Glucose Transport in Cultured Human Skeletal Muscle Cells

Regulation by Insulin and Glucose in Nondiabetic and Non-Insulin-dependent Diabetes Mellitus Subjects

Theodore P. Ciaraldi, Leslie Abrams, Svetlana Nikoulina, Sunder Mudaliar, and Robert R. Henry

Medical Research Service (9111G), San Diego Veterans Affairs Medical Center, San Diego, California 92161 and Department of Medicine (0673), University of California, San Diego, La Jolla, California 92093

Abstract

A primary human skeletal muscle culture (HSMC) system, which retains cellular integrity and insulin responsiveness for glucose transport was employed to evaluate glucose transport regulation. As previously reported, cells cultured from non-insulin-dependent diabetic (NIDDM) subjects displayed significant reductions in both basal and acute insulin-stimulated transport compared to nondiabetic controls (NC).

Fusion/differentiation of NC and NIDDM HSMC in elevated media insulin (from 22 pM to 30 μM) resulted in increased basal transport activities but reduced insulin-stimulated transport, so that cells were no longer insulin responsive. After fusion under hyperinsulinemic conditions, GLUT1 protein expression was elevated in both groups while GLUT4 protein level was unaltered. Fusion of HSMC under hyperglycemic conditions (10 and 20 mM) decreased glucose transport in NC cells only when combined with hyperinsulinemia. Hyperglycemia alone down-regulated transport in HSMC of NIDDM, while the combination of hyperglycemia and hyperinsulinemia had greater effects. In summary: (*a*) insulin resistance of glucose transport can be induced in HSMC of both NC and NIDDM by hyperinsulinemia and is accompanied by unaltered GLUT4 but increased GLUT1 levels; and (*b*) HSMC from NIDDM subjects demonstrate an increased sensitivity to impairment of glucose transport by hyperglycemia. These results indicate that insulin resistance in skeletal muscle can be acquired in NC and NIDDM from hyperinsulinemia alone but that NIDDM is uniquely sensitive to the additional influence of hyperglycemia. (*J. Clin. Invest.* 1995. 96:2820–2827.) Key words: insulin action • hyperglycemia • hyperinsulinemia • cell culture • diabetes • glucose transport • skeletal muscle

Introduction

Skeletal muscle represents the principal tissue involved in insulin-stimulated glucose disposal and contributes significantly to the insulin regulation of glycemia (1, 2). Impairments in the ability of skeletal muscle to use glucose, especially in response to insulin, are major contributors to the glucose intolerance prevalent in type 2 (non–insulin-dependent, NIDDM)[1] diabetes mellitus and obesity (2–4). Under most conditions, it is the transport of glucose across the plasma membrane that is rate-limiting for glucose utilization in muscle (5). The nearly universal presence of defects in skeletal muscle glucose transport in NIDDM subjects (6), primarily in response to insulin, support the importance of this process, although defects in subsequent steps in glucose metabolism have also been shown to occur (7).

Glucose transport into most tissues occurs through the action of the members of a family of facilitative diffusion glucose transport proteins designated GLUT1–7 (8, 9). GLUT1 is widely distributed and appears to serve the role of a constitutive transport protein (8). Another major transporter of interest is GLUT4, expressed in adipose tissue, cardiac, and skeletal muscle, which is responsible for the major portion of insulin stimulation of glucose transport in these tissues (8, 9). While the signaling events specific for glucose transport stimulation are still uncertain, it has been established that the majority of cellular GLUT4 protein resides in specific intracellular pools (10–12). Insulin induces translocation of a portion of these intracellular GLUT4 proteins to the cell surface, where enhanced transport activity is expressed (11, 13). Impairment in the ability of insulin to stimulate glucose transport could involve a number of abnormalities including a reduction in the intracellular stores of GLUT4 proteins, impaired ability of GLUT4-containing vesicles to translocate to the plasma membrane, a reduction in the intrinsic activity of GLUT4, as well as defects in the earlier steps of signal transduction.

Most of the observations about regulation of insulin-stimulated glucose transport in humans have been made in freshly isolated fat cells, which are readily available, highly insulin sensitive and responsive, as well as amenable to independent manipulation of multiple variables. The specific nature of insulin resistant glucose transport in skeletal muscle has been more difficult to ascertain, although in vivo studies (4, 14) and the use of isolated muscle strips have been revealing (15–17). An isolated muscle cell system, either freshly prepared or in primary culture, offers a number of additional advantages including full exposure of cells to media, fiber type homogeneity, technical ease for assay of initial rates of transport, and independent manipulation of variables. We have recently characterized glucose transport activity in such a human skeletal muscle cell (HSMC) culture system, and have found that impaired glucose transport is retained in cells grown from NIDDM subjects (18).

Address correspondence to Dr. Robert R. Henry, VA Medical Center, San Diego (9111G), 3350 La Jolla Village Drive, San Diego, CA 92161. Phone: 619-552-8585 x3648; FAX: 619-534-6653.

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1. *Abbreviations used in this paper:* HSMC, human skeletal muscle culture; NC, nondiabetic controls; NIDDM, non–insulin-dependent diabetes mellitus.

In the current report, we have used and extended this approach to evaluate glucose transport regulation in nondiabetic and NIDDM subjects after manipulation of in vitro exposure to insulin and glucose.

Methods

Subjects. 26 male subjects provided muscle tissue for these studies. Glucose tolerance was determined from a 75-gram oral glucose tolerance test (19). The experimental protocol was approved by the Committee on Human Investigation of the University of California, San Diego. Informed written consent was obtained from all subjects after explanation of the protocol.

Materials. Human biosynthetic insulin was kindly supplied by Dr. Ron Chance of Eli Lilly, Inc. (Indianapolis, IN). Cell culture materials were purchased from Irvine Scientific (Irvine, CA) except for skeletal muscle basal medium (SkGM), which was obtained from Clonetics Corp. (San Diego, CA). Fetal calf serum was purchased from Irvine Scientific. Bovine serum albumin (BSA, Cohn fraction V) was supplied by Boerhinger Mannheim (Indianapolis, IN). 2-[1,2-^{3}H]-deoxy-D-glucose and L-[1-^{14}C]glucose were purchased from New England Nuclear (Boston, MA). Polyclonal antisera against GLUT1 (RaGLUTRANS) and GLUT4 (RaIRGT) were purchased from East Acres Biologicals (Cambridge, MA). An anti–rabbit IgG conjugated with horseradish peroxidase and the ECL chemiluminescence kit were obtained from Amersham (Arlington Heights, IL). Pepstatin, leupepin, phenylmethylsulfonyl fluoride (PMSF), 2-deoxyglucose and L-glucose were purchased from Sigma Chemical Co. (St. Louis, MO).

Tissue biopsy and cell isolation. The method for muscle cell isolation and growth has been detailed previously (18). Briefly, percutaneous muscle biopsies were obtained from the lateral portion of the vastus lateralis muscle using a 5-mm side-cutting needle. The method used for clonal growth of human skeletal muscle cells is a modification of those of Blau and Webster (20) and Sarabia et al (21). Tissue was dissociated by treatment with 0.05% trypsin/EDTA. Muscle cells were then plated and grown in a 5% CO_2 incubator in the specially formulated human skeletal muscle growth media (SkGM) as described by Ham et al. (22). Modifications in the media insulin concentrations during the growth phase are described in the text and figure legends.

Myoblast fusion and differentiation. Upon attaining confluency, the media was changed to alpha minimal essential media containing penicillin (100 U/ml), streptomycin (100 μg/ml) and 2% FCS for 3–5 d. Media was changed every 2–3 d. The media insulin and glucose concentrations were also manipulated during this period. Unless noted, cells were grown in media containing 10% FCS; the measured insulin concentration was 108 pM. The standard media insulin level during differentiation was 22 pM. Any changes from these values are indicated in the figures or legends.

The extent of muscle differentiation was monitored by visual estimates of multinucleated myotubes from fluorescent stained plates (23). Five fields from each well were observed under a magnification of 40 and the percentage of multinucleated cells determined. This evaluation was performed on parallel plates of cultures used for glucose transport studies.

Glucose transport assay. The mechanics of glucose transport measurement were modified from those described by Klip et al. for L6 myocytes (24). Cells were grown in 12-well plates. When HSMC had fully differentiated (4 d after confluence) they were rinsed 2× with alpha-MEM, supplemented with antibiotics, and glucose at the same concentration as regular culture media (5.5 mM) plus 0.1% BSA, pH 7.4. Media was added to the cells together with insulin (0–33 pM) and the cells incubated for 60–90 min in a 5% CO_2 incubator. Plates were then removed from the incubator and wells rapidly washed 4× with room temperature reaction buffer; 150 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM $CaCl_2$, 2.5 mM NaH_2PO_4, 10 mM Hepes, 0.1% BSA, pH 7.4. 1 ml of this buffer was added to each well and the transport reaction started by the addition of 10 μl substrate (^{3}H-2-deoxyglucose/^{14}C-L-glucose, 0.1 μCi, final concentration = 0.01 mM). Reactions were halted after 15 min by aspirating off the reaction mixture and rapidly rinsing each well 5× with 4°C PBS. Cells were solubilized by addition of 0.5 ml 0.1N NaOH and incubated with shaking. An aliquot (100 μl) of the suspension was removed for protein analysis using the Bradford method (25). After solubilization, 400 ml of the suspension was placed in a scintillation vial, neutralized with 1.0N HCl and scintillation fluid added. L-glucose was used to correct each sample for the contribution of diffusion.

Membrane preparation. Cells for membrane preparation were grown in 100 mm dishes and total membranes prepared by the method developed by Walker et al for L6 cells (26). Cells were scraped from dishes with 3 ml of the following buffer: 250 mM sucrose, 5 mM NaN_3, 2 mM EGTA, 200 μM PMSF, 1 μM leupeptin, 1 μM pepstatin, 20 mM Hepes, pH 7.4. All steps were performed at 4°C. Cells were collected by centrifugation and homogenized with a Dounce homogenizer. After centrifugation at 750 *g* for 3 min, the pellet was rehomogenized, recentrifuged, and the supernatants combined. Centrifugation of the supernatant at 190,000 *g* for 60 min produced a total membrane pellet. The membranes were resuspended in homogenization buffer and protein content determined.

Detection of glucose transporter proteins. Membrane preparations were diluted 1:1 in 2× Lamelli's buffer without β-mercaptoethanol (27) and heated for 5 min at 90°C. Proteins were separated on 10% SDS-PAGE gels and then transferred to nitrocellulose (28). GLUT1 was identified using a rabbit polyclonal antisera against the rat brain glucose transporter (RaGLUTRANS, East Acres Biologicals) which also recognizes human GLUT1. A polyclonal antisera specific for GLUT4 (RaIRGT) was also employed. The second antibody was anti–rabbit IgG conjugated with horseradish peroxidase. Immune complexes were detected using an enhanced chemiluminescence kit. Exposure was limited to the linear range of density as determined by concentration curves established with human skeletal muscle total membranes, included as an internal control. Quantitation was performed with a scanning laser densitometer (Stratoscan 7000; Stratagene Cloning Systems, San Diego, CA).

Statistical analysis. Statistical significance was evaluated using Student's *t* test and two-tailed *P* values calculated. Paired analysis was performed for comparisons of acute and chronic insulin and glucose exposures in the same sets of cells. Significance was accepted at the $P < .05$ level.

Results

Cell characterization. Satellite cells from muscle grew readily in an elongated, fiber-like configuration. Fusion and differentiation of myoblasts into myotubes was initiated by changing the media of near-confluent cells from the complete basal muscle cell growth media to α-MEM containing 2% FCS. Full biochemical and morphological characterization of the cultured muscle cells has been described in detail previously (18). Greater than 90% of the differentiated cells expressed the multinucleated status characteristic of mature myotubes. Differentiation was complete by 4 d after media change and this period was uniformly employed in all cases. The extent of differentiation was monitored for all variations in the culture conditions. The extent and time course of differentiation was similar in HSMC from control and NIDDM subjects.

Glucose transport. In our initial report characterizing the HSMC system, cells were found to have specific, carrier-mediated glucose transport that was stimulated by insulin (18). HSMC were prepared from additional nondiabetic control and NIDDM subjects whose clinical characteristics are summarized in Table I; the number of subjects studied in each series of experiments is given in the legends to the figures. As reported

Table I. Subject Characteristics

Group	Age	BMI	Insulin	FPG	2 HR OGTT Glucose	2 HR OGTT Insulin
	yr	kg/M2	pM	mM	mM	pM
Nondiabetic (15)	42±2	27.9±1.5	75±14	5.2±0.1	6.1±0.3	424±84
NIDDM (11)	50±2§	31.6±1.0*	137±17§	10.2±1.6‡	16.8±2.1‡	371±106

BMI, body mass index; *FPG*, fasting plasma glucose; *OGTT*, 75-gram oral glucose tolerance test; subjects studied from each group are indicated in parentheses. * $P < 0.05$; § $P < 0.01$; ‡ $P < 0.0005$.

previously (18), there were significant differences in glucose transport activity between HSMC from normal and NIDDM subjects. For the sets of cultures used in the current studies, basal transport activity in NIDDM cells (10.5±1.6 pmol/mg protein/min) was reduced to 50–60% of the value in normal cells (17.9±3.9, $P < 0.0005$). Insulin-stimulated transport activity was also lower in NIDDM cells (16.7±1.9 vs 22.0±1.1, $P < .005$). Thus, HSMC from NIDDM subjects had a reduced capacity to transport glucose but were still able to respond to insulin, though the absolute magnitude of the response was significantly reduced. The insulin-stimulated increments in glucose transport above basal activity were 10.5±1.5 vs 6.0±1.3, $P < .025$) in NC and NIDDM cells, respectively.

Insulin regulation of glucose transport activity and transporter protein. Impaired transport activity and reduced GLUT1 expression in HSMC from NIDDM subjects, both of which have been reported previously (18), could be the result of a number of factors. Two possibilities include acquired defects from the hyperinsulinemia and hyperglycemia present in NIDDM (Table 1). We exploited the ability to independently manipulate variables in the cultured cell system to study the effect of insulin level on transport activity. HSMC from NC subjects (n = 4) were grown in SkGM media of varying insulin concentrations until they became confluent and began to fuse. Insulin concentrations tested were: 22 pM, 108 pM, (supplied by 2 and 10% FCS, respectively), and 30 μM, the level designated in Ham's original complete media (22). Cell growth was slightly slower at the lowest insulin level, requiring an additional 12–24 h before cells began to fuse. The time course and extent of differentiation (defined by percentage of multinucleated cells) was not influenced by variations in media insulin. A fivefold increase in media insulin during the growth stage (from 22 to 108 pM) had only minor effects on basal transport, while differentiation in 30 μM insulin caused a doubling (102±6% increase above 22 pM control, $P < 0.025$). Acutely insulin-stimulated transport behaved in the opposite manner, decreasing as media insulin was raised (to 58±9% of 22 pM control at 30 μM, $P < .05$). These divergent effects on transport were not readily reversible, for reducing media insulin from 30 μM to 22 pM during the differentiation period (4 d) made no difference in final transport activity, either basal or acutely insulin stimulated.

An elevation in basal transport, as noted above, coupled with the finding of reductions in insulin-stimulated transport activity would be expected to impair insulin responsiveness. This possibility was confirmed by the results presented in Fig. 1, which compares the extent of maximal acute insulin stimulation under the conditions of varying media insulin levels. There was a progressive, concentration-dependent decrease in insulin responsiveness. Cells grown at the highest insulin level (30 μM) were insulin resistant with regard to glucose transport (6±9% stimulation above basal transport). The failure to respond to acute insulin treatment was not due to persistent insulin receptor occupancy, for reducing the media insulin level (from 30 μM to 22 pM) during the 4-d differentiation period did not reverse the resistance.

Insulin regulation of glucose transport was explored further by growing additional sets of HSMC from NC and NIDDM subjects in SkGM-10% FCS with final insulin concentrations of either 108 pM or 30 μM. The media insulin concentration for all cultures during the fusion period was 22 pM. As was noted above, varying media insulin had no effect on the extent of multinucleation. With culture of NC cells in high insulin, basal glucose transport activity was increased to 161±22% ($P < 0.025$) of that in cells in low insulin (28.5±8.1 vs 17.9±3.9,

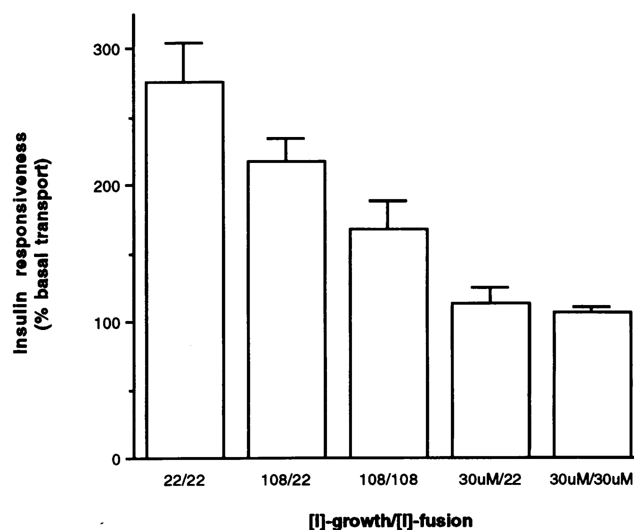

Figure 1. Effect of media insulin on insulin responsiveness in HSMC from nondiabetic control subjects. Cells were grown in media with the indicated insulin concentrations and changed to alpha-MEM 2% FCS supplemented with insulin (when indicated) for 4 ds. Conditions indicated as—$[\text{Insulin}]_{\text{growth}}/[\text{Insulin}]_{\text{fusion}}$. Unless noted otherwise all concentrations are given as pM. Cells were incubated±insulin (33 nM, 90 min) before 2-deoxyglucose (DOG) assay. Results are expressed as insulin responsiveness normalized against the basal transport activity in each experiment in cells grown under the same conditions. Results are mean±SEM, $n = 4$.

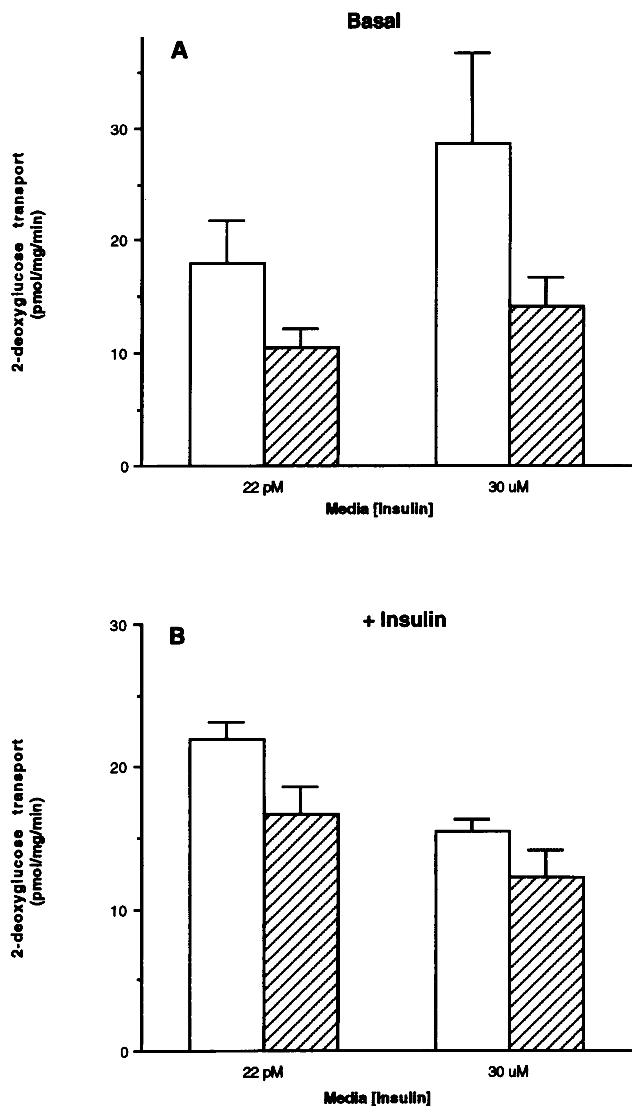

Figure 2. Effect of media insulin on glucose transport activity in HSMC from NC (*open bars*) and NIDDM (*hatched bars*) subjects. Cells were grown in low (108 pM) or high (30 μM) insulin containing media and then fused in media containing 22 pM insulin prior to DOG transport assay. (*A*) Basal (no added insulin) transport activity. (*B*) Acute insulin (33 nM)-stimulated transport. Results are mean±SEM, $n = 7$ for NC, $n = 9-13$ for NIDDM.

$P < .05$; Fig. 2 *A*). Insulin-stimulated transport in HSMC cultured in high insulin was reduced to 73±5% of the value in low insulin NC cells (22.0±1.1 vs 15.5±0.8, $P < 0.025$; Fig. 2 *B*). Cells from nondiabetic subjects grown in low insulin were insulin responsive (90±35% stimulation) while cells grown in high insulin were essentially insulin resistant (10±9% stimulation, $P < 0.05$), the expected result of opposing changes in basal and insulin-stimulated transport activities.

Regulation of glucose transport in HSMC from NIDDM subjects by media insulin was similar to that in normal cells (Fig. 2). Growth in high insulin increased basal transport by 43±15% compared to low insulin ($P < 0.01$). Insulin-stimulated transport was decreased after growth in high insulin to 74±7% of the value in low insulin ($P < 0.005$). The major effect of culture in high insulin on NIDDM cells was to reduce

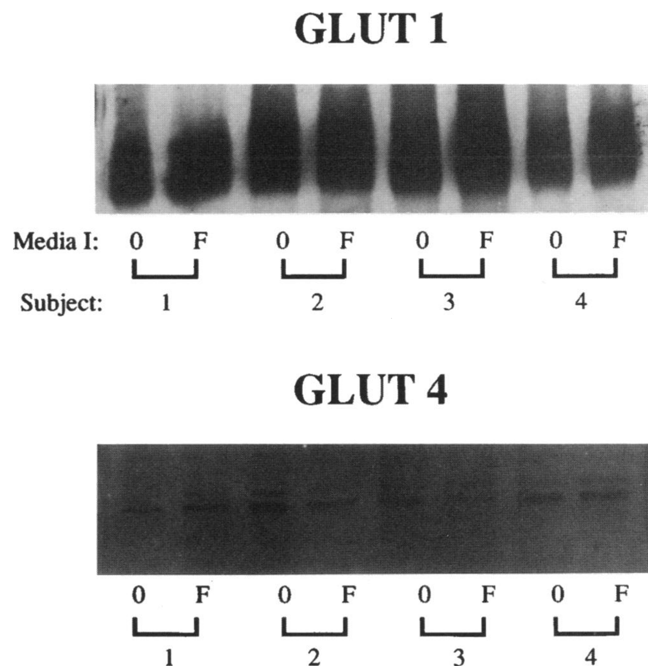

Figure 3. Effect of chronic insulin exposure on glucose transporter isoform expression in HSMC. Cells were grown as described in the legend to Fig. 2, total membranes prepared, followed by SDS-PAGE, Western blotted with polyclonal antibodies against GLUT1 and GLUT4 and visualized by chemiluminescence. Media [I] = 108 pM—0; 30 mM—F, cells from four separate subjects.

insulin responsiveness; from 84±20% stimulation in low insulin to 12±6% ($P < 0.025$). Thus, the pattern and magnitude of changes in NIDDM cells was similar to that in nondiabetic cells.

The effects of media insulin on glucose transporter expression were explored in total membranes prepared from parallel plates cultured at the same time as those for transport assays. As shown in Fig. 3, membranes were Western blotted with polyclonal antisera specific for GLUT1 and GLUT4, detected by enhanced chemiluminescence and quantitated by scanning densitometry. Quantitation of the results are presented in Fig. 4. Membranes from NC cells cultured in high insulin media displayed a 62±19% increase in GLUT1 protein expression compared to low insulin media. The magnitude of this change was similar to that seen in basal glucose transport activity (Fig. 2 *A*). GLUT4 protein levels were not influenced by media insulin (Fig. 4 *B*), even though insulin-stimulated activity declined by 27% in these cells. Thus, at least for nondiabetic control HSMC, changes in GLUT1 levels could, in large part, account for changes in basal GT activity, but a dissociation was present between changes in insulin-stimulated activity and GLUT4 protein expression. As these analyses were performed in total membrane preparations, no information about transporter localization was obtained.

The pattern of insulin effects on glucose transporter isoform expression in NIDDM cells was similar to that in NC cells (Fig. 4). GLUT1 protein level increased by 64±6% with culture in high insulin ($P < 0.05$), but remained significantly lower than that in NC cells under both growth conditions ($P < 0.05$). GLUT4 expression was unaltered by chronic elevation of media

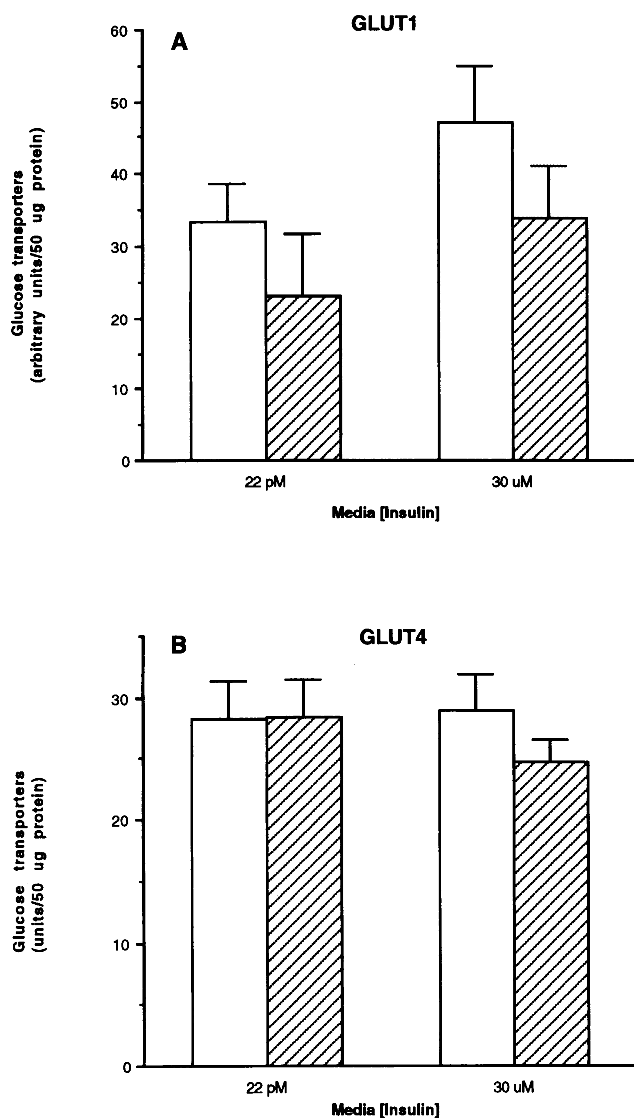

Figure 4. Quantitation of insulin effect on glucose transporter isoform expression in HSMC. Cells were grown in low (108 pM/22 pM) or high (30 μM/22 pM) insulin containing media as described in Fig. 3. Quantitation of Western blots was as described in Methods. Results are mean±SEM on membranes prepared from HSMC of NC (*open bars, n* = 5) or NIDDM (*hatched bars, n* = 6) subjects probed for GLUT1 or GLUT4.

insulin (Fig. 4 *B*), and, again, was not different between the two groups.

Glucose regulation of glucose transport activity. The independent ability of glucose to regulate glucose transport was investigated by culturing HSMC under euglycemic (5 mM) and hyperglycemic (10 and 20 mM) conditions at fixed insulin concentrations. Media glucose concentrations were varied during the 4-d period of fusion and differentiation. The combined effects of glucose and insulin were also studied by changing the media insulin level during this period. Cells were exposed to the lower glucose level (5 mM) during the 60–90-min insulin treatment. With this protocol media glucose had no influence on the extent of differentiation or cellular protein content.

For HSMC from normal subjects grown in the low insulin containing media, culture under hyperglycemic conditions had no influence on either basal or insulin-stimulated glucose transport rates (Table II). To reduce the effect of variation between subjects, which would obscure differences in individual cultures, results were normalized against the transport activity in cells grown at 5 mM glucose for each set of cells. Total membrane levels of GLUT1 were not influenced by hyperglycemia (20 mM, not shown). Insulin responsiveness was also not significantly altered by hyperglycemia (70±18% stimulation in 5 mM glucose vs 54±9% in 10 mM and 67±18% in 20 mM). Differentiation in high insulin containing media down-regulated insulin-stimulated transport, rendering the cells essentially insulin resistant (17±8% stimulation at 5 mM glucose, 12±12% at 10 mM and 12±8% at 20 mM). The combination of hyperinsulinemia and hyperglycemia also significantly reduced both basal and insulin-stimulated transport activities (Table II).

Table II. Glucose Regulation of Glucose Transport in HSMC

Group	Media [G]	Media [I]			
		22 *pm*		30 μM	
		Basal	+Ins	Basal	+Ins
	mM				
NC (6)	10	100±11	105±12	85±12	95±8
(8)	20	99±7	96±5	82±8*	80±6§
NIDDM (3)	10	98±9	80±5*	98±17	82±9*
(8)	20	91±3‖	85±1‖	72±9†	76±10*

HSMC were fused and differentiated in 5, 10, or 20 mM glucose at the indicated media insulin concentrations, followed by deoxyglucose transport assay after acute treatment in the absence (basal) or presence of insulin (33 nM, 90 min). Transport activities in cells grown in 10 or 20 mM glucose are expressed as percent of activities in paired cells in 5 mM glucose. Numbers of independent cultures studied are given in parentheses. Results are mean±SEM: * $P < 0.05$; ‡ $P < 0.025$; § $P < 0.01$; ‖ $P < 0.005$ against 5 mM controls.

A somewhat different pattern of behavior for the effects of glucose and insulin were observed in HSMC from NIDDM subjects. Culture in high glucose containing media (20 mM) caused a small, but statistically significant, decrease in basal transport when a low insulin level (22 pM) was maintained (Table II); there was no effect seen at 10 mM. In addition, unlike the case with NC cells, there was an even greater decrease in insulin-stimulated transport activity after exposure to elevated glucose. Insulin responsiveness was reduced from a 57±8% stimulation at 5 mM glucose to 30±16% at 10 mM and 32±8% after differentiation in 20 mM ($P < 0.005$). The combination of high glucose and insulin in the media resulted in further decreases in both basal and insulin-stimulated transport activity. As expected, the cells showed no acute insulin response (8±8% stimulation at 5 mM glucose, 0±15% at 10 mM, 5±4% at 20 mM). Thus, NIDDM cells were more sensitive than NC cells to the detrimental effect of glucose alone to regulate transport activity, with insulin-responsive transport the most sensitive. However, glucose was able to act together with insulin to down-regulate transport in both normal and NIDDM cells.

Discussion

Even though the importance of skeletal muscle in insulin resistance and impaired glucose tolerance is well recognized, it has

been difficult to elucidate cellular mechanisms of impaired glucose uptake in this tissue. We have modified and characterized a culture system of human skeletal muscle cells that has proved useful in addressing some of these difficult questions. Our previous report (18) presented evidence that the HSMC culture protocol produces cells with the morphological, biochemical and metabolic properties of differentiated skeletal muscle. On a morphological basis, HSMC taken through the fusion/differentiation protocol express the multinucleated phenotype characteristic of mature skeletal muscle. Upon differentiation, HSMC also show large increases in the expression of the muscle specific isozyme of creatine kinase as well as sarcomeric α-actin (18). Differentiated HSMC also express the fat/muscle specific, insulin-regulatable glucose transport isoform, GLUT4, which is an additional marker of the muscle phenotype (9, 29). The most significant metabolic property of differentiated HSMC is that they display insulin responsiveness of glucose transport, as well as insulin-stimulated pyruvate dehydrogenase and glycogen synthase activities (18), which are all crucial insulin-sensitive processes in muscle. Our previous data also revealed that HSMC from NIDDM subjects displayed decreased glucose transport activity. Therefore, the transport defect in HSMC was reflective of differences in peripheral glucose disposal measured in vivo (3, 7). There was also impaired insulin-stimulated glucose transport activity, in the presence of normal total GLUT4 content (18), a finding that is also in agreement with numerous in vivo studies (6, 30–33), providing additional evidence that HSMC are truly reflective of the status of skeletal muscle. The retention of this glucose transport defect, even after extended time in culture, suggests that impaired glucose transport in NIDDM is, at least in part, the result of either a genetic or a non-reversible acquired defect and not necessarily the sole result of the abnormal hormonal and metabolic milieu.

In addition to possible genetic factors, the roles of hyperinsulinemia and hyperglycemia in influencing muscle glucose transport have been difficult to separate in NIDDM. We exploited the ability to independently vary these levels in cultured muscle cells to determine their individual effects and to ascertain if the same regulatory mechanisms were active in NC and NIDDM cells. Growth of cells from nondiabetic subjects in the presence of elevated insulin levels and normoglycemia resulted in an increase in both basal glucose transport and GLUT1 protein. Such a finding was to be expected, as insulin has been shown to have chronic effects to elevate GLUT1 expression in a number of tissues (34) and cell systems (35). Hyperinsulinemia also increased basal transport and GLUT1 expression in cells from NIDDM subjects. The results suggest that the same mechanism(s) appears operative in normal and NIDDM cells to respond to hyperinsulinemia by increasing GLUT1 levels. In fact, culture under hyperinsulinemic conditions increased GLUT1 expression in NIDDM cells to nearly the same level as in normal controls grown in low insulin-containing media. However, despite this compensatory response NIDDM cells were not able to normalize transport and basal transport remained impaired, suggesting that additional defects in GLUT1 function or localization could also exist in NIDDM.

Divergence between chronic insulin effects on basal and insulin stimulated transport have been demonstrated in vivo (36), in isolated muscles (37, 38), in cultured muscle cells (39–41), and in primary cultured adipocytes (42). A similar pattern was present in the HSMC system. While hyperinsulinemia increased basal transport, insulin-stimulated transport was down-regulated by 30–40%. This decrease, and the resultant progressive loss of insulin responsiveness, is consistent with hyperinsulinemia as a cause of impaired glucose transport in NIDDM. The use of a pharmacologic insulin concentration (30 μM) in the hyperinsulinemia studies was determined in part by the composition of the original SkGM media (22). It was also reasoned that such an elevated level might be more likely to reveal effects on transport over the limited treatment period (4 d); longer treatment periods reflective of chronic hyperinsulinemia in NIDDM might reveal effects at more physiologic insulin levels. A strikingly similar finding to that in HSMC, that prolonged hyperinsulinemia could increase basal activity while impairing insulin stimulation, was reported by Del Prato et al for whole body glucose disposal and nonoxidative disposal determined from in vivo clamp studies (36). This convergence of results between in vivo observations and measurements in HSMC provide further support that HSMC are reflective of the behavior of skeletal muscle.

Growth under hyperinsulinemic conditions could also explain the modest insulin responsiveness (27% stimulation) reported in HSMC from normal subjects by Sarabia et al. (21). Indeed, it is interesting to note that absolute rates of insulin-stimulated transport in normal cells are reduced after hyperinsulinemic culture to the same level as in NIDDM cells. The failure of hyperinsulinemia to alter GLUT4 levels in total membranes, even as insulin-stimulated transport is impaired, is the same behavior as seen in NIDDM cells and also suggests a central role for insulin. In addition, however, NIDDM cells can down-regulate stimulated transport even further. This would suggest that the transport defect in NIDDM cells has multiple causes.

Another fact mitigating against an exclusive role of hyperinsulinemia to cause impaired transport is that the transport defect is retained in NIDDM cells even after passage and an extended time of culture in low insulin levels. Hyperinsulinemia and NIDDM may represent two circumstances where GLUT4 protein levels alone cannot explain reduced insulin-stimulated transport activity. The current data, where GLUT4 is measured in total membranes, does not permit us to distinguish between the other major possibilities of impaired translocation or defects in GLUT4 intrinsic activity. Several investigators have reached the same conclusion that little or no relationship exists between muscle insulin-stimulated glucose transport and GLUT4 content in insulin resistant states and after experimental manipulations such as insulin infusion (31, 32, 43). Therefore, factors other than total GLUT4 protein level must be important in determining insulin-stimulated glucose uptake in skeletal muscle. The decreased expression of GLUT1, reported previously (18) and confirmed in the present report might be one such factor.

Glucose transport is also regulated by the media glucose concentration, although the response to glucose is different in HSMC from NC and NIDDM subjects. Hyperglycemia alone is able to down-regulate both basal and insulin-stimulated transport in NIDDM cells, a behavior shared with L6 myocytes (41) and incubated soleus muscle (44). However, transport in NC HSMC was not altered by maintenance under hyperglycemic conditions, as has been reported for incubated epitrochlearis muscle (45). The physiologic consequences of a small reduction in glucose transport activity in NIDDM cells in response to hyperglycemia is uncertain. It is possible that prolonged hyperglycemia, extending beyond the period currently employed in the HSMC system could result in greater changes. However,

the greater sensitivity of NIDDM cells to elevated glucose, especially insulin stimulated transport, would result in even greater impairments in transport activity under the hyperglycemia characteristic of NIDDM. The combination of hyperglycemia and hyperinsulinemia had progressively greater effects to down-regulate transport in HSMC from both NC and NIDDM subjects. These results are similar to those reported in primary cultured adipocytes (46) and hindlimb (37, 47) exposed to elevated glucose and insulin levels. It is obvious that the hyperglycemic and hyperinsulinemic milieu characteristic of diabetes has the potential to significantly exacerbate the impaired glucose transport exhibited by skeletal muscle cells of NIDDM subjects.

In summary, the HSMC culture system has shown itself to be a tool with high utility to increase our understanding of regulation of glucose transport and metabolism in skeletal muscle. This system meets the needs described by several investigators for studies on insulin action to be performed in classic insulin target tissues (9, 48). Not only do HSMC maintain the morphological, biochemical and metabolic properties of skeletal muscle, but, at least with regard to glucose transport, they are reflective of in vivo defects that occur in NIDDM (18). An advantage of the HSMC system is that it is possible to independently study the contributions of NIDDM, insulin and glucose to regulation of glucose transport and metabolism. These factors cannot easily be separated in either in vivo studies or those using isolated muscle. The current studies reveal that NIDDM, insulin and glucose can each contribute to impairments of glucose transport function. There is also an interesting difference in HSMC from NIDDM subjects compared to non-diabetic subjects in addition to impaired glucose transport; an increased sensitivity to transport down-regulation by hyperglycemia. All of these differences may contribute to the glucose intolerance present in NIDDM, together with the demonstrated ability of hyperinsulinemia to also down-regulate insulin-stimulated transport.

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