Cytokine-induced glucose uptake in skeletal muscle: redox regulation and the role of α-lipoic acid

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Khanna, Savita, Sashwati Roy, Lester Packer, and Chandan K. Sen. Cytokine-induced glucose uptake in skeletal muscle: redox regulation and the role of α-lipoic acid. *Am. J. Physiol.* 276 (*Regulatory Integrative Comp. Physiol.* 45): R1327–R1333, 1999.—In L6 myotubes, glucose uptake stimulated by interferon (IFN)-γ or lipopolysaccharides (LPS) and a combination of LPS, IFN-γ, and tumor necrosis factor (TNF)-α was inhibited by the antioxidant pyrrolidinedithiocarbamate and potentiated in reduced glutathione (GSH)-deficient cells. Also, the stimulatory effect of LPS and IFN-γ individually, and of a combination of LPS, IFN-γ, and TNF-α, on glucose uptake was associated with an increased level of intracellular oxidants (dichlorofluorescein assay) and loss of intracellular GSH. Study of the individual effects of LPS, IFN-γ, and TNF-α as well as of a combination of the three activators provided evidence against a role of nitric oxide in mediating the stimulatory effect of the above-mentioned agents on glucose uptake. We also observed that the insulin-mimetic nutrient α-lipoic acid (LA; R-enantiomer) is able to stimulate glucose uptake in cytokine-treated cells that are insulin resistant. This study shows that cytokine-induced glucose uptake in skeletal muscle cells is redox sensitive and that, under conditions of acute infection that is accompanied with insulin resistance, LA may have therapeutic implications in restoring glucose availability in tissues such as the skeletal muscle.

antioxidant; immune system; infection; metabolism; nitric oxide; thioctic acid

CYTOKINES, THE SECRETORY products of macrophages, monocytes, and natural killer cells, have been suggested to mediate the effects of infection on glucose metabolism, particularly in the skeletal muscle (5–7, 21, 26, 46). In sepsis, for example, carbohydrate dyshomeostasis is a characteristic feature (4, 8, 13, 39, 40). Acute infection in sepsis is known to be associated with insulin resistance, as indicated by decreased glucose tolerance, hyperinsulinemia, and impaired insulin action on peripheral glucose disposal (38, 42). On the other hand, septic patients have an increased rate of basal glucose clearance (3, 25, 44). This response is thought to be cytokine mediated (5–7, 21, 26, 46).

The lymphokine interferon (IFN)-γ is known to influence glucose metabolism (22). Cooperative action of tumor necrosis factor (TNF)-α and IFN-γ has been observed in the regulation of various cellular responses, such as inducible nitric oxide (NO) production (1, 9). Lipopolysaccharides (LPS), bacterial endotoxins that are known to induce septic shock, may also enhance cellular glucose uptake (12, 25). In sepsis, TNF-α, IFN-γ, and LPS may directly stimulate cellular glucose uptake but impair insulin regulation of peripheral glucose disposal. A mechanism-based explanation of how cytokines influence muscle glucose metabolism is currently lacking. Such information is necessary to develop effective approaches to control irregularities in glucose metabolism that is associated with infection. In a recent study (1) in which the effect of a combination of TNF-α, IFN-γ, and LPS on glucose uptake by L6 myotubes was investigated, it was concluded that cytokines modulate skeletal muscle glucose uptake by an NO-dependent mechanism. In that work, however, the effect of individual cytokines on muscle glucose uptake was not considered (1). In the present study, we tested the hypothesis that cytokine-induced glucose uptake in skeletal muscle cells is redox sensitive. To achieve this goal, the effect of TNF-α, IFN-γ, and LPS individually, as well as in combination, on glucose uptake by L6 myotubes was investigated. Previous evidence suggests that cytokines confer insulin insensitivity to L6 myotubes (1). Thus we also sought to test whether the insulin-mimetic nutrient α-lipoic acid (LA) would increase skeletal muscle glucose uptake in cytokine-treated cells that are known to be insulin resistant. LA, or thioctic acid, is an effective modulator of cellular redox status (29) that is known to stimulate skeletal muscle glucose uptake by the favorable redistribution of glucose transporters (10, 16). It has been successfully used clinically for the treatment of diabetic polyneuropathies (2, 47, 48).

MATERIALS AND METHODS

Materials

Rat thigh skeletal muscle-derived L6 cells, known to retain the morphological, metabolic, and biochemical characteristics of skeletal muscle (45), were obtained from American Type Culture Collection (Bethesda, MD). Dulbecco's phosphate-buffered saline (DPBS) and DMEM supplemented with high glucose, L-glutamine, pyridoxine hydrochloride, and 110 mg/l sodium pyruvate were obtained from GIBCO BRL (Life Technologies). Horse serum (HyClone Laboratories, Logan, UT), FCS, and other reagents for the culture medium were obtained from the cell culture facility of the University of California at San Francisco. Recombinant rat IFN-γ and TNF-α were purchased from R & D Systems (Minneapolis, MN). LPS isolated from *Escherichia coli*, L-buthionine-[*S*,*R*]-sulfoximine (BSO), the ammonium salt of pyrrolidinedithiocarbamate (PDTC), cytochalasin B, sulfanilamide, and naphthylethylenediamine dihydrochloride were purchased from Sigma (St. Louis, MO). Asta Medica (Frankfurt, Germany)

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provided the R-enantiomer of LA (R-LA), which is the naturally occurring form, as kind gift. R-LA was dissolved in dimethyl sulfoxide to obtain a stock concentration of 0.1 mol/l. Anhydrous dextrose was obtained from Fisher Biotech (Fair Lawn, NJ). N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) was purchased from Alexis (San Diego, CA). Dichlorodihydrofluorescein diacetate (DCFH-DA) was from Molecular Probes, Eugene, OR. 2-[^{14}C(U)]deoxy-D-glucose, with a specific activity of 300–350 mCi/mmol, was purchased from New England Nuclear Life Science Products (Boston, MA).

Methods

Experimental design. L6 myoblasts grown in 75-cm^2 culture flasks were harvested and split to six wells of a six-well plate as described below. Thus cells in all six wells were derived from the same source. L6 myoblasts were then grown in six-well plates and differentiated to myotubes as described below. Myotubes prepared as such were used for all measurements reported in this study. Thus, for each experiment, data from control and treated cells were obtained from the same batch of myotubes prepared from the same stock of myoblasts under exactly similar conditions. For glucose uptake studies, two wells of each plate were utilized as control samples. Myotubes in one of these two wells were not treated with any agent to obtain basal glucose uptake values. Cells in the other control well were treated with cytochalasin B as described below to control for non-carrier-mediated transport of radiolabeled 2-[^{14}C(U)]deoxy-D-glucose. Before glucose uptake studies were done, cells in the other four wells were either treated or not with agents described in the legends to Figs. 1–3.

Cell culture and differentiation. Differentiated myotubes (30) obtained as described below were used in this study. For experiments, cells were seeded at a concentration of 0.03×10^6 cells per well in a six-well, flat-bottom, tissue culture-treated polystyrene plate (Falcon, Becton Dickinson Labware). In each well, cultures were grown in 3 ml of DMEM supplemented with 10% FCS, 5 mM glutamine, 0.5% D-glucose, 100 U/ml penicillin, and 0.1 mg/ml streptomycin, in humidified air containing 5% CO_2 at −37°C. On the fifth day from the date of seeding, the culture medium was changed. The new culture medium that was meant to stimulate cell differentiation was serum deprived, containing 2% horse serum in place of 10% FCS. The culture medium was changed again on *day 8* and *day 11* after cell seeding. Glucose uptake experiments were carried out either on *day 14* or *15* after cell seeding. Before each experiment, formation of multinucleated myotubes and abundance were verified by nuclear staining.

Cell incubations and glucose transport assay. L6 myotubes were incubated with or without TNF-α (50 ng/ml), IFN-γ (500 ng/ml), and LPS (10 μg/ml) as indicated in the legends to Figs. 1–3 for 24 h. Treatment of myotubes with these inflammatory mediators did not influence cell viability or membrane integrity as measured by lactate dehydrogenase release from cells to the medium as well as standard trypan blue exclusion assays (not shown). Where indicated, L-NAME (2 mM) or PDTC (0.2 mM) was added 5 min or 2 h before cytokine or LPS treatment, respectively. For the glucose transport assay, cells in each well were washed three times with 1 ml of transport buffer (140 mM NaCl, 5 mM KCl, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$, in 20 mM Tris-HEPES, pH 7.4). One milliliter of transport buffer was added to each well. In experiments in which the effect of LA was studied, 2.5 μl of a 0.1 M stock solution of LA in DMSO or a matched volume of DMSO was added to each well for 30 min at 37°C before the start of glucose transport assay. Transport assay was started by the addition of a mixture of D-glucose (5 mM) and 2-[^{14}C(U)]deoxy-D-glucose (2 μCi/well) to each well and transferring the plate to an incubator maintained at 37°C. Ten minutes after the start of glucose transport assay, the six-well plate was placed on ice and 3 ml of ice-cold transport buffer was added to each well to stop the transport assay. After aspiration of the cold transport buffer, each well was washed with 2 ml of ice-cold transport buffer three times. After this, 0.5 ml of 10% sodium dodecyl sulfate or 50 mM NaOH was added to each well to disrupt and collect the cells. The cell lysate was collected in a glass scintillation vial containing 5 ml of a scintillation cocktail (Econo-Safe; Research Products International, Mount Prospect, IL). After proper vortexing of the cell lysate and the scintillation cocktail, each vial was subjected to ^{14}C scintillation counting. Glucose uptake values were normalized against total protein values measured from lysates extracted in NaOH. The values were also corrected for non-carrier-mediated transport by measuring glucose uptake in the presence of 0.01 mM of cytochalasin B added just before the start of the transport assay.

Cellular reduced glutathione measurement. Cells in monolayer were washed three times with DPBS and then treated with 0.5 ml of 4% monochloroacetic acid. The extract was mixed by resuspending and transferred to an Eppendorf tube that was snap frozen in liquid nitrogen. Before HPLC analysis, the extract was centrifuged (16,000 *g*, 5 min) and the supernatant filtered using 0.45-μM microfilterfuge tubes fitted with nylon membrane (Rainin, Woburn, MA). The sample pellet was dissolved in 1 N NaOH for determination of total protein using a BCA protein assay kit (Pierce, Rockford, IL).

Reduced glutathione (GSH) measurements were performed using a HPLC system coupled with a electrochemical coulometric detector (ESA, Chelmsford, MA). A C_{18} column (150 mm × 4.6 mm, 5-μm pore size; Alltech, Deerfield, IL) was used for GSH separation as described previously (20). GSH levels were expressed as nanomoles per milligram protein.

Nitrite and nitrate determinations. For the determination of nitrite and nitrate from cell culture medium, the medium was first deproteinized by adding 290 μl of 0.3 M NaOH to 400 μl of the culture medium as described previously (14). After incubation of the mixture for 5 min at room temperature, 290 μl of 5% (wt/vol) $ZnSO_4$ was added. The mixture was then allowed to stand for another 5 min, after which it was centrifuged at 2,800 *g* for 20 min. The resulting supernatant was filtered through 0.45-μM microfilterfuge tubes fitted with a nylon membrane (Rainin). Nitrite and nitrate levels were detected from the deproteinized supernatant (100 μl) using an automated NOx analyzer (model TCI-NOX 1000; Tokyo Kasei Kogyo, Tokyo, Japan). This analyzer employs the technique of automated flow injection analysis (14). Nitrite reacts with Greiss reagent (1% wt/vol sulfanilamide and 0.1% wt/vol naphthylethylenediamine dihydrochloride in 2.5% wt/vol H_3PO_4) and forms a diazo compound. The absorbance of this compound is measured at 540 nm using a flow-through visible spectrophotometer (model S/3250; Soma Kogaku, Tokyo, Japan) connected to a chart recorder. Nitrate was determined by reducing it to nitrite using an A7200 copper-cadmium reduction column (Tokyo Kasei Kogyo) and then quantified as described above. Both sodium nitrite and nitrate solutions were used as standards. The volume of culture medium and total cellular protein in each well were not significantly different, allowing direct comparison of results obtained.

Determination of intracellular peroxides. Intracellular peroxides were detected using DCFH-DA as described previously (33). Myotubes were treated with the cytokines or LPS for 24 h as indicated in the legend to Fig. 5. After the treatment

time, culture medium was aspirated from each well and the myotubes were washed once with DPBS at room temperature. A solution (1 ml) of DCFH-DA (50 μM) in DPBS was added to each well, and myotubes were incubated with the probe for 40 min at 37°C in dark. After the incubation, the DCFH-DA uptake and staining process was stopped by adding 3 ml of ice-cold DPBS to each well. After the entire overlay buffer was aspirated, 0.5 ml of fresh ice-cold DPBS was added to each well. Myotubes were detached from the monolayer using a disposable cell lifter (Fisher Scientific, Pittsburgh, PA). Dichlorofluorescein (DCF) fluorescence in cells was detected using a 488-nm argon ion laser for excitation in a flow cytometer (XL; Coulter, Miami, FL), and the 530 nm emission was recorded in fluorescence channel 1. Data were collected from at least 10,000 gated cells.

Statistical analyses. Differences between means of groups were determined by analysis of variance. The minimum level of significance was set at $P < 0.05$.

RESULTS

Glucose Uptake by L6 Myotubes

Compared with the basal rate in nontreated cells, treatment of L6 myotubes with IFN-γ or LPS significantly increased glucose uptake. TNF-α treatment did not influence glucose uptake in the myotubes, however (Fig. 1). LA treatment of L6 myotubes also resulted in significant increase of glucose uptake compared with the rate in nontreated control cells (Fig. 1). The enhancing effects of IFN-γ and LPS on glucose uptake in L6 myotubes was additive with the corresponding effect of LA. The stimulatory effect of a combination of LPS or IFN-γ with LA on glucose uptake in L6 myoblasts was markedly suppressed in cells that were pretreated with the antioxidant PDTC (Fig. 1, *A* and *B*). Under resting conditions, the level of GSH in L6 myotubes was 94.17 ± 7.4 nmol/mg protein. Inhibition of intracellular GSH synthesis by treatment of cells with BSO for 48 h decreased cellular GSH content to 17.82 ± 2.92 nmol/mg protein. In GSH-depleted cells, the stimulatory effects of both IFN-γ and LPS on glucose uptake by L6 myotubes were significantly higher (Fig. 2). TNF-α alone did not influence glucose uptake even in GSH-depleted cells (not shown). In combination, TNF-α, IFN-γ, and LPS significantly increased glucose uptake in L6 myotubes compared with the corresponding basal rate. Treatment of myotubes with L-NAME, a NO synthase inhibitor, did not influence the glucose uptake stimulatory effect of the cytokine and LPS combination (Fig. 3). The enhancing effect of a combination of TNF-α, IFN-γ, and LPS on glucose uptake in L6 myotubes was additive with the corresponding effect of LA. The effect of a combination of TNF-α, IFN-γ, LPS, and LA on L6 myotube glucose uptake was not influenced by L-NAME treatment (Fig. 3). Results are presented as means ± SD of at least three separate experiments.

Inducible NO Generation

As evidence for the generation of NO production, nitrite and nitrate content of the cell culture medium were determined. When L6 myotubes were treated with any one of TNF-α, IFN-γ, or LPS, NO production by cells was not increased. When used in combination, however, TNF-α, IFN-γ, and LPS treatment resulted in a marked increase in nitrite and nitrate content in the cell culture medium (Fig. 4*A*). Pretreatment of the myotubes with L-NAME, but not D-NAME (not shown), completely abolished the induced generation of NO in response to a combination of TNF-α, IFN-γ, and LPS treatment (Fig. 4*B*). L-NAME treatment alone slightly increased basal production of nitrate and nitrite by L6 myotubes. This observation is consistent with previous data showing that L-NAME treatment alone resulted in

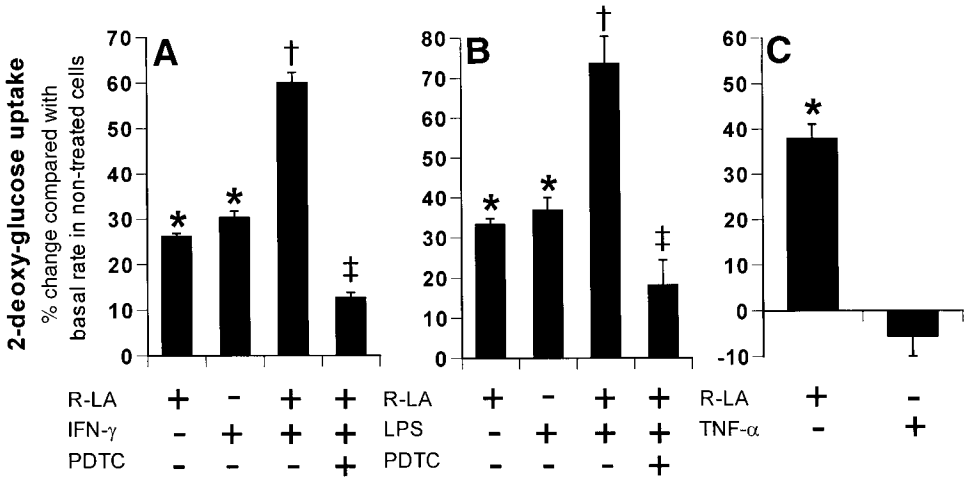

Fig. 1. Regulation of glucose uptake by cytokines, endotoxin, α-lipoic acid (LA), and pyrrolidinedithiocarbamate (PDTC). L6 myotubes were incubated with (+) or without (−) interferon (IFN)-γ (500 ng/ml; *A*), lipopolysaccharide (LPS; 10 μg/ml; *B*), and tumor necrosis factor (TNF)-α (50 ng/ml; *C*) as indicated for 24 h. When indicated, PDTC (0.2 mM) was added 2 h before cytokine or endotoxin treatment. R-enantiomer of LA (R-LA; 0.25 mM) was added 30 min before determination of glucose transport. Glucose uptake values were corrected for non-carrier-mediated transport by measuring glucose uptake in presence of 0.01 mM of cytochalasin B added just before start of transport assay. *$P < 0.05$, significantly higher than basal glucose uptake. †$P < 0.05$, significantly higher than R-LA- or IFN-γ-stimulated glucose uptake. ‡$P < 0.05$, significantly lower than all other bars in panel.

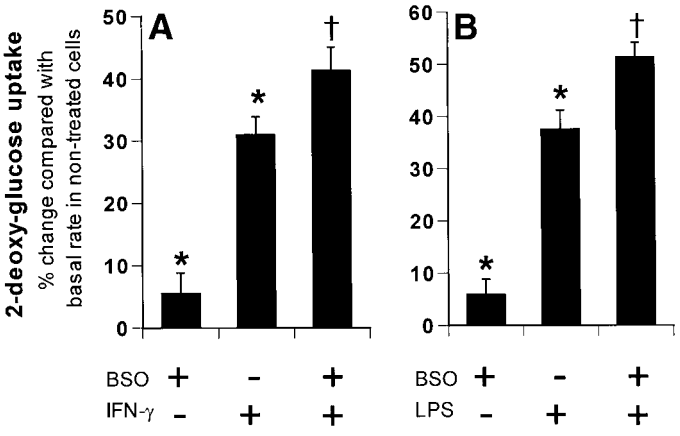

Fig. 2. Enhancement of basal glucose uptake and potentiation of stimulated glucose uptake in reduced glutathione (GSH)-deficient cells. GSH-deficient cells were prepared by treatment with 0.25 mM L-buthionine-[*S*,*R*]-sulfoximine (BSO) for 48 h before IFN-γ (*A*) or LPS (*B*) treatment. All other details are as described in legend of Fig. 1. $*P < 0.05$, significantly higher than basal glucose uptake. $\dagger P < 0.05$, significantly higher than IFN-γ- or LPS-stimulated glucose uptake.

an 80% increase in basal nitrite production in L6 myotubes (1). Results are presented as means ± SD of at least three separate experiments.

Intracellular Peroxides and GSH

Treatment of L6 myotubes with IFN-γ or LPS increased the level of intracellular peroxides as estimated by the development of DCF fluorescence. TNF-α treatment did not influence intracellular peroxide level, however. A combination of TNF-α, IFN-γ, and LPS also increased the level of intracellular peroxides and decreased the level of intracellular GSH compared with the corresponding levels in nontreated cells (Fig. 5). Results are presented as means ± SD of at least three separate experiments.

DISCUSSION

This study shows that in skeletal muscle cells, cytokine-induced upregulation of glucose uptake is mediated by a redox-dependent mechanism. In support of this, it has been shown that such effect of LPS and IFN-γ individually, and of a combination of LPS, IFN-γ, and TNF-α, is *1*) inhibited by treatment of cells with the antioxidant PDTC and *2*) potentiated in GSH-deficient cells with impaired antioxidant defenses. Also, the stimulatory effect of LPS and IFN-γ individually, and of a combination of LPS, IFN-γ, and TNF-α, on glucose uptake in L6 myotubes was associated with increased accumulation of intracellular peroxides and loss of intracellular GSH. A recent study concluded that a combination of LPS, IFN-γ, and TNF-α upregulates glucose uptake in L6 myotubes by an NO-dependent mechanism (1). The individual effects of LPS, IFN-γ, and TNF-α on glucose uptake by L6 myotubes and cellular NO production were not reported (1). In the present study, results obtained from experiments studying the individual effects of LPS, IFN-γ, and TNF-α as well as a combination of the three activators provide evidence against a role of NO in mediating the stimulatory effect of the above-mentioned agents on glucose uptake in L6 myotubes.

Redox-dependent mechanisms have been shown to regulate a wide variety of cellular function and response (35, 37). Our current observation that cytokine-induced glucose uptake by L6 myotubes is redox regulated and perhaps reactive oxygen species mediated is consistent with a previous report showing that oxidants may indeed stimulate skeletal muscle glucose uptake via increased expression of GLUT-1 mRNA and protein (23, 24). Exposure of L6 myotubes to prolonged low-grade oxidative stress results in increased GLUT-1 expression at both the protein and mRNA levels, leading to elevated glucose transport activity. Oxidative stress increased GLUT-1 transcription rate by activating activator protein 1 binding to enhancer 1 of the GLUT-1 gene. (24). Inhibition of intracellular GSH synthesis impairs the antioxidant defense system, resulting in increased accumulation of intracellular peroxides (15, 27). Previously we have shown that in L6 cells, BSO treatment markedly depletes cellular GSH and potentiates TNF-α-induced activation of the peroxide-inducible transcription factor nuclear factor κB (36). The stimulatory effect of IFN-γ or LPS on glucose uptake was potentiated in GSH-deficient cells, suggesting that the infection-related agonists may have functioned via an oxidant-dependent mechanism. Indeed, treatment of L6 cells with IFN-γ or LPS increased the level of intracellular peroxides as detected by DCF fluorescence. Moreover, treatment of cells with the antioxidant PDTC inhibited LPS- or IFN-γ-induced glucose uptake. PDTC is a reducing agent that, when

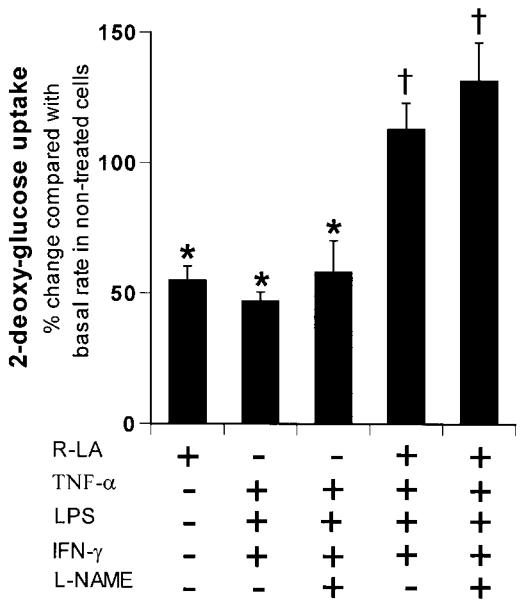

Fig. 3. Enhancement of basal glucose uptake by combination of cytokines and endotoxin is not mediated by enhanced nitric oxide (NO) production. When indicated, 2 mM N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) was added 5 min before activation of L6 myotubes with cocktail of TNF-α, IFN-γ, and LPS. All other details are as described in legend of Fig. 1. $*P < 0.05$, significantly higher than basal glucose uptake. $\dagger P < 0.05$, significantly higher than R-LA- or cocktail-stimulated glucose uptake.

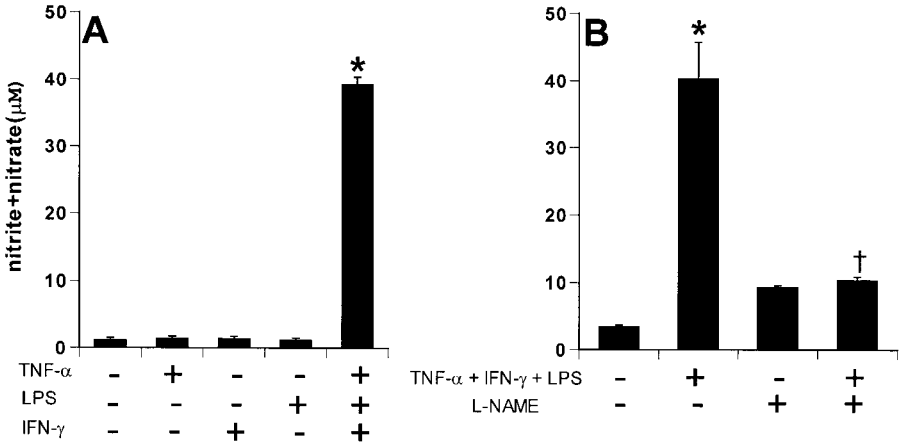

Fig. 4. NO production in resting and activated cells. *A*: when treated to L6 myotubes, individual cytokines or LPS did not induce NO production, but combination of TNF-α, IFN-γ, and LPS markedly increased NO production. *B*: L-NAME, but not D-NAME (not shown), potently inhibited NO production in response to combination of TNF-α, IFN-γ, and LPS treatment. Cell treatment and other details are described in legends of Figs. 1 and 3. $*P <$ 0.05, significantly higher than that in nonactivated cells or cells treated with TNF-α, IFN-γ, or LPS individually. $\dagger P < 0.05$, significantly lower than that in cells treated with combination of TNF-α, IFN-γ, and LPS.

treated at a concentration and duration as was used in this study, has been shown to enhance antioxidant defenses in L6 cells (36). All of these findings support the conclusion that both LPS and IFN-γ increase glucose uptake in L6 myotubes by a reactive oxygen species-dependent mechanism. In L6 myotubes, TNF-α treatment alone did not influence the level of intracellular peroxides as well as the rate of glucose uptake. In combination, LPS, IFN-γ, and TNF-α markedly enhanced glucose uptake in L6 myotubes. This effect was accompanied with oxidative stress, as indicated by increased level of intracellular peroxide and loss of intracellular GSH. The ability of cytokines and endotoxins to induce oxidative stress has been previously demonstrated (11, 17, 32, 43).

Recently it has been reported that in L6 myotubes a combination of LPS, IFN-γ, and TNF-α markedly enhances NO production by inducing the activity of inducible NO synthase (1). The same treatment also increased glucose uptake in those cells. On the basis of a set of experiments studying the combined effect of LPS, IFN-γ, and TNF-α on glucose uptake but not that of the individual cell activating agents, it was concluded that LPS, IFN-γ, and TNF-α enhance glucose uptake by an NO-dependent mechanism (1). Our observations from the additional study of the individual effects of LPS, IFN-γ, and TNF-α provide evidence against a role of NO. Consistent with previous reports (1), we have observed that neither LPS, IFN-γ, nor TNF-α is able individually to induce NO synthesis in L6 myotubes. Despite this, both LPS and IFN-γ are able to stimulate glucose uptake in these cells. When treated to cells in combination, LPS, IFN-γ, and TNF-α markedly enhanced NO production as well as glucose uptake in L6 myotubes. However, this effect of a combination of LPS, IFN-γ, and TNF-α on skeletal muscle glucose uptake was not influenced by inhibition of inducible NO synthase activity. This line of evidence further confirms our contention that NO is not involved in mediating the effect of a combination of LPS, IFN-γ, and TNF-α on skeletal muscle glucose uptake. L-NAME is a commonly used inhibitor of inducible NO synthase activity. That this inhibitor was potent is clearly evident from our results showing that increased NO production in response to treatment of a combination LPS, IFN-γ, and TNF-α was completely abrogated in L-NAME-treated cells.

The development of malnutrition is often rapid in critically ill patients with sepsis and severe trauma. Hypermetabolism, associated with protein and fat catabolism, negative nitrogen balance, hyperglycemia, and resistance to insulin, constitutes the hallmark of

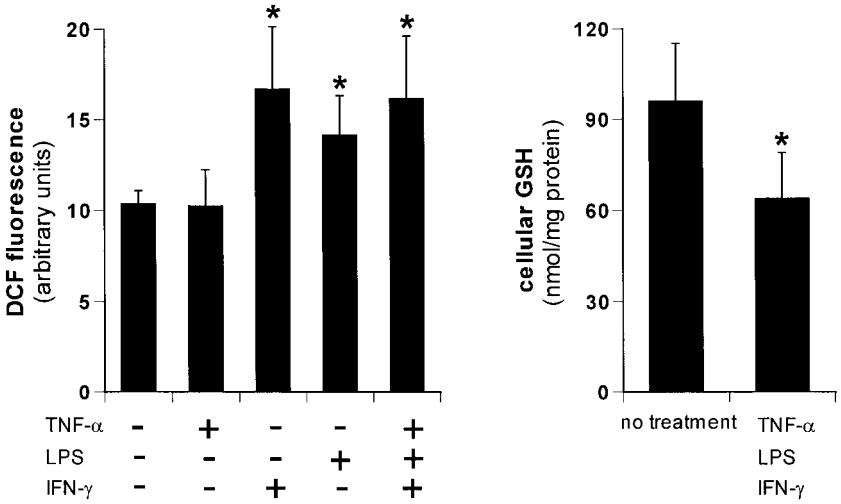

Fig. 5. Oxidative stress markers in nontreated and activated L6 myotubes. *Left*: relative level of intracellular peroxides expressed as mean fluorescence per cell. DCF, dichlorofluorescein. $*P < 0.05$, significantly higher than that in nonactivated cells or cells treated with TNF-α above. *Right*: treatment of cells with combination of TNF-α, IFN-γ, and LPS resulted in loss of intracellular GSH. Cell treatment and other details are as described in legend of Fig. 1. $*P < 0.05$, significantly lower than in nontreated cells.

this response (4). A common property of LPS, IFN-γ, and TNF-α is that individually each of these three agents may cause insulin resistance. TNF-α is overexpressed in the adipose tissue of obese rodents and humans and is associated with insulin resistance (34). Insulin resistance caused by TNF-α has been thought to be implicated in disorders such as obesity and non-insulin-dependent diabetes mellitus (28, 41). Consistent with this, neutralization of TNF-α in obese *fa*/*fa* rats was observed to cause a significant increase in the peripheral uptake of glucose in response to insulin. (18). Many viral infections cause insulin resistance by inducing IFN production (19, 22). In skeletal muscle tissue, endotoxin shock is known to cause insulin resistance (31). In L6 myotubes, LA treatment has been shown to be associated with an intracellular redistribution of GLUT-1 and GLUT-4 glucose transporters, similar to that caused by insulin, with minimal effects on GLUT-3 transporters. On the basis of these observations, it has been proposed that elements of the insulin-signaling pathway mediate the effect of LA on glucose uptake (10). In a more recent study, however, it was determined that although a portion of LA action on glucose transport in mammalian skeletal muscle is mediated via the insulin signal transduction pathway, a majority of the direct effect of LA on skeletal muscle glucose transport is insulin independent (16). Results of the current study agree with this contention because, although a combination of LPS, IFN-γ, and TNF-α causes decreased insulin sensitivity (1), such treatment is not able to influence the ability of LA to stimulate glucose uptake in skeletal muscle cells. These observations lead to the hypothesis that under conditions of acute infection that is accompanied with insulin resistance, LA may have therapeutic implications in restoring glucose availability in tissues such as the skeletal muscle.

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