Effects of cellular ATP depletion on glucose transport and insulin signaling in 3T3-L1 adipocytes

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Kang, Jione, Emma Heart, and Chin K. Sung. Effects of cellular ATP depletion on glucose transport and insulin signaling in 3T3-L1 adipocytes. Am J Physiol Endocrinol Metab 280: E428–E435, 2001.—Glucosamine induced insulin resistance in 3T3-L1 adipocytes, which was associated with a 15% decrease in cellular ATP content. To study the role of ATP depletion in insulin resistance, we employed sodium azide (Na3N) and dinitrophenol (DNP), which affect mitochondrial oxidative phosphorylation, to achieve a similar 15% ATP depletion. Unlike glucosamine, NaN3, and DNP markedly increased basal glucose transport, and the increased basal glucose transport was associated with increased GLUT-1 content in the plasma membrane without changes in total GLUT-1 content. These agents, like glucosamine, did not affect the early insulin signaling that is implicated in insulin stimulation of glucose transport. In cells with a severe 40% ATP depletion, basal glucose transport was similarly elevated, and insulin-stimulated glucose transport was similar in cells with 15% ATP depletion. In these cells, however, early insulin signaling was severely diminished. These data suggest that cellular ATP depletion per se does not induce insulin resistance in 3T3-L1 adipocytes.

Insulin resistance, defined as an impairment of insulin action in the stimulation of glucose transport, is a common characteristic of both type 2 and poorly controlled type 1 diabetes (2, 17). Previous studies have demonstrated that hyperglycemia in diabetic humans (4, 6, 16) and rats (8) could induce or worsen insulin resistance, which was reversed by normalization of blood glucose. A number of studies have suggested that glucose and glucosamine share a common pathway to induce insulin resistance and that an increased flux through the hexosamine biosynthetic pathway may play a role in their induction of insulin resistance in insulin-sensitive tissues in vivo and in vitro (9, 12). In cells, glucosamine enters the hexosamine biosynthetic pathway distal to the first rate-limiting step that is catalyzed by glutamine, fructose-6-P amidotransferase, is metabolized to produce UDP-N-acetylglucosamines, and has been proven to be more potent than glucose in induction of insulin resistance (9, 11).

We have previously demonstrated (5) that, in 3T3-L1 adipocytes, glucosamine could induce insulin resistance in a time- and dose-dependent manner. In glucosamine-treated 3T3-L1 adipocytes, there was impaired insulin-stimulated trafficking of the major insulin-sensitive glucose transporter GLUT-4. In these cells, however, there was no significant impairment of early insulin signaling, including insulin receptor functions, phosphorylation of insulin receptor substrates (IRS)-1/2, and phosphatidylinositol 3-kinase (PI 3K) activation (5). In these cells, insulin stimulation of GLUT-1 trafficking was normal. In contrast to our observations, Hresko et al. (7) reported that induction of insulin resistance by glucosamine in 3T3-L1 adipocytes was due to a severe cellular ATP depletion and impairment of all insulin-signaling pathways.

In the present study, we investigated the role of cellular ATP depletion in the induction of insulin resistance and early insulin signaling in 3T3-L1 adipocytes. For study, we employed sodium azide (Na3N) and dinitrophenol (DNP), which affect mitochondrial oxidative phosphorylation and subsequently lower cellular ATP content. We failed to induce insulin resistance in 3T3-L1 adipocytes with Na3N and DNP. Our data suggest that cellular ATP depletion per se does not account for induction of insulin resistance by glucosamine in 3T3-L1 adipocytes.

Experimental Procedures

Materials and reagents. Monoclonal antibodies to phosphotyrosine (α-PY) were purchased from Upstate Biotechnology (Lake Placid, NY); rabbit polyclonal anti-phospho-Akt1 (Ser473) antibody (α-phospho-Akt1) and rabbit polyclonal anti-phospho-ERK (Thr202/Tyr204) antibody (α-phospho-ERK) were from New England Biolabs (Beverly, MA); rabbit polyclonal anti-p70S6 kinase antibody (α-p70S6k) were from Santa Cruz Biotechnology (Santa Cruz, CA); and rabbit polyclonal anti-GLUT-4 antibody (α-GLUT-4) were from Biogenesis (Sandown, NH). Rabbit polyclonal anti-GLUT-1 antibody (α-GLUT-1) was a generous gift of Dr. Amira Klip (Toronto, Canada). 2-deoxy-d-[1,2-3H]glucose (2-[3H]DG, 26.2 Ci/mol) was synthesized as described previously (10). 2-[3H]DG was dissolved in DMSO at a concentration of 1 M. The carrier-free 2-[3H]DG was stored at -20°C before use. Deoxyglucose uptake was measured as described previously (2). Glucose uptake was determined by HPLC analysis of the 2-[3H]DG released from cellular 2-[3H]DG in 3T3-L1 adipocytes grown on coverslips (5).

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mmol), 3-O-[methyl-3H]glucose (3-[3H]OMG, 81.5 Ci/mmol), and [γ-32P]ATP (26.2 Ci/mmol) were purchased from NEN Life Science Products (Boston, MA). Other chemicals were from Sigma (St. Louis, MO), unless specified otherwise.

Maintenance and treatment of 3T3-L1 adipocytes. 3T3-L1 fibroblasts obtained from Dr. Howard Green at Harvard University (Boston, MA) were grown and differentiated into 3T3-L1 adipocytes, following the procedure of García de Herreros and Birnbaum (3) with some modifications. For experiments, cells were used on 10–14 days of postdifferentiation, at which time >90% of the cells exhibited the fatty phenotype as judged by phase contrast microscopy.

ATP analysis. To study the effects of NaN3, DNP, and glucosamine on cellular ATP contents in 3T3-L1 adipocytes, we incubated cells in 6-well plates for 5 h with serum-free DMEM containing varying concentrations of NaN3, DNP, and glucosamine. For glucosamine incubation, d-mannitol was used as a control sugar and was added to cells to compensate for the final osmolality. Cells were washed with ice-cold PBS and solubilized in 0.6 M NaOH, followed by heating and neutralization with 1 M HCl. These cell lysates were then incubated in MIX solution (50 mM Tris-HCl, pH 8.1, 0.02% BSA, 1 mM MgCl2, 0.5 mM dithiothreitol (DTT), 0.1 mM glucose, 0.1 mM NADP+ p, 2 μl/ml hexokinase, 0.5 μM/mg leuconostoc mesenteroides glucose-6-phosphate dehydrogenase) for 20 min at room temperature to generate NADPH (10). Then, the excess NADP+ was destroyed by heating in 0.7 N NaOH at 60°C for 20 min, followed by heating in FES solution (6 N NaOH, 10 mM imidazole base, 0.01% H2O2) at 60°C for 20 min to enhance the fluorescence signal of NADPH. The fluorescence was measured at 340 nm excitation and 460 nm emission and was converted to actual ATP content (μM) by use of an ATP standard curve. ATP concentrations were normalized for protein concentration determined by the Bradford method.

Measurements of 2-DG uptake and 3-OMG transport. To study the effects of NaN3, DNP, and glucosamine on 2-DG uptake, we preincubated cells with specified concentrations of NaN3, DNP, and glucosamine in serum-free medium for 5 h. For glucosamine study, d-mannitol was added to media to compensate for the same final 50-mM sugar concentration. Cells were washed with warm PBS, placed in buffer A (1.47 mM K2HPO4, pH 7.4, 140 mM NaCl, 1.7 mM KCl, 0.9 mM CaCl2, 0.9 mM MgSO4, 0.1% BSA) and incubated for 5 min with or without 100 nM insulin at 37°C. Next, 2-[3H]DG (0.2 μM, 0.5 μCi) was added, and incubation was continued for 5 min. Non-specific 2-DG uptake was measured in the presence of 10 μM cytochalasin B, which accounted for ~10% of basal 2-DG uptake and was subtracted from all experimental data (5). Cells were washed in ice-cold PBS and solubilized for 30 min at 0°C. Aliquots were measured for radioactivity in a liquid scintillation β-counter and for protein content by the Bradford method (5).

Employing 3-OMG, we also measured glucose transport. Similar procedures were used as for 2-DG uptake, except that cells were treated with 3-[3H]OMG (0.1 mM, 0.5 μCi) for 2 min.

Preparation of cell lysates and Western blot analysis. 3T3-L1 adipocytes were preincubated for 5 h with the specified concentrations of NaN3, DNP, and glucosamine in serum-free medium. Cells were then incubated for 5 min with or without 100 nM insulin, solubilized for 30 min in 1% NP-40 lysis buffer (20 mM Tris, pH 8.0, 1% NP-40, 137 mM NaCl, 1 mM CaCl2, 1 mM MgCl2, 10% glycerol, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 2 mM Na3VO4, 10 mM NaF, 10 mM Na2P2O7, 1 μg/ml leupeptin, 1 μg/ml aprotinin). After microcentrifugation for 15 min at 15,000 g, the supernatant (cell lysates) was assayed for protein content. The cell lysates (20–60 μg) were resolved by 8% SDS-PAGE followed by electrophoretic transfer of proteins onto Hybond-P membranes (Amersham, Buckinghamshire, England). The membranes were then probed with α1-PY (1:10,000), α-phospho-Akt1 (1:2,000), α-p70S6k (1:4,000), and α-phospho-ERK (1:2,000, respectively (5). PI 3K enzyme assay. Cell lysates (0.5 mg) prepared in 1% NP-40 lysis buffer as described above were incubated for 3 h with α1-PY (1 μg) at 4°C followed by 2-h incubation with protein A-Sepharose (1:1 slurry). α1-PY immunoprecipitates were then assayed for 10 min at 25°C in reaction mixture containing phosphatidylinositol (0.2 mg/ml) and [γ-32P]ATP (40 μM, 5 μCi) followed by thin-layer chromatography and autoradiography (5).

Subcellular fractionation. Cells in 150-mm dishes were treated for 5 h in serum-free medium with either NaN3 or DNP, and insulin was added during the last 30-min incubation. Cells were then homogenized, and a subcellular fractionation procedure was carried out to prepare the plasma membrane and low-density membranes as previously described (5). Plasma membranes and low-density membranes were resolved by Western blot analysis with α-Glut-1 (1:1,000) and α-Glut-4 (1:1,000).

RESULTS

Effects of NaN3, DNP, and glucosamine on cellular ATP contents in 3T3-L1 adipocytes. We had previously demonstrated (5) that glucosamine induced insulin resistance in 3T3-L1 adipocytes in a time- and dose-dependent manner. In those cells, insulin stimulation of GLUT-4 trafficking was impaired, resulting in impaired insulin stimulation of glucose transport. To study the effects of glucosamine on cellular ATP content, we treated cells with 0–50 mM glucosamine for 5 h and measured cellular ATP content as described in EXPERIMENTAL PROCEDURES. Glucosamine at 25 mM decreased cellular ATP content by 15%, which level was maintained with ≤50 mM glucosamine (Fig. 1).

To study the role of cellular ATP depletion in the induction of insulin resistance, we employed a mitochondrial oxidative phosphorylation uncoupler, DNP, and an inhibitor of electron transport, NaN3. These agents inhibit mitochondrial ATP production and thus lower cellular ATP content. Cells were treated for 5 h with varying concentrations of these agents and were solubilized and measured for cellular ATP content as described in EXPERIMENTAL PROCEDURES. Both NaN3 (0–10 mM) and DNP (0–0.2 mM) decreased cellular ATP content in a dose-dependent manner with a maximal decrease of 40% at 10 and 0.2 mM, respectively (Fig. 1). When cells were treated for 5 h with 3 mM NaN3, 0.05 mM DNP, or 50 mM glucosamine, there was a similar 15% decrease in cellular ATP content (15.6 ± 1.3, 16.1 ± 1.7, and 14.4 ± 1.0%, respectively). A severe 40% decrease in cellular ATP content was obtained with 10 mM NaN3 (40.3 ± 1.0%) and 0.2 mM DNP (38.2 ± 1.1%).

Effects of NaN3, DNP, and glucosamine on glucose transport in 3T3-L1 adipocytes. To study the relationship between cellular ATP content and glucose transport, we treated cells for 5 h with NaN3, DNP, and glucosamine. After the cells were washed, they were...
incubated for 5 min with or without 100 nM insulin and were further incubated for 5 min with 2-[^3H]DG. Next, cells were solubilized, and cell-associated radioactivity was measured (5). When cells were treated for 5 h with 50 mM glucosamine, there was an ~50% decrease in insulin-stimulated 2-DG uptake compared with control cells (Fig. 2A). In cells treated with either 3 mM NaN₃ or 0.05 mM DNP to achieve 15% ATP depletion, basal 2-DG uptake was increased fourfold (Fig. 2A). In these cells, total 2-DG uptake after insulin treatment of cells (i.e., insulin-stimulated 2-DG uptake) was rather higher than in control cells treated

**Fig. 1.** Effects of sodium azide (NaN₃), dinitrophenol (DNP), and glucosamine (GLN) on cellular ATP content in 3T3-L1 adipocytes. 3T3-L1 adipocytes in 6-well plates were incubated for 5 h in serum-free DMEM containing various concentrations of NaN₃ (0, 1, 3, 5, 10 mM), DNP (0, 0.01, 0.03, 0.05, 0.2 mM), and GLN (0, 25, 30, 40, 50 mM). For GLN study, D-mannitol was used as a control sugar and was added to cells to compensate for the final same osmolarity. Cells were then solubilized and assayed for ATP content as described in EXPERIMENTAL PROCEDURES. Data are presented as nmol ATP/mg protein and as means ± SE of 2 or 4 separate experiments.

**Fig. 2.** Effects of NaN₃, DNP, and GLN on 2-deoxyglucose (2-DG) uptake (A) and 3-O-methyl-D-glucose (3-OMG) transport (B) in 3T3-L1 adipocytes. A: 3T3-L1 adipocytes in 24-well plates were incubated for 5 h in serum-free DMEM containing NaN₃ (0, 3, 10 mM), DNP (0, 0.05, 0.2 mM), and GLN (0 and 50 mM). Cells were then washed to remove agents and were incubated for 5 min with or without 100 nM insulin followed by additional 5-min incubation with 2-[^3H]DG (0.2 mM, 0.5 μCi). Next, cells were solubilized for 30 min in 0.03% SDS followed by liquid scintillation β-counting. Data are presented as %basal 2-DG uptake ± SE of 3 separate experiments. Basal 2-DG uptake was taken as 100%. Basal 2-DG uptake was 0.09 nmol 2-DG uptake·mg protein⁻¹·min⁻¹. B: after incubation of cells with various agents as in (A), cells were incubated for 2 min with or without 3[^3H]OMG (0.1 mM, 0.5 μCi). Next, cells were processed as in (A). Data are presented as %basal 3-OMG transport ± SE of 3 separate experiments. Basal 3-OMG transport was taken as 100%. Basal 2-DG uptake was 0.85 nmol 2-DG uptake·mg protein⁻¹·min⁻¹.
with vehicle only, by 35 and 15%, respectively. In cells treated with 10 mM NaN₃ and 0.2 mM DNP to achieve 40% ATP depletion, basal 2-DG uptake was increased fourfold, similarly to cells treated with low concentrations of NaN₃ and DNP. These data demonstrate that the pattern of 2-DG uptake, both basal and insulin-stimulated, is different in cells treated with NaN₃, DNP, and glucosamine. Furthermore, these data suggest that the use of NaN₃ and DNP to mimic the insulin resistance by glucosamine failed.

When taken up by the cell, 2-DG is first phosphorylated by hexokinase. If hexokinase is altered in cells and thus the 2-DG inside the cell is not effectively phosphorylated, 2-DG uptake will be subsequently inhibited. To study the effects of ATP depletion on glucose transport independent of hexokinase, we carried out a similar experiment with nonmetabolizable 3-[³H]OMG (Fig. 2B). As expected, insulin stimulated 3-OMG transport by threefold. In glucosamine-treated cells, there was an ~50% decrease in insulin-stimulated 3-OMG transport. In cells treated with NaN₃ and DNP, basal 3-OMG transport was increased three- to fourfold, and insulin stimulation of 3-OMG was slightly higher than basal 3-OMG transport. Similar data obtained with 2-DG uptake and 3-OMG transport strongly suggest that agents used in the study primar-
ily affect glucose transport independently of hexokinase. These data also suggest that cellular ATP depletion per se does not induce insulin resistance, and ATP depletion by NaN₃ and DNP, unlike glucosamine, increased basal glucose transport.

**Effects of NaN₃, DNP, and glucosamine on insulin-signaling proteins in 3T3-L1 adipocytes.** The insulin receptor is a tyrosine kinase requiring cellular ATP for its activation and subsequent signal transduction within the cell (15). Thus it is possible that ATP depletion within the cell, regardless of the degree of depletion, may have adverse effects on insulin receptor functions and insulin signaling proteins downstream of the insulin receptor. To address this issue in our study, we pretreated cells with 3 mM NaN₃, 0.05 mM DNP, or 50 mM glucosamine and then treated the cells for 5 min with or without insulin. These cells were next studied by Western blot analysis for phosphorylation of IRS-1/2, Akt1, p70⁵⁶⁶, and ERK1/2 and by in vitro enzyme assays for PI 3K activity. As expected, insulin greatly stimulated phosphorylation of all insulin-signaling proteins and PI 3K activity. In cells treated with glucosamine, insulin effects on most early insulin-signaling proteins were normal except for p70⁵⁶⁶, whose phosphorylation was partially diminished as determined by a decreased band shift (Fig. 3A). In cells treated with either 3 mM NaN₃ or 0.05 mM DNP, insulin's effects on most early insulin-signaling proteins were normal except for ERK1/2, whose phosphorylation was diminished by 70–80 and 35–40%, respectively (Fig. 3B). p70⁵⁶⁶ and ERK1/2 have previously been reported to play little role in insulin stimulation of glucose transport. These data suggest that a moderate 15% cellular ATP depletion does not affect the phosphorylation of early insulin-signaling proteins that were reported to play a role in insulin stimulation of glucose transport.

When cells were treated with NaN₃ (10 mM) and DNP (0.2 mM) to achieve a severe 40% ATP depletion, insulin stimulation of all insulin-signaling proteins tested was greatly diminished. These include phosphorylation of the insulin receptor, Akt1, p70⁵⁶⁶, and ERK1/2 (Fig. 4).

**Effects of NaN₃ and DNP on GLUT-1 content in the plasma membrane.** Basal glucose transport in insulin target cells such as 3T3-L1 adipocytes is mediated mainly by GLUT-1. To study whether increased basal glucose transport by NaN₃ and DNP is due to an increased amount of GLUT-1 in the plasma membrane, we treated cells for 5 h with these agents, and during the last 30 min of incubation, insulin was added. Cells were then homogenized and fractionated to prepare both plasma membranes and low-density membranes as previously described (5). These membranes were next resolved by Western blot analysis with α-GLUT-1. At basal states, both NaN₃ and DNP significantly increased GLUT-1 content in the plasma membrane with few changes in GLUT-1 content in low-density membranes (Fig. 5A). Insulin stimulation of cells increased GLUT-1 content in the plasma membrane, whereas it significantly decreased GLUT-1 content in low-density membranes, as previously reported (5). NaN₃ and DNP did not significantly affect insulin-induced GLUT-1 translocation from low-density membranes to the plasma membrane.

**Fig. 4.** Effects of high concentrations of NaN₃ and DNP on phosphorylation of the IR, Akt1, p70⁵⁶⁶, and ERK1/2 in 3T3-L1 adipocytes. 3T3-L1 adipocytes in 100-mm dishes were incubated for 5 h in serum-free DMEM containing either vehicle, 10 mM NaN₃, or 0.2 mM DNP. Cells were then incubated for 5 min with or without 100 nM insulin, followed by solubilization and Western blot analysis as described in Fig. 3. Intensities of various protein phosphorylations were quantified by laser scanner. All data except those for p70⁵⁶⁶ are presented as % maximum ± SD of 2 experiments. Responses at 100 nM insulin in control cells were taken as maximal.
concentrations of agents (10 mM NaN₃ and 0.2 mM DNP) for 5 h in serum-free DMEM containing either vehicle, NaN₃ (3 and 10 mM), or DNP (0.05 and 0.2 mM). Cells were next incubated for 30 min with or without 100 nM insulin, followed by subcellular fractionation as described in EXPERIMENTAL PROCEDURES. Membranes were next resolved by Western blot analysis with anti-GLUT-1 antibody (α-GLUT-1; A). Data are presented as %basal ± SE of 3–4 experiments. GLUT-1 content at basal states was taken as 100%. PM, plasma membranes; LDM, low-density membranes; L, low concentrations of agents (3 mM NaN₃ and 0.05 mM DNP); H, high concentrations of agents (10 mM NaN₃ and 0.2 mM DNP). B: no effects of NaN₃ and DNP on total GLUT-1 content. Total cell homogenates were studied for total GLUT-1 content by Western blot analysis with α-GLUT-1. NaN₃ (L), 3 mM; NaN₃ (H), 10 mM; DNP (L), 0.05 mM; DNP (H), 0.2 mM.

plasma membrane (Fig. 5A). These agents at low concentrations did not affect total GLUT-1 content, whereas at high concentrations they may have increased it slightly, if at all (Fig. 5B). Employing the same subcellular fractions, we also performed Western blot analysis with α-GLUT-4. As expected, insulin stimulated GLUT-4 translocation from low-density membranes to the plasma membrane. However, pretreatment of cells with NaN₃ and DNP did not affect GLUT-4 content in the plasma membrane, either at basal or after insulin stimulation of cells (Data not shown).

DISCUSSION

In this study, we investigated the role of cellular ATP depletion in insulin resistance in 3T3-L1 adipocytes. When cells were treated with either glucosamine, NaN₃, or DNP to lower cellular ATP content to a similar extent, differential effects on basal and insulin-stimulated glucose transport were obtained. NaN₃ and DNP markedly raised basal 2-DG uptake, whereas glucosamine lowered it slightly, if at all. Total 2-DG uptake obtained after insulin treatment of cells was decreased only in glucosamine-treated, but not in NaN₃- or DNP-treated cells. When cellular ATP content was severely depleted by 40% with either NaN₃ or DNP, basal 2-DG uptake remained similarly elevated. We observed similar data with 3-OMG transport, suggesting that NaN₃, DNP, and glucosamine affected glucose transport independently of hexokinase. Differential effects of these agents on glucose transport strongly suggest that cellular ATP depletion per se does not induce insulin resistance in 3T3-L1 adipocytes and does not fully account for glucosamine-induced insulin resistance in these cells.

Insulin stimulates glucose transport by stimulating GLUT-4 trafficking from the intracellular low-density microsomes to the plasma membrane (15). In glucosamine-treated 3T3-L1 adipocytes, there was impaired GLUT-4 trafficking in response to insulin (5). To study whether the impaired GLUT-4 trafficking in these cells was due to impaired insulin signaling, we (5) had previously investigated insulin-signaling proteins that were reported to play a role in insulin action on glucose transport. Under our experimental conditions, there was no significant impairment of early insulin-signaling proteins that played a role in insulin stimulation of glucose transport. These included insulin receptor functions, tyrosine phosphorylation of IRS-1/2, and PI 3K activity, although insulin stimulation of Akt1 was slightly impaired in these cells. However, impaired Akt1 activation by insulin did not correlate directly with the time course for glucosamine-induced impairment of 2-DG uptake, suggesting that induction of insulin resistance by glucosamine could occur without impaired Akt1 activity (5). In the present study, in fact, we achieved an ~50% decrease in insulin stimulation of glucose transport without impaired Akt1 activity. It should be noted that, in glucosaminetreated cells, insulin stimulation of p70S6k was significantly impaired. Because insulin stimulation of PI 3K/Akt1 upstream of p70S6k was normal in these cells, these data may suggest the presence of a PI 3K/Akt-independent pathway leading to activation of p70S6k. A PI 3K/Akt-independent pathway for p70S6k activation has previously been reported in L6 myocytes (13).

Under our experimental conditions, glucosamine at 25–50 mM decreased cellular ATP content by up to 15%. Normal insulin signaling involves multiple enzymes that require cellular ATP for their activation. To study whether ATP depletion by other means affects insulin signaling proteins, we employed NaN₃ and DNP, which affect mitochondrial oxidative phosphorylation and decrease mitochondrial ATP production, resulting in decreases in cellular ATP content. Both NaN₃ and DNP decreased cellular ATP content in a dose-dependent manner by up to 40% under our experimental conditions.

In NaN₃- and DNP-treated cells for a 15% cellular ATP depletion, there was no significant impairment of early insulin signaling proteins that were reported to play a role in insulin stimulation of glucose transport. These data suggest that a moderate 15% ATP depletion is not sufficient to impair insulin-induced phosphory-
lation and/or activation of most insulin-signaling proteins. In these cells, however, insulin stimulation of ERK1/2 was greatly impaired, with effects of NaN₃ being more prominent. Together with selective inhibitory effects of glucosamine on insulin stimulation of p70S6k, these data suggest that cellular ATP depletion per se does not fully explain the differential effects of these agents on ERK1/2 and p70S6k. In this regard, it should be noted that glucosamine, unlike NaN₃ and DNP, decreased cellular ATP content most likely as a result of rapid phosphorylation by hexokinase of newly transported glucosamine (7). This difference may, at least in part, account for their differential effects on certain insulin-signaling proteins and/or glucose transport in 3T3-L1 adipocytes.

When cellular ATP was further depleted by 40%, insulin-induced phosphorylation and/or activation of all insulin-signaling proteins studied were markedly impaired; yet both basal and insulin-stimulated glucose transport in these cells were similar to those in cells with 15% ATP depletion. These data suggest that, under our experimental conditions, glucose transport in cells pretreated with either NaN₃ or DNP was independent of insulin signaling. It should be noted that cells treated with high concentrations of NaN₃ or DNP (i.e., cells with 40% ATP depletion) did not adhere to tissue culture plates as well as control cells, suggesting possible cytotoxicity.

Previously, Hresko et al. (7) reported that glucosamine treatment of 3T3-L1 adipocytes severely decreased cellular ATP content by 60–80% and inhibited all insulin functions, including phosphorylation of the insulin receptor and IRS-1/2, activation of PI 3K and Akt1, and trafficking of GLUT-4 and GLUT-1. They concluded that glucosamine induction of insulin resistance in 3T3-L1 adipocytes was due to impaired insulin signaling associated with severe cellular ATP depletion. Because 40% ATP depletion was sufficient to severely impair all insulin signaling proteins tested in our study, it is not surprising that a more severe 60–80% ATP depletion impaired all insulin signaling proteins and GLUT-1/4 trafficking. Although it is unclear, the differences between our study and ours may be due, at least in part, to the different experimental conditions employed. For glucosamine treatment of cells in our study, we included 5 mM glucose and excluded exogenous insulin from incubation media. These experimental conditions required a longer incubation time to induce insulin resistance by glucosamine when compared with those of Hresko et al. In addition, a different degree of adipocyte differentiation might be partially responsible for the differences.

An increase in basal glucose transport in response to impaired mitochondrial function by NaN₃ and DNP was previously reported to be part of the adaptive responses of the cell, probably to compensate for the increased rate of glycolysis (1). Furthermore, increased basal glucose transport by NaN₃ and DNP was reported to be due to elevated GLUT-1 but not GLUT-4 levels in the plasma membrane (1) and was independent of insulin signaling in L6 myotubes (14). We found that, in 3T3-L1 adipocytes, pretreatment of cells with NaN₃ and DNP increased GLUT-1 content in the plasma membrane without detectable, concomitant decreases in GLUT-1 content in low-density membranes. In these cells, NaN₃ and DNP did not alter total GLUT-1 content. Currently, it is unclear where the increased GLUT-1 pool in the plasma membrane came from after treatment of cells with NaN₃ and DNP. We speculate that there may exist a readily available GLUT-1 pool in the vicinity of the plasma membrane that responds to certain stimuli (i.e., NaN₃ and DNP) to move to the plasma membrane. In cells treated with glucosamine, however, basal glucose transport and basal GLUT-1 content in the plasma membrane were not increased as previously reported (5). The increase in basal glucose transport by NaN₃ and DNP was not associated with stimulation of insulin-signaling proteins as assessed by phosphorylation and/or activation of IRS-1/2 and PI 3K and Akt1 activity. These data are consistent with the fact that GLUT-1 is mainly responsible for basal glucose transport whereas GLUT-4 is mainly responsible for insulin-stimulated glucose transport. In addition, these data are consistent with the fact that increased basal glucose transport by NaN₃ and DNP is independent of insulin signaling.

Taken together, our study clearly demonstrate that ATP depletion by NaN₃, DNP, and glucosamine cause differential effects on basal and insulin-stimulated glucose transport. Furthermore, ATP depletion by NaN₃ and DNP failed to induce insulin resistance, unlike glucosamine. These data also suggest that a moderate 15% cellular ATP depletion does not significantly alter insulin stimulation of early insulin-signaling proteins, whereas a severe 40% cellular ATP depletion markedly inhibits all insulin-signaling proteins tested.

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