1998

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Digital Object Identifier (DOI)
10.1210/endo.139.12.6368

0013-7227/98/$03.00/0
Endocrinology

Vol. 139, No. 12
Printed in U.S.A.

Effects of Tumor Necrosis Factor-α on Glucose Metabolism in Cultured Human Muscle Cells from Nondiabetic and Type 2 Diabetic Subjects*

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ABSTRACT

The effects of tumor necrosis factor-α (TNFα) on glucose uptake and glycogen synthase (GS) activity were studied in human skeletal muscle cell cultures from nondiabetic and type 2 diabetic subjects. In nondiabetic muscle cells, acute (90-min) exposure to TNFα (5 ng/ml) stimulated glucose uptake (73 ± 14% increase) to a greater extent than insulin (37 ± 4%; $P < 0.02$). The acute uptake response to TNFα in diabetic cells (51 ± 6% increase) was also greater than that to insulin (31 ± 3%; $P < 0.05$). Prolonged (24-h) exposure of nondiabetic muscle cells to TNFα resulted in a further stimulation of uptake (152 ± 31%; $P < 0.05$), whereas the increase in cells from type 2 diabetics was not significant compared with that in cells receiving acute treatment. After TNFα treatment, the level of glucose transporter-1 protein was elevated in nondiabetic (4.6-fold increase) and type 2 (1.7-fold) cells. Acute TNFα treatment had no effect on the fractional velocity of GS in either nondiabetic or type 2 cells. Prolonged exposure reduced the GS fractional velocity in both nondiabetic and diabetic cells. In summary, both acute and prolonged treatment with TNFα up-regulate glucose uptake activity in cultured human muscle cells, but reduce GS activity. Increased skeletal muscle glucose uptake in conditions of TNFα excess may serve as a compensatory mechanism in the insulin resistance of type 2 diabetes. (*Endocrinology* **139:** 4793–4800, 1998)

THE INSULIN resistance present in both obesity and type 2 diabetes is multifactorial in origin. A common feature of both situations is an elevation in the cytokine tumor necrosis factor-α (TNFα). Expression of TNFα is increased in adipose tissue and muscle, the major insulin target tissues, of insulin-resistant humans (1–3) and animals (4), suggesting that elevated TNFα is a causative or contributory factor in the development of insulin resistance, especially in obesity and diabetes (reviewed in Ref. 5). Infusion of animals and humans with TNFα can lead to whole body insulin resistance, localized to both the liver and peripheral tissues, especially muscle (6–8). Meanwhile, infusion of insulin-resistant animals with a soluble TNFα-binding protein improved insulin action *in vivo* (5).

At the cell level, extensive investigation has occurred in adipocytes, where TNFα treatment results in insulin resistance for stimulation of glucose transport (9, 10). Several potential mechanisms for TNFα-induced insulin resistance have been suggested, including down-regulation of the glucose transporter-4 (GLUT4) glucose transporter (10) and impaired insulin signaling (11, 12). However, it is muscle that represents the major site of glucose disposal, especially in response to insulin, and this tissue has not been studied in the same depth. Elevation of TNFα levels can lead to insulin resistance for both glucose disposal into muscle (6, 7) and suppression of hepatic glucose output (6, 7). Paradoxically, in the fasting (basal) state, hypoglycemia can occur, and glucose uptake into muscle is increased after TNFα treatment (6, 7, 13).

TNFα is a potent lipolytic agent in adipose tissue (9), leading to the possibility that both TNFα and FFA released from adipose tissue could be acting as paracrine factors influencing metabolism and insulin action in adjacent skeletal muscle. As FFA can inhibit insulin stimulation of glucose transport in both fat (14) and muscle (15, 16), it is possible that the effects of TNFα on muscle might be indirect in nature. To investigate the direct effects of TNFα on skeletal muscle glucose metabolism, we used the human skeletal muscle culture system. These cells express the morphological, biochemical, and metabolic properties of differentiated muscle, including insulin responsiveness (17). Most importantly, cells obtained from type 2 diabetic subjects display defects in glucose transport (18) and glycogen synthase (GS) (19) that reflect the impaired function observed *in vivo* and in muscle biopsies. In this way we could determine whether there were any differences between normal and diabetic muscle with regard to the influence of TNFα.

Received July 1, 1998.

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* This work was supported by funds from the American Diabetes Association, the Whittier Institute for Diabetes Research, Medical Research Service, the Department of Veterans Affairs, and the Veteran Affairs Medical Center-San Diego (to R.R.H.); Grant MO1-RR-00827 from the General Clinical Research Branch, Division of Research Resources, NIH, and the Medical Research Service, Department of Veterans Affairs and Veteran Affairs Medical Center-Little Rock (to P.A.K.); and NIH Grant DK-39176.

Materials and Methods

Materials

Human biosynthetic insulin was supplied by Dr. Ron Chance of Eli Lilly & Co., Inc. (Indianapolis, IN). Cell culture materials were purchased from Irvine Scientific (Irvine, CA) except for skeletal muscle basal medium, which was obtained from Clonetics Corp. (San Diego, CA). FBS was purchased from Gemini (Calabasas, CA). BSA (Cohn fraction V) was supplied by Boehringer Mannheim (Indianapolis, IN). 2-[1,2-^{3}H]Deoxy-D-glucose, L-[1-^{14}C]glucose, D-[U-^{14}C]glucose, and UDP-[^{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). A monoclonal antiserum against sarcomeric-specific α-actin was obtained from Sigma Chemical Co., Inc. (St. Louis, MO). Antirabbit and antimouse IgGs conjugated with horseradish peroxidase and the enhanced chemiluminesence kit were obtained from Amersham (Arlington Heights, IL). Protein assay kits and electrophoresis chemicals were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA). Pepstatin, leupeptin, phenylmethylsulfonylfluoride, 2-deoxyglucose, L-glucose, glycogen, TNFα, and other reagents were purchased from Sigma Chemical Co.

Human subjects

Nondiabetic and type 2 diabetic subjects were recruited for muscle biopsy. Glucose tolerance was determined from a 75-g oral glucose tolerance test (20). Insulin action was analyzed by a 3-h hyperinsulinemic (300 mU/m^2·min) euglycemic (5.0–5.5 mM) clamp; the glucose disposal rate was determined during the last 30 min of the clamp (21). Subject characteristics are summarized in Table 1. The diabetic group was significantly older and more obese than the nondiabetics. Impairments of the maximally insulin-stimulated glucose disposal rate confirmed the insulin resistance of the diabetic group (Table 1). The experimental protocol was approved by the committee on human investigation of the University of California-San Diego (La Jolla, CA). Informed written consent was obtained from all subjects after explanation of the protocol. Biopsy of the vastus lateralis muscle was performed according to previously described procedures (21).

Human muscle cell cultures

The method for skeletal muscle cell isolation and clonal growth has been described in detail previously (17). At confluence, cells were fused for 4 days in αMEM containing 2% FBS, 1% fungibact, 100 U/ml penicillin, and 100 μg/ml streptomycin. Fusion medium was changed every 2 days to reduce glucose depletion. All studies were carried out on first passage cultures. The extent of differentiation was established by fluorescent microscopic observation of multinucleation and striation patterns of skeletal myosin fast (heavy chain) and induction of α-sarcomeric actin protein as described in previous reports (17). When indicated, TNFα was added to the medium 24 h before acute hormone exposure. TNFα treatment had no effect on these markers of muscle differentiation.

Glucose uptake assay

The procedure for glucose uptake measurement has been described previously (18). Medium was added to the cells together with insulin (0 or 33 nM) and/or TNFα (0–5 ng/ml), and the cells were incubated for 90 min in a 5% CO_2 incubator before washing and transport assay. An aliquot of the suspension was removed for protein analysis using the Bradford method (22). The uptake of L-glucose was used to correct each sample for the contribution of diffusion.

TABLE 1. Subject characteristics

	Nondiabetic	Type 2 diabetic
Number of subjects	20	15
Age (yr)	38.6 ± 2.0	52.3 ± 2.0^a
BMI (kg/m^2)	26.8 ± 1.0	32.1 ± 0.8^a
HbA1c (%)	5.0 ± 0.1	8.2 ± 0.6^a
Fasting glucose (mM)	4.8 ± 0.1	8.7 ± 0.6^a
Fasting insulin (pM)	36 ± 8	121 ± 32^a
2 h OGTT:		
Glucose	5.7 ± 0.3	15.2 ± 1.0^a
Insulin	196 ± 35	316 ± 52
GDR (mg/kg · min)	11.1 ± 1.0	6.7 ± 0.5^a

GDR, Glucose Disposal Rate. Results are average ± SEM.
^{a} $P < 0.05$ compared with nondiabetic.

Membrane preparation

Cells for membrane preparation were grown in 100-mm dishes and treated as described for activity assays. Total membranes were prepared as described previously (18). Cells were scrapped from dishes, collected by centrifugation, and homogenized with a Dounce homogenizer (Kontes Co., Vineland, NJ). After centrifugation at 750 × *g* for 3 min, the pellet was rehomogenized and recentrifuged, and the supernatants were 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 the protein content was determined.

Detection of glucose transporter proteins

Membrane preparations were diluted 1:1 in 2 × Lamelli's buffer without β-mercaptoethanol (23) and heated for 5 min at 90 C. Proteins were separated on 10% SDS-PAGE gels and then transferred to nitrocellulose (24). GLUT1 was identified using a rabbit polyclonal antiserum against the rat brain glucose transporter (RaGLUTRANS, East Acres Biologicals). A polyclonal antiserum specific for GLUT4 (RaIRGT) was also employed. The second antibody was antirabbit IgG conjugated with horseradish peroxidase. Immune complexes were detected using an enhanced chemiluminescence kit. Quantitation was performed with a scanning laser densitometer (ScanAnalysis, Biosoft, Ferguson, MO).

GS activity

GS activity was measured as described in detail previously (19). GS activity was assayed at a physiological concentration of substrate (0.3 mM UDP-[^{14}C]glucose) in parallel incubations with 0.1 and 10 mM glucose-6-phosphate. GS activity is expressed as nanomoles of UDP-[^{14}C]glucose incorporated into glycogen per min/mg total protein or as fractional velocity (FV), a percentage of the ratio of activity at 0.1 mM glucose-6-phosphate/10 mM glucose-6-phosphate.

Glycogen synthesis

Glycogen synthesis was determined in differentiated myotubes as [^{14}C]D-glucose incorporation into glycogen during a 1-h incubation at 37 C (25). Treated cells were washed free of TNFα before incubation with or without insulin (33 nM). After incubation, cells were rinsed four times with PBS at 4 C and solubilized with 1 N NaOH at 55 C for 1 h. An aliquot (100 μl) of the lysate was removed for protein analysis. Lysates were neutralized with 10 N HCl, boiled for 30 min, and then cooled on ice. Glycogen was precipitated with 95% ethanol, and the pellet was washed by resuspension and precipitation. The final glycogen pellets were resuspended in 0.5 ml H_2O and mixed with scintillation fluid, and radioactivity was determined by liquid scintillation counting. Results are expressed as nanomoles of glucose converted to glycogen per h/mg protein.

Immunoblotting

Western blot analysis was performed by the method detailed previously (19). GS was identified using an affinity-purified polyclonal antibody raised in rabbits against an oligopeptide (12-mer) specific for the carboxyl-terminal sequence of GS (a gift from Dr. L. Groop, Malmo, Sweden). The secondary antibody was antirabbit IgG conjugated with horseradish peroxidase. Sarcomeric α-action was detected with a monoclonal antibody and antimouse IgG conjugated to horseradish peroxidase. Proteins were visualized with the enhanced chemiluminescence Western blot detection kit (Amersham) and exposed to autoradiograph film (XAR-5, Eastman Kodak Co., Rochester, NY). The intensity of the bands was quantified by scanning laser densitometry.

Statistical analysis

Statistical significance was evaluated using Student's *t* test and two-tailed *P* values were calculated. Paired analysis was performed for comparisons of insulin action and acute and prolonged TNFα exposures in the same sets of cells. Results presented as the percent change were obtained from comparisons with paired controls. Significance was accepted at $P < 0.05$.

Results

Influence of TNFα on glucose uptake

In vivo, TNFα infusion has been shown to have rapid effects on insulin-stimulated glucose disposal (7, 13). The acute effects of TNFα on glucose uptake in cultured muscle cells were tested by treating cells for 90 min in the absence or presence of insulin (33 nM), conditions shown to reveal maximal insulin stimulation of uptake (18). In cells from nondiabetic subjects, insulin treatment caused an approximately 50% increase over basal activity (paired comparison, $P < 0.05$). TNFα treatment led to a dose-dependent increase in uptake activity (not shown). Stimulation due to a maximal dose of TNFα (5 ng/ml; 75 ± 14%) was greater than that due to insulin (Fig. 1; $P < 0.02$). The combination of insulin and TNFα (77 ± 16% increase) did not cause any greater stimulation than that seen with TNFα alone.

Glucose uptake in type 2 diabetic muscle cells was impaired, compared with that in the nondiabetic group, in both the absence ($P < 0.05$) and presence ($P < 0.01$) of insulin (Fig. 1). However, similar results for the effects of TNFα were observed for glucose uptake in muscle cells from type 2 diabetic subjects. The stimulation resulting from insulin treatment (31 ± 4%) was less than that observed after TNFα exposure (51 ± 6%; $P < 0.05$). There was no additive or synergistic effect of combining insulin and TNFα (72 ± 18% increase; P = NS compared with TNFα alone).

Many effects of TNFα to cause insulin resistance in cultured cells require more prolonged exposure to the cytokine (5). To investigate the influence of extended TNFα exposure on muscle cell glucose uptake, cells were treated for 24 h with a maximal dose (5 ng/ml). The cells were then washed free of TNFα before the acute insulin treatment. Control cells retained insulin responsiveness (Fig. 1). In nondiabetic cells, prolonged TNFα treatment stimulated uptake activity to 152 ± 31% over basal, a greater extent than after acute treatment (74%; $P < 0.05$). There was a small additional effect of acute insulin stimulation in TNFα-treated cells (173 ± 35% stimulation; $P < 0.05$ compared with chronic TNFα alone). Prolonged TNFα treatment also elevated uptake in diabetic cells (76 ± 10%). However, unlike the case in nondiabetic cells, there was no further significant increase over that due to acute exposure (51%). Acute insulin exposure of chronically TNFα-treated diabetic cells resulted in a small further stimulation to 100 ± 13% of the basal level ($P < 0.05$ compared with chronic TNFα alone).

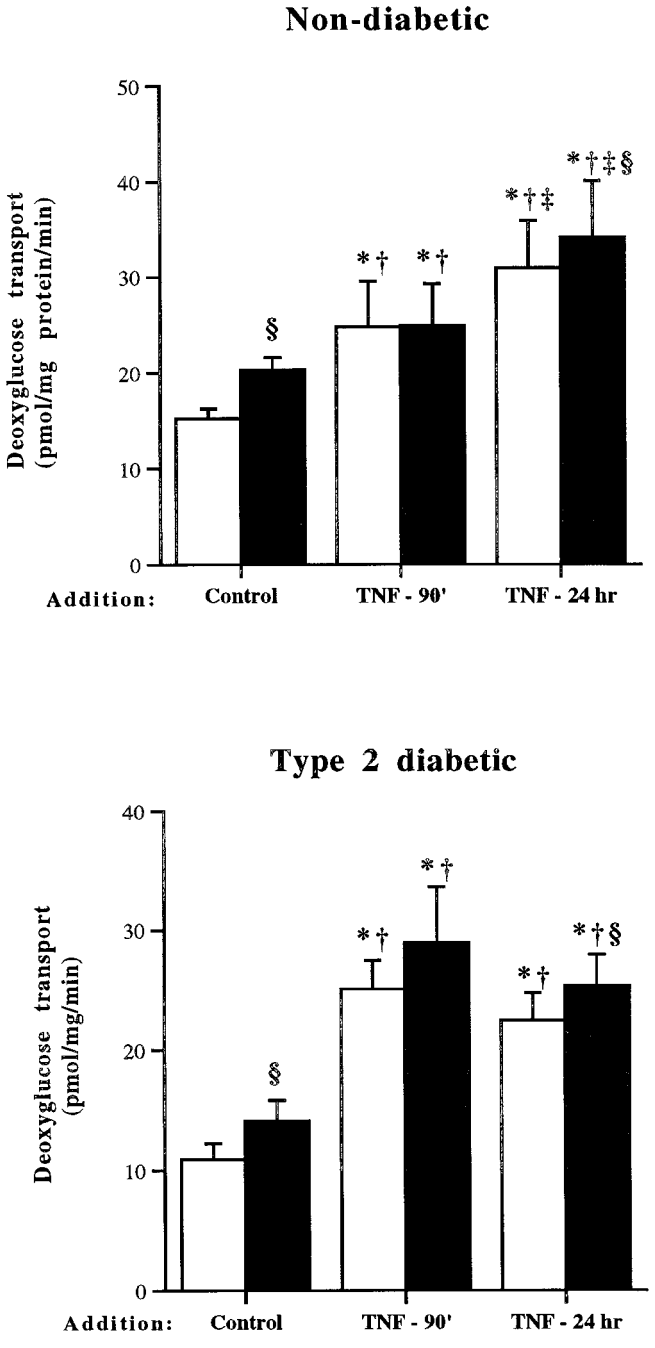

FIG. 1. Effect of TNFα exposure on glucose transport activity in cultured muscle cells from nondiabetic (*top panel*) and diabetic (*bottom panel*) subjects. Cells were treated for the indicated times in the absence (control) or presence of TNFα (5 ng/ml). *Open bars*, Basal transport; *solid bars*, insulin-stimulated transport (33 nM for 90 min). Results are the average ± SEM. *, $P < 0.05$ *vs.* paired control; †, $P < 0.05$ *vs.* insulin-treated control; §, $P < 0.05$ *vs.* paired basal values; ‡, $P < 0.05$ *vs.* 90-min TNF treatment.

Regulation of glucose transporter expression

One possible mechanism by which TNFα could modulate glucose transport activity would be through control of glucose transporter protein levels. Total membranes were prepared from muscle cells treated for 24 h in the absence or presence of 5 ng/ml TNFα. Transporter protein expression was determined by Western blotting. A representative autoradiogram for GLUT4 along with quantitation of such measurements performed on a larger set of cells are presented in Fig. 2A. GLUT4 protein levels were similar in nondiabetic and diabetic cells, confirming our previous results (17) and

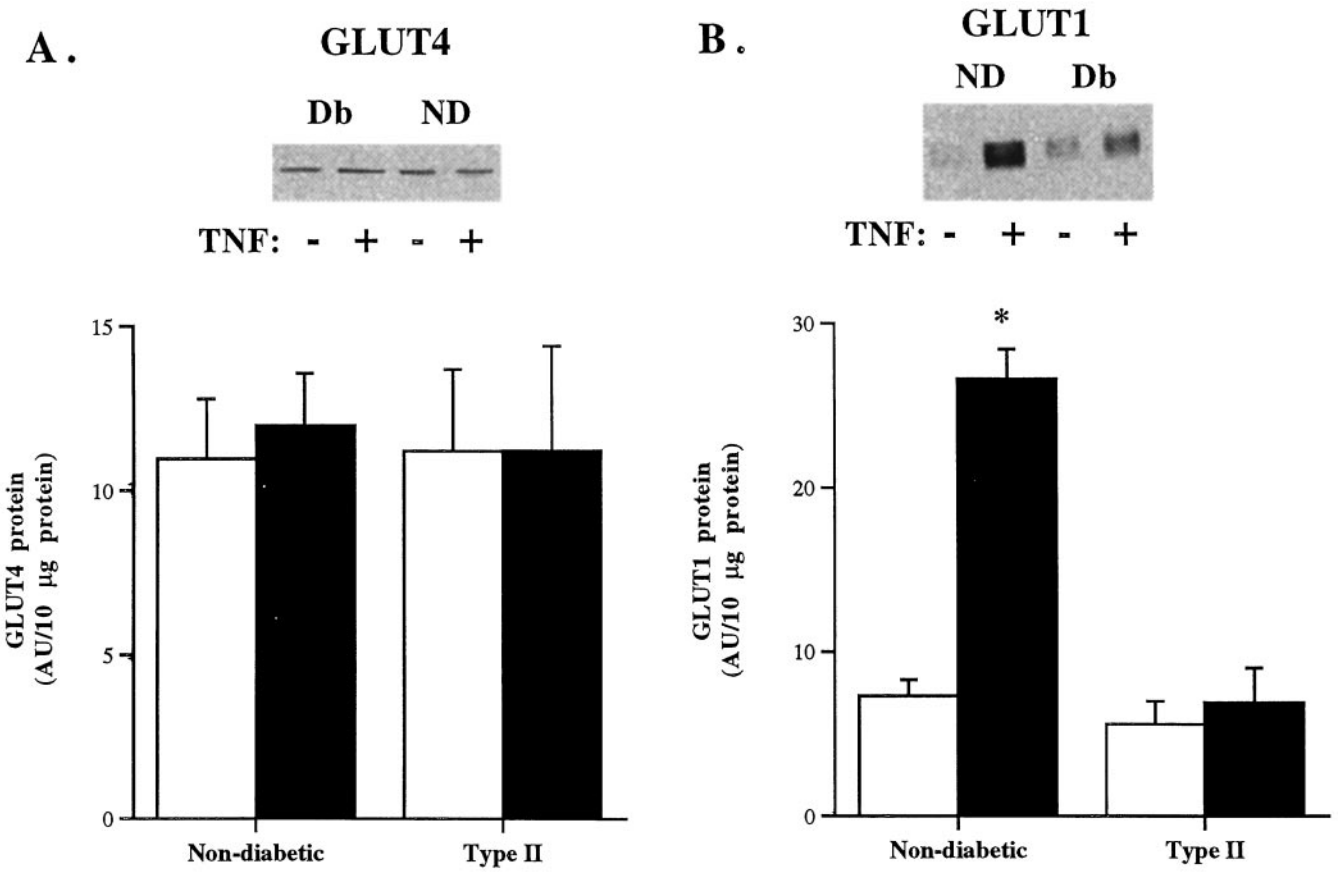

FIG. 2. Effect of prolonged TNFα exposure on total membrane glucose transporter protein in cultured muscle cells from nondiabetic (ND) and diabetic (Db) subjects. Cells were treated for 24 h in the absence (*open bars*) or presence (*solid bars*) of TNFα (5 ng/ml) before membrane preparation. A, *Top panel*, Representative autoradiogram of Western blot of GLUT4; *bottom panel*, quantitation of GLUT4 protein expression in nondiabetic (n = 7) and type 2 (n = 11) cells. B, *Top panel*, Representative autoradiogram of Western blot of GLUT1; *bottom panel*, quantitation of GLUT1 protein expression in nondiabetic and type 2 cells. *, $P < 0.05$ *vs.* control.

in agreement with findings in muscle biopsies (26). Prolonged TNFα treatment had no effect on GLUT4 protein expression in either nondiabetic or diabetic cells. Such a result might explain the lack of any increase in insulin stimulation of transport over the TNFα effect.

Total membrane GLUT1 protein expression in nondiabetic cells was increased 4.6 ± 1.3-fold ($P < 0.05$) after chronic TNFα treatment (Fig. 2B). The effect of chronic TNFα treatment on GLUT1 expression (1.7 ± 0.2-fold increase; $P < 0.05$) was much less in type 2 diabetic cells, consistent with the lesser up-regulation of transport activity (Fig. 1). It should be noted that only total membrane transporter content was measured in these studies; any potential additional effects of TNFα on transporter localization, such as translocation to the plasma membrane, would not be apparent from this analysis.

Influence of TNFα on GS and glycogen production

A major fate of glucose, once transported into skeletal muscle, is storage as glycogen. The activity of the rate-limiting enzyme for this process, GS, was monitored after acute treatment with insulin (33 nM) and/or TNFα. Insulin stimulated the FV of synthase by 57 ± 14% ($P < 0.05$) in nondiabetic cells. TNFα, when added to cells for 90 min, had no significant effect on synthase activity in either the absence or presence of insulin (Fig. 3); insulin responsiveness was retained in the presence of TNFα. Although both basal and insulin-stimulated synthase FVs were lower in diabetic muscle cells than in those of nondiabetics ($P < 0.05$; Fig. 3), similar behavior was observed in diabetic cells; the insulin response (70 ± 19% stimulation) was not altered by TNFα treatment (61 ± 18% stimulation). Basal synthase activity in diabetic cells was also not influenced by acute exposure to TNFα.

Longer term (24-h) treatment with TNFα reduced the GS FV (Fig. 3). In nondiabetic cells a similar relative decrease was seen for both basal (to 64 ± 11% of control; $P < 0.05$) and insulin-stimulated (71 ± 10% of control) activities. Insulin responsiveness was retained after TNFα treatment. Diabetic muscle cells displayed a smaller response to prolonged TNFα exposure; basal activity was 83 ± 11% of the control value ($P < 0.05$), and insulin-stimulated activity was reduced to 77 ± 7% of the control value. Again, synthase remained responsive to insulin. These reductions in synthase activity occurred with no change in either total enzyme activity or enzyme protein expression (Table 2) and most likely represent alterations in the activation state of the enzyme.

The consequence of the opposing effects of TNFα on glucose transport (positive) and of GS (none or negative) on the portion of glucose metabolism in muscle cells represented by

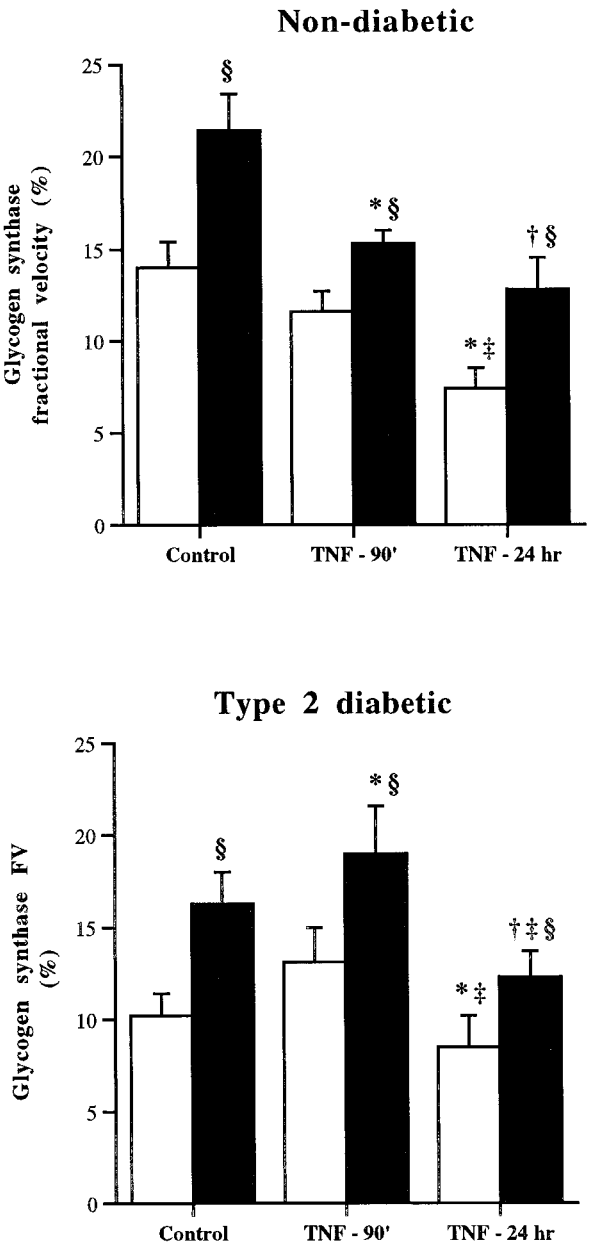

FIG. 3. Influence of TNFα exposure on GS activity in cultured muscle cells from nondiabetic and diabetic subjects. Cells were treated for the indicated times in the absence (control) or presence of TNFα (5 ng/ml). Cells were treated for 90 min in the absence (basal; *open bars*) or presence of insulin (*solid bars*; 33 nM) before cell extraction and GS assay. *Top panel*, Muscle cells from nondiabetic subjects (n = 11). *Bottom panel*, Cells from type 2 diabetic subjects (n = 9). Results are the average ± SEM. *, $P < 0.05$ *vs.* paired control values; †, $P < 0.05$ *vs.* insulin-treated controls; §, $P < 0.05$ *vs.* paired basal values; ‡, $P < 0.05$ *vs.* 90-min TNF treatment.

storage in glycogen was investigated in several ways. With regard to the total glycogen content of nondiabetic cells, neither acute nor prolonged TNFα treatment had any effect on glycogen stores (Table 3). The glycogen content of control diabetic cultures was lower than that in nondiabetics ($P < 0.01$), but again TNFα had no effect after either acute or prolonged exposure.

TABLE 2. Effect of chronic TNF-α treatment on glycogen synthase

	Nondiabetic	Type 2
Glycogen synthase total activity (nmol/mg protein/min)	(n = 11)	(n = 9)
Control	2.40 ± 0.20	2.89 ± 0.37
TNF-α treated	2.43 ± 0.17	2.77 ± 0.24
Glycogen synthase protein (AU/10 μg protein)	(n = 6)	(n = 5)
Control	10.8 ± 2.1	7.4 ± 3.8
TNF-α treated	12.9 ± 1.6	8.4 ± 3.8

Cells were treated ± TNF-α (5 ng/ml) for 24 h before assay of total activity of glycogen synthase or Western blotting for glycogen synthase protein. Results are average ± SEM.

TABLE 3. Influence of TNF-α treatment on glycogen stores

Treatment	Nondiabetic (n = 6)	Type 2 (n = 5)
Control	2.04 ± 0.34	0.93 ± 0.08[a]
TNF-α: 90 min	1.65 ± 0.19	1.01 ± 0.05[a]
TNF-α: 24 h	1.90 ± 0.40	0.82 ± 0.05[a]

Cells were treated ± TNF-α (5 ng/ml) for the indicated times before measurement of total glycogen content (mg glycogen/mg protein). Results are average ± SEM.

[a] $P < 0.05$ *vs.* ND.

Glucose incorporation into glycogen represents the net balance of glucose uptake, glycogen synthesis, and glycogenolysis. In nondiabetic cells, this activity was unaltered after either acute or prolonged TNFα treatment (Fig. 4). Insulin responsiveness was also retained. The large increases in transport activity seen under the same conditions did not direct more glucose toward glycogen. The same result was obtained in diabetic muscle cells (Fig. 4); both basal and insulin-stimulated glycogen synthesis were unaltered after TNFα treatment.

Discussion

Although TNFα is primarily secreted from macrophages and has a major role in mediating inflammatory responses (27), it is also produced in muscle (3) and adipose tissue (1) and can influence glucose and lipid metabolism (28). Recently, several lines of evidence have been advanced that support the hypothesis that TNFα is involved in the development of insulin resistance in obesity and type 2 diabetes (2, 5). Expression of TNFα is elevated in obese and diabetic humans (2, 3) as well as in animal models of obesity-linked insulin resistance (4). TNFα expression is also elevated in adipose tissue (1) and muscle (3) of insulin-resistant humans, is correlated with obesity, and is reduced with weight loss (2). There are also numerous reports that TNFα treatment of rats (6, 7) or dogs (13) led to insulin resistance for both peripheral glucose disposal, mainly into muscle, and suppression of hepatic glucose output. Neutralization of TNFα by infusion of a soluble TNFα-binding protein ameliorated insulin resistance in *fa/fa* rats (5).

Mechanisms by which TNFα might cause insulin resistance have been elucidated by studies in cultured cells. In 3T3-L1 adipocytes, incubation with TNFα resulted in down-regulation of GLUT4 (10, 29). Impairments of insulin signaling were observed as reductions in insulin effects on receptor

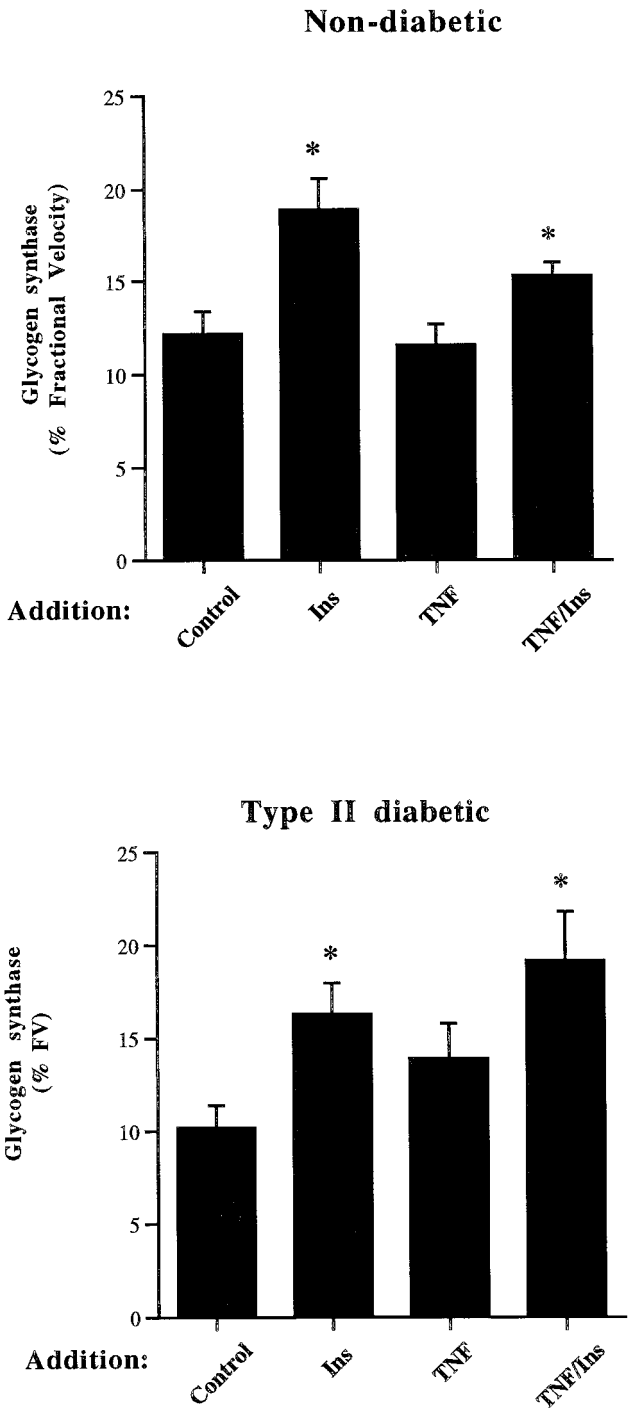

FIG. 4. Effect of TNFα treatment on net glycogen synthesis in muscle cells. No insulin (basal; *open bars*) or insulin (33 nM; *solid bars*) and [U-^{14}C]glucose were added together for 60 min. TNFα (5 ng/ml) treatment was given for 90 min or 24 h before insulin and glucose addition; TNFα remained present during the glycogen synthesis assay. *Top panel*, Muscle cells from nondiabetic subjects (n = 5). *Bottom panel*, Cells from type 2 diabetic subjects (n = 5). Results are the average ± SEM. *, $P < 0.05$ *vs.* basal values for the same condition.

autophosphorylation and insulin receptor substrate-1 phosphorylation (11). These effects occurred together with increased serine phosphorylation of insulin receptor substrate-1 (11). Complicating factors in evaluating the effects of TNFα on adipocytes include evidence that the cytokine is a potent lipolytic agent (9) and also causes adipocyte dedifferentiation (10, 29).

The literature concerning the effects of TNFα on glucose metabolism and insulin action in skeletal muscle is more mixed. Although there is a report in L6 myocytes that TNFα treatment reduced glucose transport and glycogen synthesis, possibly through impairment of protein phosphatase-1 (30), other investigators have found no effect in the same cell line (31). Incubation of isolated soleus muscles with TNFα also failed to have any effect on glucose uptake (32). Multiple investigators have found that infusion of TNFα, although generating insulin resistance (6–8, 13), also elevated basal glucose disposal and uptake into muscle and adipocytes (33, 34).

Stimulatory effects of acute TNFα treatment on glucose metabolism *in vivo* (13) are similar to our finding of the ability of TNFα to rapidly stimulate glucose uptake in human skeletal muscle cells (Fig. 1), although the greater potency than insulin was an unexpected finding. The lack of additivity of insulin and TNFα after acute exposure suggests either that both agents work through the same pathways or that TNFα stimulates transport to the maximal capacity of the system. However, the failure of TNFα to cause significant stimulation of GS (Fig. 4) suggests that TNFα replicates only some of the actions of insulin. TNFα might be acting at a point where signaling to transport diverges from that for GS activation.

Although the mechanism for the acute transport stimulatory effect of TNFα is unknown, the major cause of the chronic increase in transport activity seems clear; up-regulation of the GLUT1 protein (Fig. 2). Up-regulation of GLUT1 has also been seen in human adipocytes cultured with TNFα (9). In addition, a recent report in L6 cells found that 24-h TNFα treatment in combination with interferon-γ up-regulated basal glucose transport and GLUT1 protein expression (35); these changes were linked to the induction of inducible nitric oxide synthase (NOS) and nitric oxide production. In 3T3 fibroblasts, TNFα has been shown to cause GLUT1 messenger RNA stabilization through synthesis of a factor that binds to the 3′-UTR (untranslated region) of the gene (36); either or both of these mechanisms may be active in skeletal muscle. Diabetic muscle cells showed smaller changes in both transport activity and GLUT1 protein in response to TNFα. This difference may be indicative of partial TNFα resistance, at least for one response, in diabetic muscle. This would be in addition to the impairments of glucose transport activity preserved in cultured human muscle cells. If inducible NOS is involved in mediating the GLUT1 response to chronic TNFα exposure (35), then the reduced response in diabetic cultured muscle cells may indicate an impairment in the NOS system in diabetes.

The response of GLUT4 to TNFα appears to be tissue specific. GLUT4 protein levels in muscle are unaltered in animals with elevated TNFα levels (37, 38), similar to our finding. Meanwhile, decreases are common in TNFα-treated adipocytes (10, 29, 29). Although dedifferentiation of adipocytes in response to TNFα could account for some loss of GLUT4, this would not occur in terminally differentiated

primary adipocytes; some adipocyte-specific factor might be involved in the differential response.

Beyond effects on final metabolic responses, TNFα has also been shown to generate insulin resistance both *in vivo* (6, 7) and in cell systems (9–12). Due to the TNFα-induced elevations in basal glucose uptake, it is difficult to draw any conclusions about the effect of TNFα on insulin action for this response. Is there little or no further insulin stimulation because the cells are now insulin resistant or because the maximal capacity of the system has been attained? Yet GS and glucose incorporation into glycogen remain normally insulin responsive after TNFα treatment, suggesting that under these experimental conditions, TNFα alone is not causing insulin resistance.

The additional glucose entering muscle cells after either acute or prolonged TNFα treatment was not directed toward storage in glycogen, as seen by the lack of change in glycogen content or net glycogen synthesis. A similar lack of change in glycogen content was seen in isolated soleus strips after incubation with TNFα (32). Elevations of basal glucose uptake and GLUT1 protein, seen in L6 cells after cytokine treatment (35) and presumably linked to elevations in TNFα, were accompanied by increases in lactate release, suggesting that the additional glucose might be directed toward glycolysis and away from glycogen synthesis. The slight reduction in GS activity could also contribute to glycolysis being preferred over storage in glycogen. This scenario is conjecture, as glycolytic intermediates were not measured in our studies.

As both basal and insulin-stimulated GS FVs are reduced, even as total activity and enzyme protein expression are unaltered, it is the activation state of the enzyme that must be influenced by TNFα. Insulin regulates synthase activity by dephosphorylation of the enzyme. However, this is a rapid response and occurs within the time frame where there was no acute effect of TNFα on synthase activity. The more delayed effect of TNFα to reduce synthase activity could be due to the accumulation of an intracellular intermediate that would exert allosteric effects on the synthase enzyme. Conversely, TNFα could gradually alter the expression and activity of phosphatases or kinases that activate or deactivate the enzyme, respectively.

The opposing effects of TNFα on metabolism in adipose tissue and skeletal muscle may serve several roles. One would be to divert substrates from adipose tissue, by lipolysis or inhibition of glucose transport, to meet the energy needs of muscle. This would preserve substrate stores within the muscle at the expense of adipose tissue. TNFα stimulation of muscle glucose transport, which would be greater in the presence of the higher cytokine levels characteristic of insulin-resistant states, could also partially compensate for impaired glucose transport and insulin resistance due to either elevated FFA levels or the defects intrinsic to skeletal muscle in type 2 diabetes. The elevated glucose and insulin levels present in diabetes and other insulin-resistant states could also influence the final effect of TNFα; the current studies investigated direct effects of TNFα under normal glycemic, insulinemic, and triglyceridemic conditions and might not be fully reflective of the more complicated situation present in type 2 diabetes. In addition, resistance to the chronic ability of TNFα to up-regulate glucose transport and GLUT1 expression would represent an additional impairment in type 2 diabetic skeletal muscle and limit whatever effects TNFα might have to compensate for impaired glucose uptake in diabetes.

There is compelling evidence that TNFα can lead to skeletal muscle insulin resistance in the *in vivo*, whole body context. What the current results, obtained in an isolated muscle cell culture system, suggest is that this response may be an indirect one. Although TNFα may act directly on skeletal muscle to increase glucose uptake and promote glucose utilization, possibly toward oxidation, the cytokine would also be activating lipolysis in adipose tissue. The resulting elevated FFA levels could contribute to insulin resistance in the adjacent muscle. This supposition is supported by the finding that antidiabetic thiazolidinediones, which lower FFA levels (39), can improve TNFα-induced insulin resistance both *in vivo* (8) and in adipocytes (10, 40). More direct evidence comes from a report that thiazolidinedione treatment of 3T3-L1 adipocytes can block the lipolytic effects of TNFα (41). However, the hypothesis that elevated FFA levels may be causing insulin resistance also needs to be tested directly in cultured human muscle cells. The smaller changes in diabetic muscle cells in glucose transport and GLUT1 up-regulation to TNFα suggest that in addition to insulin resistance, type 2 diabetics may have an impaired response to this potential compensatory effect of TNFα.

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