 with growth medium (Ham's F-10 with 20% FBS, 1% PeSt). The remaining tissue was digested for a further 20 min at 37 °C with fresh digestion solution. The resultant supernatant was pooled with the previous cells and centrifuged for 10 min at 350 g. The cell pellet was resuspended in 5 ml Ham's F-10/20% FCS and incubated in a non-coated (bacteriological) petri dish for 1 h to selectively promote adherence of non-myogenic cells. The supernatant was then transferred and cells were seeded in 150-cm^2 Costar culture flasks. Medium was changed every 2–3 days. At confluence (>80%), cells were trypsinised and subcultured. This first flask (after the first trypsinisation) was designated 'passage 1'. For experimental assays, myoblasts were allowed to reach >80% confluence before initiation of the differentiation protocol.

To initiate differentiation into myotubes, Ham's-F10/20% FBS was removed from cells and DMEM containing 1% PeSt/4% FBS added for 48 h. After this, medium was changed to DMEM containing 1% PeSt/2% FBS. Fusion and multinucleation of cells was observed at day 3 after initiation of differentiation. All studies were carried out on the second or third passage cultures.

Giemsa/Wright staining

To assess the extent of differentiation, myotubes were fixed in methanol (10 min), 1:10 Giemsa (15 min) and 1:10 Wright (20 min). Cells were washed with double-distilled H_2O and mono- or multinucleated cells were observed under a phase contrast inverted light microscope.

Analysis of mRNA expression of GLUT4, GLUT1 and β_2-microglubolin

Cells were cultured in 100-mm dishes as described. Cultures were washed three times with RNase-free PBS, and harvested directly for RNA extraction (RNAeasy mini kit; Qiagen, Crawley, UK). All RNA was DNase treated before reverse transcription (RQ1 RNase-free DNase; Promega, Southampton, UK). Three microlitres cDNA (corresponding to 0.15 µg of total RNA) was amplified with 1 × Taqman buffer, 5 mM $MgCl_2$, 200 µM each dNTP, 200 µM each primer, 1.25 pM of probe, 0.25 U Amp-Erase uracil N-glycosylase, 1.25 U AmpliTaq gold (PE Applied Biosystems, Foster City, Calif.) in a real-time quantitative polymerase chain reaction (RTQ-PCR), using an ABI PRISM 7700 sequence detection system instrument and software (PE Applied Biosystems). The nucleotide sequences for human β_2-microglobin and GLUT1 primer/probe sets for real-time PCR were designed using the manufacturer's software and sequences available in GenBank. GLUT4 primer/probe sequences have been described [23]. All sequences are reported in table 2. cDNA specificity of each primer pair was verified by RT-PCR using both genomic DNA and cDNA as a template. For normalisation of RNA loading, control samples were run using the β_2-microglobulin housekeeping gene. Expression levels were quantified by generating a six-point serial standard curve.

Glucose transport

Six-well cultures from day 0 and 5 days post-differentiation, were pre-incubated in serum-free DMEM for 6 h. Cells were then incubated in serum and glucose free DMEM without or with insulin (120 nM) for 40 min at 37 °C with 5% CO_2, followed by addition of 5 µM [^{3}H]2-deoxyglucose (0.33 µCi per well), for 10 min. 1 nM insulin stimulation led to a 1.3 ± 0.04-fold increase in glucose uptake ($p < 0.05$); however, this concentration of insulin did not lead to consistent stimulation of glycogen synthesis, and thus we selected a higher insulin concentration for the current studies. After incubation with [^{3}H]2-deoxyglucose, medium was rapidly aspirated and cells washed three times with ice-cold PBS and lysed in 1 ml 0.03% SDS of which 0.85 ml was transferred to scintillation liquid. Radioactivity was determined by liquid scintillation counting (1214 Rackbeta; Wallac, Turku, Finland). The remaining suspension was used for protein concentration determination using a commercial kit (Pierce, Rockford, Ill.). To determine non-specific uptake, parallel incubations were performed in the presence of 50 µM cytochalasin B, and results were subtracted from the respective incubations in the absence of cytochalasin B. Cytochalasin B exposure reduced glucose uptake by approximately 60%. Each experiment was carried out on triplicate wells.

Glycogen synthesis

Six-well cultures from day 0 and 5 days post-differentiation were serum starved for 6 h, stimulated without or with 120 nM insulin, or addition of compound (Cpd) 2 or 3 as indicated, for 30 min at 37 °C and incubated with 5 mM glucose DMEM, supplemented with D-[U-^{14}C] glucose (1 µCi/ml; final specific activity 0.18 µCi/µmol) for 90 min. In CHO cells overexpressing human insulin receptor, an EC_{50} of 0.3 µM was reported for Cpd 2 [20]. However, cultured human skeletal muscle cells have a lower insulin receptor expression than the transfected CHO cell line. Thus, a higher concentration of Cpd 2 was required to detect a measurable response. Following incubation, monolayers were washed with ice-cold PBS, and lysed in 1 ml 0.03% SDS. 0.85 ml of the suspension was transferred to 10-ml tubes and 100 µl (2 mg/sample) carrier glycogen was added. The remaining cell suspension was used for protein concentration determination. Samples were heated to 95 °C for 30 min. Glycogen was precipitated by addition of 95% ethanol and incubated overnight at 4 °C with slight agitation. Glycogen pellets were collected by centrifugation for 35 min at 1700 g, washed once with 70% ethanol and resuspended in 200 µl distilled water. Radioactivity was determined by liquid scintillation counting (1214 Rackbeta; Wallac). Each experiment was carried out on triplicate wells.

Western blot analysis

Cell monolayers were washed once in ice-cold PBS and harvested directly by scraping into ice-cold lysis buffer

Table 2. Primers and probes for real-time quantitative PCR.

Gene	Forward primer	Reverse primer	Probe
β_2-microglubolin	5′-GCCTGCCGTGTGAACCAT-3′	5′-TTACATGTCTCGATCCCAC-TTACCTATC- 3′	5′-FAM-TGACTTTGTCACAGC-CCA-TAMRA-3′
GLUT1	5′-CCTGTGGGAGCCTGCAAA-3′	5′-TCTATACACAGGGCAGGA-GTCT-3′	5′-FAM-CACTGCTCAA-GAAG-AC-TAMRA-3′
GLUT4 *	5′- GCTACCTCTACATCATCC-AGAATCTC-3′	5′-CCAGAAACATCGGCCCA-3	5′-FAM-CTGCCAGAAAGAGTCT-GAAG GCCT-TAMRA-3′

* The primers and probe sequences have been described [23].

[135 mM NaCl, 1 mM $MgCl_2$, 2.7 mM KCl, 20 mM Tris pH 8.0, 0.5 mM Na_3VO_4, 10 mM NaF, 1% Triton X-100, 10% v/v glycerol, 0.2 mM phenylmethylsulfonylfluoride (PMSF), 10 µg/ml leupeptin, 10 µg/ml antipain, and 10 µg/ml aprotinin]. Homogenates were rotated for 60 min at 4 °C and subjected to centrifugation (20,000 g for 10 min at 4 °C). Western blotting was performed as described elsewhere [24]. GLUT4 protein content was estimated by comparison with a dilution series of a standard rat adipocyte membrane fraction containing 60 pmol GLUT4/mg of total protein [25].

Photolabelling of cell surface glucose transporters

Skeletal muscle cells were serum starved overnight, followed by incubation without or with 120 nM insulin for 35 min at 37 °C, incubated at 18 °C for 5 min and gently rinsed twice with ambient pre-gassed (5% CO_2) Krebs-Henseleit bicarbonate buffer (KHB) supplemented with 5 mM Hepes and 0.1% BSA. Bio-LC-ATB-BGPA (4,4′-*O*-[2-[2-[2-[2-[2-[6-(biotinylamino)hexanoyl] amino]ethoxy]ethoxy]ethoxy]-4-(1-azi-2,2,2,-trifluoroethyl) benzoyl] amino-1,3-propanediyl bis-D-glucose [26] was diluted to a final concentration of 100 µM, in 2 ml KHB supplemented without or with 120 nM insulin. The assay mixture was added to the respective dish and incubated for 8 min in a dark room, with gentle shaking at 18 °C. Dishes were UV irradiated for 3 min (shaking every 20 s). Thereafter, cells were washed with PBS, solubilised and scraped into 1 ml PBS with 2% thesit ($C_{12}E_9$) and protease inhibitors (10 µg/ml aprotinin, 10 µg/ml antipain, 10 µg/ml leupetin, 200 µM PMSF). Solubilised cell extracts were transferred to microtubes (Sarstedt, Nümbrecht, Germany) and rotated for 60 min at 4 °C followed by 10 min centrifugation at 20,000 g. The supernatant (900 µl) was collected and mixed with 50 µl streptavidin-agarose beads [50% slurry (Pierce), pre-washed twice in PBS) to generate a streptavidin-biotin complex. The streptavidin-biotin complex was incubated overnight at 4 °C with end-to-end rotation. Beads were washed three times with PBS containing 1% thesit, three times with PBS containing 0.1% thesit, and twice with PBS. An aliquot (60 µl) of Laemmeli buffer containing 3 M urea was added to each sample, and lamp-heated for 40 min. Photolabelled glucose transporters were eluted from the beads by 10 min boiling. Samples were then applied directly to SDS-PAGE and subjected to electrophoresis and immunoblot analysis with either GLUT1 or GLUT4 antibody. Rat plasma membrane GLUT4 was used as a standard to quantify the concentration of GLUT4 in samples.

Statistics

Data are presented as the mean ± SE. Statistical differences were determined by Student's t test as appropriate. Significant differences were accepted at $p < 0.05$.

Results

Expression of GLUT1 and GLUT4 during differentiation of primary human skeletal muscle cultures

Cultures were grown to >80% confluence and then induced to differentiate. Fused multinucleated cells were apparent by day 3 post-differentiation. By day 5 post-differentiation, cultures were >90% differentiated, as assessed by Giemsa/Wright staining (data not shown). Cultures were used at day 5 following initiation of differentiation. Protein and mRNA expression of GLUT1 was reduced in myotubes compared to myoblasts ($p < 0.05$) (fig. 1A, B). In contrast, protein and mRNA expression of GLUT4 increased ($p < 0.05$) with differentiation (fig. 1C, D). Despite the differentiation-induced increase, GLUT4 expression remained lower than GLUT1; in myoblasts, GLUT1:GLUT4 expression was 37:1 and in myotubes, 12:1. Furthermore, GLUT4 in differentiated myotubes in cultured muscle was reduced ($p < 0.05$) compared with mature skeletal muscle (53 fmol/mg protein in cultured myotubes vs 1128 fmol/mg protein in rat skeletal muscle homogenates, and 4130 fmol/mg protein in human skeletal muscle [27]; data not shown).

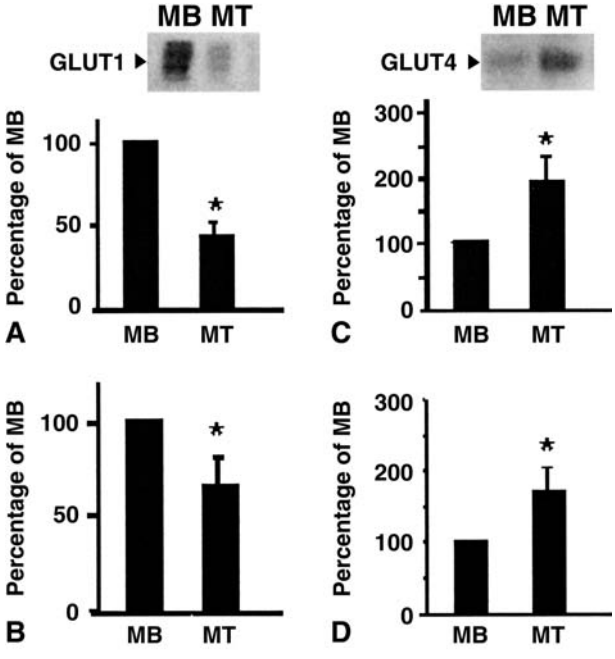

Figure 1. Expression of GLUT1 protein (*A*), GLUT1 mRNA (*B*) and GLUT4 protein (*C*) and mRNA (*D*) in human skeletal muscle cell cultures during differentiation from myoblasts (MB; day 0) to myotubes (MT; day 5). Top panel shows a representative immunoblot. The mRNA levels of GLUT1 and GLUT4 were standardised by reference to β_2-microglubolin mRNA levels. Day zero was considered as 100%. Shown are means ± SE (n = 6 subjects). *$p < 0.05$ vs myoblasts.

Insulin-stimulated glucose metabolism

Insulin-stimulated [C^{14}]glucose incorporation to glycogen was assessed after exposing cells to 120 nM insulin for 30 min, followed by addition of [C^{14}]glucose for an additional 90 min. In myoblasts, insulin increased glycogen synthesis by 2.1 ± 0.4-fold ($p < 0.05$). In myotubes, insulin treatment increased glucose incorporation to glycogen 1.9 ± 0.2-fold ($p < 0.01$). The absolute magnitude of insulin-stimulated glycogen synthesis was reduced in both basal and insulin-stimulated myotubes as compared to myoblasts (fig. 2A).

We next determined insulin-stimulated activation of glucose transport. Cultures were incubated in the absence or presence of 120 nM insulin for 40 min, followed by addition of [^{3}H]2-deoxyglucose for a further 10 min. Insulin treatment resulted in a modest, but significant increase in glucose uptake in both myoblasts and myotubes (fig. 2B). A tendency for a decrease in glucose transport was noted in muscle cells post-differentiation (fig. 2B). Insulin-stimulated glucose transport was increased 1.4 ± 0.1-fold ($p < 0.05$) in myoblasts and 1.6 ± 0.2-fold ($p < 0.05$) in myotubes. When glucose uptake rates per se were considered, the remaining GLUT1 expression continued to mask the effect. However, if the insulin-stimulated increment was considered, this increased from 3.2 pmol glucose/mg protein per minute in mytoblasts to 5.2 pmol glucose/mg protein per minute in myotubes, which corresponds to an increase of 62%.

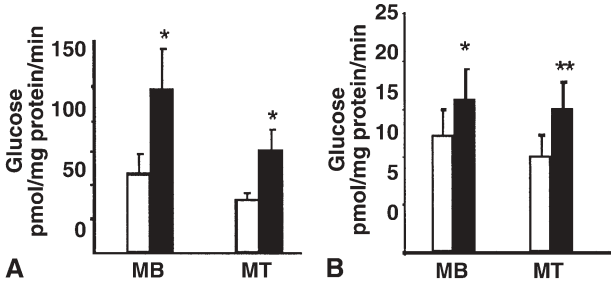

Figure 2. Insulin-stimulated glycogen synthesis (*A*) and insulin-stimulated glucose uptake (*B*) in myoblast (MB) and myotube (MT) cultures. Results are the mean ± SE for basal (□) and insulin-stimulated (■) conditions. *$p < 0.05$, **$p < 0.01$ vs basal. n = 5–6 subjects.

Assessment of glucose transporters at the cell membrane

A high expression level of GLUT1 may mask measurements of insulin-stimulated glucose uptake in cultured myotubes. To address this issue, we labelled cell surface glucose transporters with a biotinylated glucose analogue (Bio-LC-ATB-BGPA) in differentiated myotubes. Insulin treatment did not significantly increase cell surface GLUT1 content (fig. 3A). In contrast, insulin stimulation led to a significant increase (1.6 ± 0.09-fold, $p < 0.003$) in cell surface GLUT4 content (fig. 3B). The insulin-stimulated appearance of GLUT4 at the cell membrane using Bio-LC-ATB-BGPA was almost completely abolished by pre-incubation of cells with 25 mM cold glucose (data not shown).

Non-peptide insulin mimetic compounds stimulate glucose metabolism in human skeletal muscle cultures

We utilised Cpd 2 [20], a recently described analogue of L-783,281 (a small-molecule fungal metabolite with insulin-like activity) to determine the in vitro efficacy of this reagent on glucose metabolism in cultured human skeletal muscle cells. Myotubes were incubated with 3, 10 or 30 µM Cpd 2, or with 30 µM Cpd 3 (an inactive analogue [20]) for 30 min. Cpd 2 increased glucose uptake to a level 50% of that achieved with 100 nM insulin ($p < 0.05$; fig. 4A). Glucose uptake was not altered in cells incubated with inactive Cpd 3 (fig. 4A). Incubation of differentiated (day 5) human skeletal muscle cells with Cpd 2 resulted in a significant increase in glycogen synthesis ($p < 0.05$, fig. 4B). Glycogen synthesis was not altered in the presence of the inactive Cpd 3 (fig. 4B).

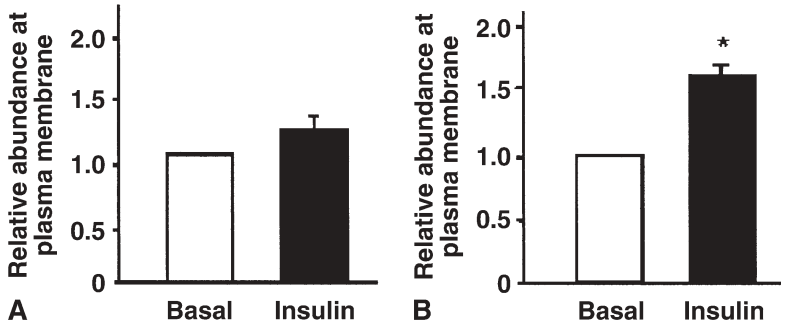

Figure 3. Membrane content of the glucose transporters GLUT1 (*A*) and GLUT4 (*B*) in human myotubes. *$p < 0.05$ vs. basal. n = 5–6 subjects.

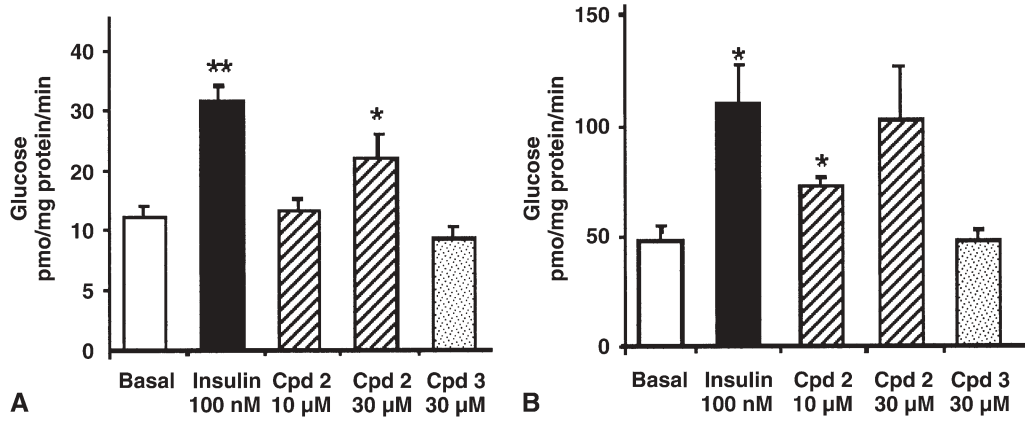

Figure 4. Glucose uptake following incubation with insulin, Cpd 2 (active) or Cpd 3 (inactive) in differentiated cells (day 5) (*A*) and glycogen synthesis following incubation with insulin, Cpd 2 or Cpd 3 in differentiated cells (day 5) (*B*) Results are the mean ± SE for basal (□), insulin (■), Cpd 2 (▨) and Cpd 3 (▩). *$p < 0.05$, **$p < 0.01$ basal vs stimulated. n = 5–6 subjects.

Discussion

Insulin resistance in skeletal muscle has been described in subjects with type II diabetes mellitus at the level of glucose transport [4, 5] and glycogen synthesis [28]. Thus, insulin action needs to be studied in skeletal muscle, not least because it is the largest insulin-sensitive organ in the body and a target for pharmacological intervention strategies aimed to improve glucose homeostasis in altered metabolic states. Human skeletal muscle cell culture provides a suitable model system in which to dissect molecular mechanisms governing insulin action [11–16, 18, 29].

A characteristic feature of adult human skeletal muscle is the expression of the insulin-responsive glucose transporter GLUT4. Cultured myoblasts and myotubes express relatively less GLUT4, and have a relatively greater expression of GLUT1, than intact muscle [11]. Interestingly in the present study, the expression of GLUT1 decreased as the cultured muscle cells differentiated. In parallel to the reduced expression of GLUT1 in myotubes, there was a reduction in the absolute magnitude of both basal and insulin-stimulated glucose uptake. A reduction in GLUT1 as the cultures remodel to myotubes should correlate with a more adult skeletal muscle phenotype, since little, if any, GLUT1 is expressed in adult human skeletal muscle [30]. However, GLUT1 protein expression remains higher in the cultured myotubes compared to adult human skeletal muscle [30, 31]. Our studies in human skeletal muscle culture show that with differentiation to myotubes, GLUT1 expression is reduced, mimicking the phenotype observed in adult skeletal muscle.

As both myotube and myoblast cultures express both GLUT1 and GLUT4, both transporters are likely play a role in glucose uptake; however, the relative contribution of each transporter to glucose transport is not known. To dissect this issue, we utilised a newly described biotinylated photolabelled glucose analogue (Bio-LC-ATB-BGPA) [26, 27, 32] to detect either GLUT1 or GLUT4 content at the cell surface before and after insulin stimulation. Using this methodology we could demonstrate that GLUT1 is not responsive to insulin, while GLUT4 is recruited to the cell surface upon insulin stimulation. This positive relationship between cell surface GLUT4 and glucose transport activity is in agreement with studies in human skeletal muscle [27]. Furthermore, our studies provide evidence to suggest that when the ratio GLUT1 : GLUT4 is high, GLUT1 can mask effects of insulin on GLUT4-mediated glucose transport. Similar effects may be seen in transgenic mice overexpressing GLUT1, which are characterised by increased basal glucose transport and reduced fold insulin-stimulated glucose compared to wild-type mice [33]. The photolabelling technique employed in the present study allows assessment of GLUT4-mediated events without confounding effects of GLUT1. Together these tools will be important in the evaluation of compounds that increase glucose uptake in skeletal muscle.

A recent report described the discovery of a small non-peptide fungal metabolite that elicits insulin-like effects in cultured cells and in animal models of diabetes [19, 34]. This novel compound directly interacts with the insulin receptor and may function by altering conformation of the protein, and partially relieve autoinhibition and increase kinase activity [20]. We investigated the effects of active and inactive analogues of this compound [20] on glucose metabolism in differentiated human skeletal muscle cultures. Stimulation of cultures with the active insulin mimetic (Cpd 2) increased glucose uptake and glucose incorporation into glycogen. This novel class of compounds has been shown to be highly selective for the insulin receptor [34], and with development may lead to powerful new therapies for treating insulin resistance and

type II diabetes mellitus. Thus, cultured human skeletal muscle cells provide a useful model system in which to dissect the action of novel and potentially therapeutic compounds.

In summary, we report a reduction in GLUT1 expression following differentiation of cultures to myotubes, accompanied by a modest increase in GLUT4 expression, indicating that myotubes, rather than myoblasts, have a phenotype that is closer to adult skeletal muscle. Despite this, GLUT1 expression is highly expressed in cultured skeletal muscle compared to skeletal muscle biopsies, pointing to an important difference in using cultured muscle cells versus adult skeletal muscle. Utilising a sensitive photolabelling technique to determine insulin-mediated effects on GLUT4 translocation to the plasma membrane, we demonstrated that GLUT4 is the predominant insulin-stimulated glucose transporter in cultured human skeletal muscle cells. Thus, cultured human myotubes can serve as a suitable experimental tool to study insulin action on glucose metabolism in physiologically relevant insulin target tissue. Since many of the components of the insulin signalling machinery to glucose transport are expressed, cultured human myotubes will be useful for providing biological and molecular validation of targets relevant for novel pharmacological agents aimed to improve glucose metabolism.

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