Stimulation of Lipolysis by Tumor Necrosis Factor-α in 3T3-L1 Adipocytes Is Glucose Dependent

Implications for Long-Term Regulation of Lipolysis

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Tumor necrosis factor-α (TNF-α) and hyperglycemia both impair insulin sensitivity in vivo. This may be secondary to stimulation of adipose tissue lipolysis and consequent increased circulating free fatty acids (FFAs). Here we report that neither TNF-α nor glucose alone has a pronounced effect on lipolysis in 3T3-L1 adipocytes. However, the combination of TNF-α plus glucose markedly stimulates lipolysis. Glucose does not affect the ability of isoproterenol to stimulate lipolysis. Alternative substrates such as acetate, pyruvate, and lactate do not allow the TNF-α effect. Mannose was almost as effective as glucose; fructose was marginally effective, but galactose was ineffective. The effectiveness of the sugars corresponded with production of lactate, i.e., the cells readily produced lactate from glucose or mannose, slightly from fructose, and not at all from galactose. The ability of TNF-α to phosphorylate extracellular signal–regulated kinase 1 (ERK1) and ERK2 and to downregulate perilipin (which has been implicated in the lipolytic effect of TNF-α) was not affected by glucose. We conclude that the lipolytic action of TNF-α is influenced by glucose in 3T3-L1 adipocytes. The findings suggest that glucose metabolism is required for the lipolytic response to TNF-α but not for early signaling events. These findings suggest novel mechanisms by which TNF-α and hyperglycemia raise FFA levels and induce insulin resistance. *Diabetes* 53:74–81, 2004

Obesity is strongly associated with the development of insulin resistance. In the past few years, the role of adipose tissue itself in development of insulin resistance has been gaining attention (1). This is thought to be the result of at least two phenomena. First, adipose tissue produces a number of hormones and cytokines, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), resistin, and adiponectin, each of which may affect insulin action (2). Second, adipose tissue contributes to excess free fatty acid (FFA) production in obese individuals, through lipolysis of adipocyte triglyceride. It has been hypothesized for many years that FFA can antagonize the effect of insulin on skeletal muscle, through the so-called "glucose-fatty acid cycle" of Randle et al. (3). More recently, this hypothesis has been demonstrated experimentally in vivo (4,5). Excess FFA can also increase hepatic glucose output (1,6) and have multiple adverse effects relevant to development of diabetes and cardiovascular disease (7–9).

Much attention has focused on the potential role of TNF-α in the insulin resistance of obesity (10–15). Recent findings in animals and humans have demonstrated that TNF-α mRNA is expressed in adipose tissue. Furthermore, adipose tissue from obese animals or obese humans contains markedly higher concentrations of this mRNA than adipose tissue from the lean. TNF-α induces insulin resistance in adipocytes (16), and infusion of TNF-α–binding proteins into obese animals improves insulin resistance (14). TNF-α has direct effects on insulin signaling (11,16,17), but in addition, we (18–20) and others (21,22) have proposed that TNF-α causes insulin resistance in vivo indirectly by increasing FFA release from adipose tissue. TNF-α increases lipolysis in human (23,24), rat (18–20), and 3T3-L1 adipocytes (21,22,25,26).

Glucose itself also induces insulin resistance in certain cells and in vivo—so-called glucose toxicity (27). Therefore, hyperglycemia may contribute to worsening insulin resistance in individuals with impaired glucose tolerance. We have reported that hyperglycemic, hyperinsulinemic conditions can chronically increase the rate of lipolysis in primary rat adipocytes (28). Therefore, hyperglycemia may worsen insulin resistance in part by increasing FFA release from adipocytes, which would induce insulin resistance as described above.

In the present report, we describe findings that demonstrate that the lipolytic effect of TNF-α is markedly influenced by glucose concentration. The findings demonstrate an absolute requirement for glucose for the effect of TNF-α on lipolysis in these cells and suggest that one or more glycolytic intermediates are required for the effect of TNF-α.

RESEARCH DESIGN AND METHODS

Materials. 3T3-L1 cells were obtained from American *Type Culture* Collection (ATCC, Manassas, VA). Glucose-containing Dulbecco's modified Eagle's medium (DMEM) and antibiotics were from Atlanta Biologicals (Norcross,

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DMEM, Dulbecco's modified Eagle's medium; ERK, extracellular signal–regulated kinase; FFA, free fatty acid; IL-6, interleukin-6; MAP, mitogen-activated protein; TNF-α, tumor necrosis factor-α.

GA), glucose-free DMEM was from Irvine Scientific (Santa Anna, CA), fetal bovine serum was from Hyclone Laboratories (Logan, UT), insulin (Humulin R7) was from Eli Lilly and Co. (Indianapolis, IN), BSA was from Intergen Co. (Purchase, NY), glutamine was from Gibco (Grand Island, NY), anti–extracellular signal–regulated kinase (ERK) 1/2 and anti-active mitogen-activated protein (MAP) kinase antibodies were from Promega (Madison, WI), and secondary antibody (donkey anti-rabbit horseradish peroxidase conjugate) was from Santa Cruz Biotechnology (Santa Cruz, CA). All other reagents were from Sigma (St. Louis, MO).

Cell culture. 3T3-L1 cells (ATCC CL-173) were plated in 24-well plates and maintained in standard medium (DMEM with high glucose, supplemented with 10% fetal bovine serum and with PSA (penicillin 100 units/ml, streptomycin 100 μg/ml, and amphotericin 0.25 μg/ml). Medium was changed every 2–3 days. At 2–4 days after confluence, differentiation into adipocytes was initiated as follows: standard medium was supplemented with 5 μg/ml insulin, 0.5 μg/ml dexamethasone, and 0.5 mmol/l 3-isobutyl-1-methylxanthine for 2 days. The medium was then changed and supplemented with insulin only for 2–3 days. Thereafter, the cells were maintained in standard medium only.

For experimental conditions without glucose, cells were incubated in DMEM without glucose, supplemented with 1% BSA, 4 mmol/l glutamine, 44 mmol/l $NaHCO_3$, 20 mmol/l HEPES, and 0.01% pyruvic acid. Stock solutions of glucose, fructose, galactose, and mannose were prepared at 500 mmol/l in DMEM without glucose.

Lipolysis assay. Lipolysis was measured as the rate of glycerol release over a 1-h period. Media were collected from the cells and heated at 65°C for 8 min to inactivate any enzymes released from the cells. Samples (25 μl) were then assayed for glycerol using 175 μl of glycerol reagent (GPO Trinder Reagent A; Sigma) in a flat-bottom 96-well plate. Absorption was measured at 540 nm on a Dynatech plate reader. In some experiments, fatty acid accumulation was determined on 20-μl samples, using a kit from Wako Chemicals (Richmond, VA).

Lactate and glucose determinations. Lactate and glucose concentrations in cellular supernatants were measured on a Roche Cobas Mira Plus analyzer.

ATP determinations. Cells were incubated as described in the figure legends, and then the cells were washed with PBS (100 μl per well). ATP concentrations were measured using the CellTiter-Glo luminescent cell viability assay kit (Promega), following directions provided by the manufacturer.

Western blots. Cells were harvested in Laemmli sample buffer (29) and aspirated with a syringe five times through a 25-G needle. The samples were then centrifuged (16,000*g*, 30 s) to remove fat and then heated at 95°C for 5 min before being resolved on SDS polyacrylamide gels (10%). Proteins were transferred to nitrocellulose. For GLUT1, membranes were blocked with 5% nonfat dry milk in TBS and 0.05% TWEEN for at least 2 h, washed, and incubated with primary antibody (affinity-purified, polyclonal antibodies against human GLUT1; Alpha Diagnostics, San Antonio, TX) overnight at 4°. The membranes were washed again and incubated with second antibody (peroxidase-conjugated affinity-purified goat anti-rabbit IgG; Jackson ImmunoResearch Laboratories, West Grove,

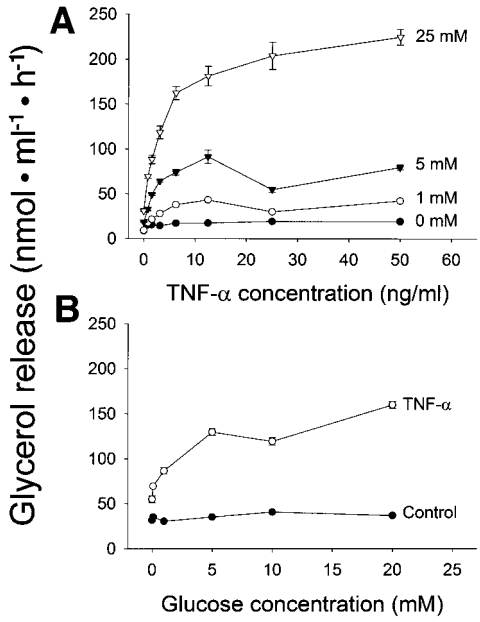

FIG. 1. Effect of TNF-α and glucose on lipolysis. Fully differentiated 3T3-L1 adipocytes were incubated overnight with or without glucose and TNF-α, as indicated. The cells were washed, and the rate of lipolysis was measured as glycerol release over a 1-h incubation period. *A*: Dose-response curve to TNF-α in the presence of 0, 1, 5, or 25 mmol/l glucose. *B*: Dose-response curve to glucose in the absence or presence of TNF-α (50 ng/ml). Data are means ± SD (n = 3).

PA) for 1 h. The membranes were washed and finally developed using a SuperSignal West Pico Chemoluminescent Kit (Pierce, Rockford, IL). Dried membranes were exposed using XFS-1 Scientific Imaging Film (Eastman Kodak, Rochester, NY) for 5 s to 3 min. Film images were digitized and analyzed using the software provided with a Bio-Rad Molecular Imager FX. For ERK and perilipin, membranes were blocked with 5% blotto, 1% BSA, or 0.2% I-Block and probed with polyclonal rabbit antibodies, anti-perilipin (gift of Dr. Andrew Greenberg), anti-ERK1/2, or anti-active MAP kinase. After incubation with anti-rabbit IgG- horseradish peroxidase, the blots were developed with ECL Plus and visualized with Hyperfilm ECL (Amersham Pharmacia Biotech, Piscataway, NJ).

RESULTS

A number of reports have demonstrated that TNF-α increases the rate of lipolysis in 3T3-L1 adipocytes (21,22,25,26). The experiment illustrated in Fig. 1 demonstrates a strong dependence for this effect on the presence of glucose in the incubation medium. Fully differentiated 3T3-L1 cells were incubated for 16 h with various concentrations of TNF-α (0–50 ng/ml) and glucose (0–25 mmol/l). The rate of glycerol release was then determined over a 1-h period as a measure of the rate of lipolysis. When there was no glucose in the incubation medium, the stimulatory effect of TNF-α was minimal. However, glucose produced a marked and dose-related increase in the TNF-α–induced rate of lipolysis (Fig. 1*A*). In the presence of glucose, TNF-α increased the rate of lipolysis approximately sevenfold, with a half-maximally effective concentration of TNF-α of ~2 ng/ml.

Conversely, as shown in Fig. 1*B*, glucose did not substantially alter the basal rate of lipolysis in 3T3-L1 cells.

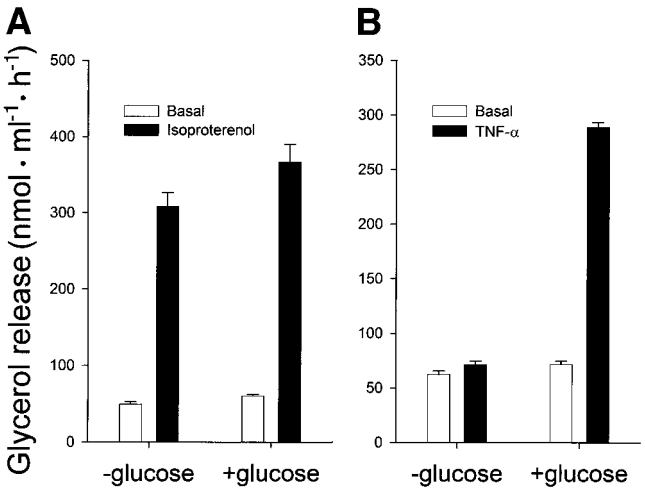

FIG. 2. Effect of isoproterenol and TNF-α in the absence and presence of glucose. *A*: Adipocytes were incubated for 16 h with or without glucose, and then the cells were washed and lipolysis was measured without or with isoproterenol (1 μmol/l) over 1 h. *B*: Adipocytes were incubated for 16 h in the absence or presence of glucose and TNF-α. The cells were then washed, and the adipocytes were incubated for an additional 1 h. Data are means ± SD (n = 3).

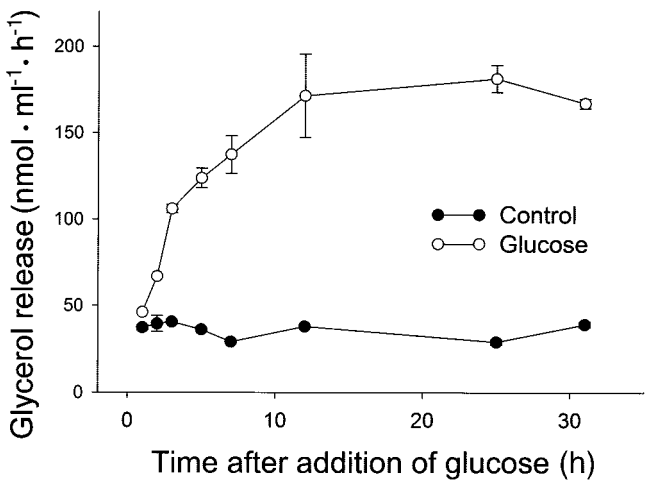

FIG. 3. Time course for glucose-induced TNF-α action. Adipocytes were incubated for 16 h with TNF-α (50 ng/ml) but no glucose. The medium was then removed and replaced with media containing TNF-α either with no glucose (●) or with 25 mmol/l glucose (○). At intervals thereafter, the cells were washed and the rate of glycerol release was determined over the next 1 h. Data are means ± SD (n = 3).

However, when the cells were incubated in the presence of TNF-α, glucose induced a dose-related increase in the rate of lipolysis. This dose-response relationship corresponded approximately to the physiologically meaningful concentration range of glucose. These findings demonstrate that the lipolytic action of TNF-α is markedly influenced by glucose concentration and that, conversely, the lipolytic effect of glucose is influenced by TNF-α.

The above findings could be interpreted as suggesting that 3T3-L1 cells require glucose to support high rates of lipolysis. Therefore, we investigated whether this requirement for glucose was necessary for the lipolytic effect of isoproterenol, another very potent lipolytic agent. This experiment (Fig. 2) demonstrated that isoproterenol is a potent stimulator of lipolysis regardless of whether the cells had been incubated with glucose. This finding demonstrates that there is no requirement for glucose for lipolysis to proceed at a high rate and that lack of glucose interferes more specifically with the lipolytic effect of TNF-α.

For determining the time required for glucose to augment the TNF-α effect on lipolysis, adipocytes were incubated for 16 h with TNF-α but no glucose (Fig. 3). Glucose was then added, and the rate of lipolysis was followed over time. As in the previous experiments, there was little effect with TNF-α alone; however, when glucose was added, the rate of lipolysis increased markedly. Lipolysis was higher than basal by ~2 h and maximal by ~12 h after addition of glucose to the incubation medium. By contrast, the rate of lipolysis remained low and constant in the cells that received TNF-α but no glucose. This finding demonstrates that the glucose effect is relatively slow to develop and also demonstrates that 16 h without glucose does not damage the cells in a manner that impairs their ability to respond to TNF-α.

To determine whether the lack of effect of TNF-α in cells incubated without glucose is simply due to a lack of energy source, we first determined whether alternative substrates could substitute for glucose. The experiment in Fig. 4 confirmed the requirement for glucose but also demonstrated that the lipolytic effect of TNF-α could not be supported by acetate, pyruvate, or lactate, suggesting that lack of an energy source in the absence of glucose was not an issue. This was further supported by measurements of cellular ATP (Fig. 5), which demonstrated that ATP concentrations were maintained in these cells, regardless of the presence or absence of glucose or TNF-α in the incubation medium.

Experiments were performed to determine whether similar results would be obtained if fatty acid release, rather than glycerol release, were used as a measure of lipolysis. In Fig. 6, 3T3-L1 cells were incubated for 16 h in the absence or presence of glucose and TNF-α as before, and then the rate of fatty acid release and glycerol release was measured on the same samples. As before, glucose and TNF-α increased the rate of glycerol release only modestly when added alone, but the combination of glucose plus TNF-α produced a marked stimulation. Similarly, glucose or TNF-α alone only modestly stimulated fatty acid release, but the combination markedly stimulated fatty acid release (Fig. 6*B*).

To determine whether other monosaccharides can support the lipolytic effect of TNF-α, we investigated effects of galactose, mannose, and fructose (Fig. 7*A*). Galactose, at concentrations up to 25 mmol/l, resulted in only a very small effect of TNF-α, similar to that seen in the absence of glucose. Fructose had a small effect but much less than that of glucose. Mannose, however, had a markedly greater effect, similar to that of glucose.

As a measure of whether the cells could metabolize these monosaccharides, we determined the amount of lactate released from cells incubated with each of the sugars (Fig. 7*B*). Two interesting findings emerged from these studies. First, there was almost no lactate production from galactose and very little from fructose but marked lactate production from both glucose and mannose. This correlates well with the ability of the sugars to allow TNF-α–induced lipolysis (Fig. 7*A*). Second, it can be seen that TNF-α increases the rate of lactate production

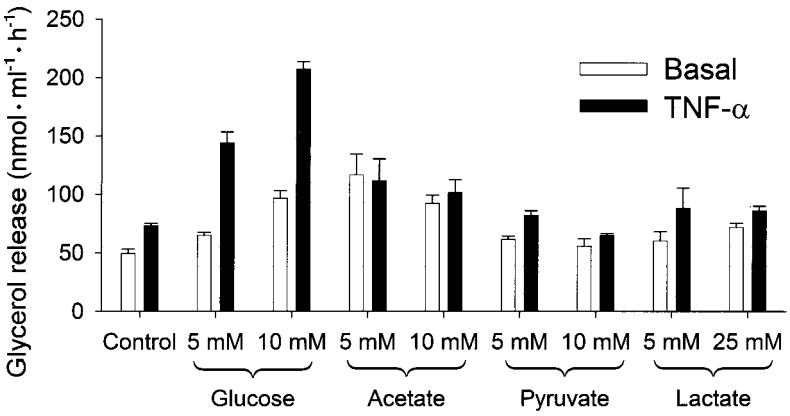

FIG. 4. Effect of alternative substrates on the lipolytic effect of TNF-α. Adipocytes were incubated for 16 h in the absence or presence of TNF-α (50 ng/ml), with no additions (control), or glucose, acetate, pyruvate, or lactate as indicated. The cells were washed, and glycerol release was determined after 1 h as described in Fig. 1. Data are means ± SD (n = 3). Two-way ANOVA showed a significant (P < 0.001) interaction between substrate (control, glucose, acetate, pyruvate, lactate) and condition (basal versus TNF-α). Post hoc comparisons showed a statistically significant (P < 0.001) difference in glycerol release between the basal and TNF-α but in the presence of glucose only.

from either glucose or mannose (Fig. 7*B*). This finding suggests that TNF-α increases the rate of monosaccharide uptake and metabolism by 3T3-L1 adipocytes and suggested the possibility that expression of GLUT1 might be affected. Western blots with a GLUT1 antibody were performed on extracts of these cells (see RESEARCH DESIGN AND METHODS), but the relative concentration of GLUT1 protein was not altered by glucose, TNF-α, or glucose plus TNF-α (data not shown).

Further experiments were performed to determine whether 3-*O*-methyl glucose could substitute for glucose to allow TNF-α–induced lipolysis (Fig. 8). This glucose analog is taken up by adipocytes but is not a substrate for hexokinase and so is not further metabolized. These experiments demonstrated that this nonmetabolizable glucose analog could not replace glucose. Similarly, L-glucose, which is neither taken up nor metabolized, could not substitute for D-glucose (data not shown).

Glucose is known to induce insulin resistance in adipocytes by a mechanism that involves the hexosamine pathway (30). This effect of glucose can be mimicked by addition of glucosamine to certain cells, especially primary rat adipocytes (31). Therefore, we investigated whether the effect of glucose that we have identified here is also mimicked by glucosamine. In Fig. 9, 3T3-L1 adipocytes were treated with glucosamine, with or without TNF-α. Glucosamine itself resulted in a small increase in the rate of lipolysis. TNF-α alone produced a small increase in the rate of lipolysis, as in the other experiments (Figs. 1–8). However, unlike glucose, glucosamine did not allow the dramatic increase in lipolysis in response to TNF-α that we observed in the presence of glucose (cf. Fig. 1). Furthermore, this small effect of TNF-α did not increase as the glucosamine concentration increased, demonstrating that glucosamine did not alter the ability of TNF-α to stimulate lipolysis in these cells.

Activation of ERK1 and ERK2 has been shown to be an early event in TNF-α signaling in human preadipocytes (24) and in 3T3-L1 cells (32). Therefore, we investigated whether these MAP kinases can be activated in the absence of glucose. Cells were incubated for 16 h in the presence or absence of glucose and were then treated for 15 min with or without TNF-α (Fig. 10). TNF-α increased phosphorylation of both ERK1 and ERK2 (Fig. 10*A*) but had no effect on total ERK1 or ERK2 (Fig. 10*B*). Furthermore, the ability of TNF-α to increase phosphorylation of these MAP kinases was not altered by the presence or absence of glucose. Similarly, glucose did not affect total cellular concentration of ERK1/2 in these cells, demonstrating that lack of glucose does not block all effects of TNF-α.

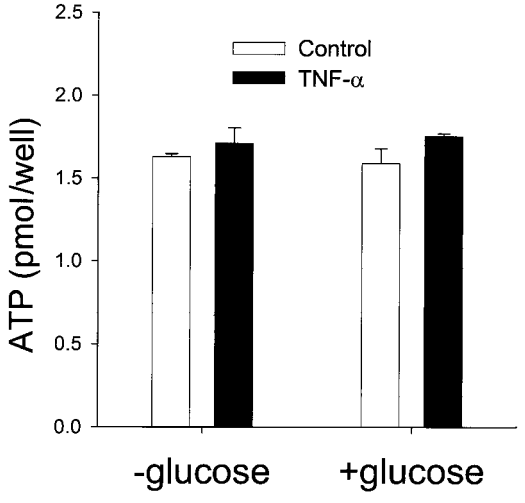

FIG. 5. Cellular ATP concentrations. Adipocytes were incubated with no additions, glucose (25 mmol/l), TNF-α (50 ng/ml), or glucose plus TNF-α, as indicated, for 16 h. The cells were washed and lysed, and ATP concentrations were determined as described in RESEARCH DESIGN AND METHODS. Data are means ± SD (n = 3).

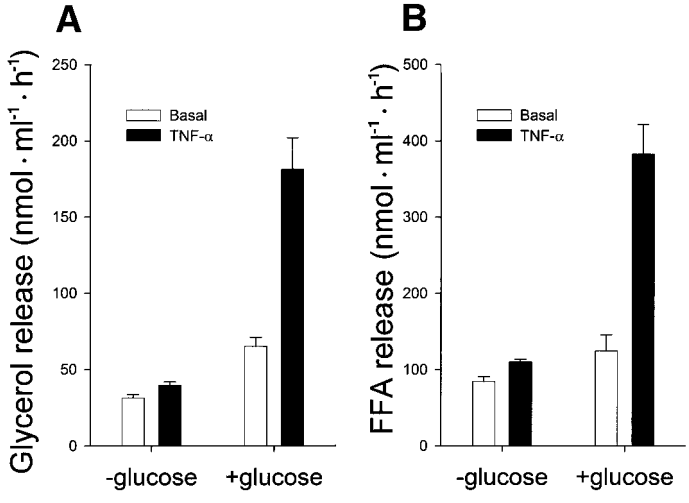

FIG. 6. Effect of glucose and TNF-α on fatty acid release. Adipocytes were incubated with or without glucose (25 mmol/l) and/or TNF-α (50 ng/ml), as indicated, for 16 h. The cells were washed, and the rate of lipolysis was measured as glycerol release (*A*) or fatty acid release (*B*) over a 1-h incubation period. Data are means ± SD (n = 3).

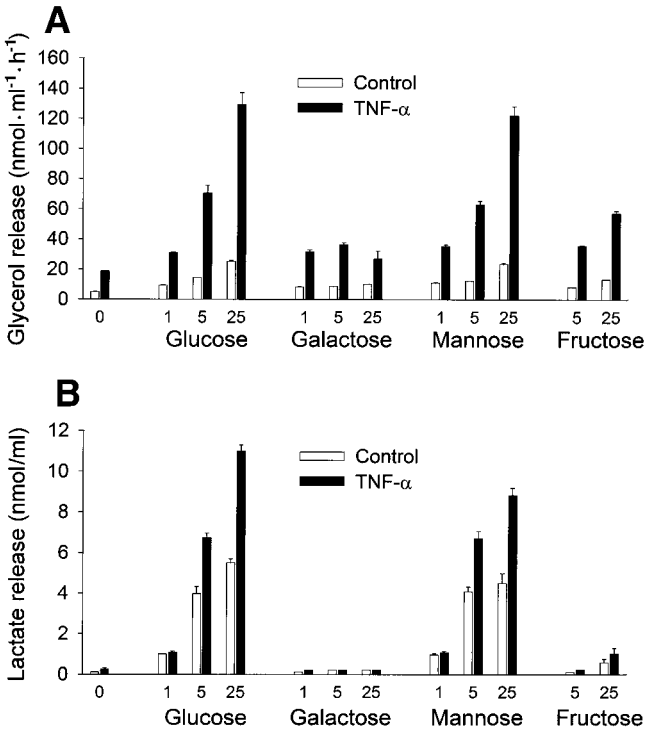

FIG. 7. Effect of other sugars on the lipolytic effect of TNF-α. Adipocytes were incubated for 16 h in the absence or presence of TNF-α (50 ng/ml) with no additions or with 1, 5, or 25 mmol/l glucose, galactose, mannose, or fructose, as indicated. After 16 h, the supernatant was removed, and the cells were washed. *A*: Glycerol release over the next 1 h. *B*: Lactate in the supernatant removed after the 16-h incubation. Data are means ± SD (n = 3).

The lipolytic effect of TNF-α in 3T3-L1 adipocytes has been reported to be secondary to downregulation of perilipin (21,22). Therefore, we determined relative concentrations of perilipin in cells treated with or without both TNF-α and glucose (Fig. 11). Adipocytes express two forms of perilipin, termed perilipin A and perilipin B, although the latter has been reported to be very weakly expressed in 3T3-L1 cells (21). Indeed, under our conditions, we were able to detect only perilipin A, which ran as a doublet, probably representing different phosphorylation

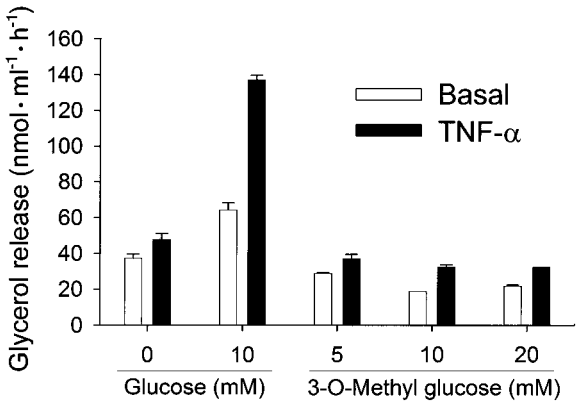

FIG. 8. Effect of glucose and 3-*O*-methyl glucose on the lipolytic effect of TNF-α. Adipocytes were incubated for 16 h in the absence or presence of TNF-α (50 ng/ml) with no additions; with glucose (10 mmol/l); or with 5, 10, or 20 mmol/l 3-*O*-methyl glucose, as indicated. The supernatant was then removed, the cells were washed, and the rate of glycerol release was measured over 1 h. Data are means ± SD (n = 3).

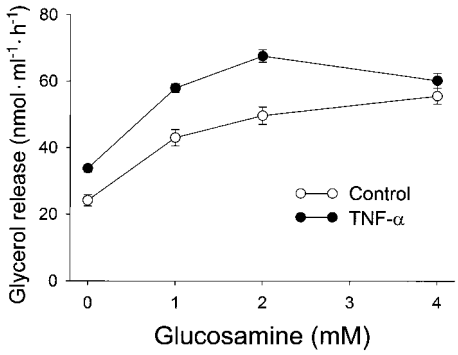

FIG. 9. Effect of glucosamine and TNF-α on lipolysis. Adipocytes were incubated with no glucose for 16 h in the absence or presence of TNF-α (50 ng/ml) and 0–4 mmol/l glucosamine as indicated. The cells were washed, and glycerol release was determined over the next 1 h. Data are means ± SD (n = 3).

states. TNF-α induced a clear downregulation of perilipin. However, the ability of TNF-α to downregulate perilipin was not affected by the presence or absence of glucose in the incubation medium. This finding demonstrates that lack of glucose does not prevent TNF-α–induced perilipin downregulation and that perilipin downregulation is not sufficient, by itself, to induce lipolysis.

DISCUSSION

Several reports have documented that TNF-α is a potent stimulus for lipolysis in primary rat adipocytes (18–20), human adipocytes differentiated in vitro (23,24), and differentiated murine 3T3-L1 cells (21,22,25,26). We have previously reported that in primary rat adipocytes, basal and isoproterenol-stimulated lipolysis can be increased by the presence of glucose in the incubation medium (28). Furthermore, this increase occurred over the physiologically significant concentration range of glucose. In the present report, we have found that the lipolytic action of TNF-α is markedly influenced by the concentration of glucose in the incubation medium, in 3T3-L1 adipocytes. Neither glucose alone nor TNF-α alone greatly affected the rate of lipolysis in these cells. However, the combination of TNF-α and glucose increased the rate of lipolysis markedly, by as much as six- to sevenfold. Furthermore, the glucose concentrations that affect the lipolytic action

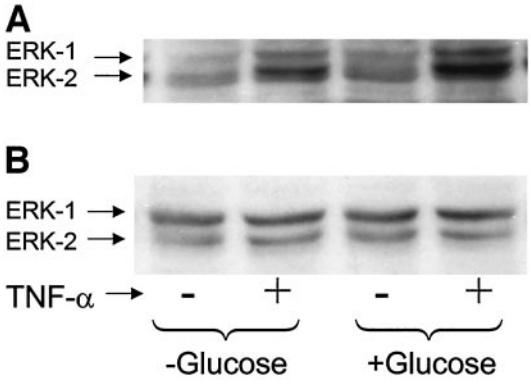

FIG. 10. Effect of glucose and TNF-α on MAP kinase phosphorylation. Adipocytes were incubated for 16 h in the absence or presence of glucose (25 mmol/l), and then TNF-α (50 ng/ml) was added as indicated. The cells were harvested 15 min later, and extracts were analyzed on Western blots with antibodies to phosphorylated ERK1/2 (*A*) or total ERK1/2 (*B*), as described in RESEARCH DESIGN AND METHODS.

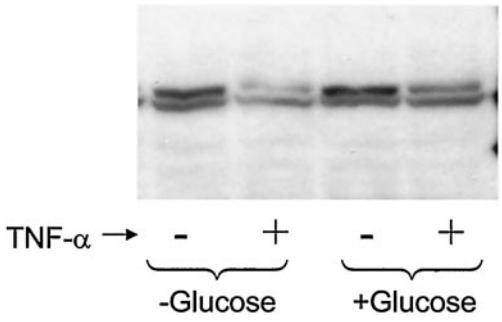

FIG. 11. Perilipin downregulation by TNF-α. Adipocytes were incubated for 16 h in the absence or presence of glucose (25 mmol/l) and TNF-α (50 ng/ml) as indicated. The cells were then extracted and analyzed on Western blots with an antibody to perilipin.

of TNF-α are within the range that would suggest physiologic importance.

Glucose itself has been implicated in the development of insulin resistance—one aspect of so-called glucose toxicity (33). High glucose concentrations induce insulin resistance in various in vitro models, in particular primary adipocytes, and in vivo. Several mechanisms have been proposed for this glucose-induced insulin resistance, including intermediates in the hexosamine pathway (27,30, 34) and defective Akt activation (35). The findings presented here suggest another mechanism: that glucose influences the action of endogenous TNF-α (and, perhaps, other cytokines) to increase adipocyte lipolysis, which would likely increase circulating FFA and thus lead to insulin resistance.

The mechanism by which glucose increases TNF-α–mediated lipolysis is unclear, but several conclusions can be reached from our findings. First, the lack of glucose does not prevent all effects of TNF-α on these cells, i.e., TNF-α activates ERK1/2, regardless of the presence or absence of glucose. ERK1/2 activation is an early event in TNF-α signaling (24,32), but a more chronic effect of TNF-α, downregulation of perilipin (21,22), is also unaffected by the absence or presence of glucose. Second, it is likely that metabolism of glucose is necessary for the lipolytic effect of TNF-α, because nonmetabolizable glucose analogs (L-glucose, 3-*O*-methyl glucose) are ineffective. Similarly, mannose, which was metabolized by these cells to lactate, was effective, whereas galactose, which was not metabolized to lactate, was ineffective. Consistent with this proposed relationship between sugar metabolism and potentiation of TNF-α action, fructose was converted to lactate at a much lower rate than either glucose or mannose, and its effect on lipolysis was modest. Finally, the slow onset of the glucose effect suggests that changes in the expression of genes involved in regulation of lipolysis may be important. Glucose is known to affect the transcription of many genes important in metabolic regulation (rev. in 36).

A recent report (37) demonstrated that various lipogenic enzymes, including fatty acid synthase and acetyl CoA carboxylase, are upregulated by glucose in primary rat adipocytes. It is interesting that these genes were upregulated by the same sugars as we have found to increase the lipolytic action of TNF-α. That is, glucose and mannose were effective, fructose was less effective, and galactose was ineffective. These authors speculated that the effective sugars were more readily metabolized than the ineffective sugars. Our findings on rates of lactate production support this speculation. In addition, this previous report presented evidence that products of the hexosamine biosynthetic pathway are responsible for effects of glucose on expression of these lipogenic enzymes. By contrast, our finding that glucosamine did not greatly affect the lipolytic action of TNF-α would argue against a role of the hexosamine biosynthetic pathway in regulation of lipolysis. Similarly, the effect of glucose on lipolysis that we have previously reported in primary rat adipocytes was unaffected by the presence or absence of glutamine (28), which also argues against a role for the hexosamine biosynthetic pathway in regulation of lipolysis. Thus, it seems that products of the hexosamine biosynthetic pathway are important in regulation of lipogenesis but not lipolysis. Further studies will be required to determine how and whether TNF-α and glucose interact to regulate expression of genes involved in lipolysis.

The mechanism by which TNF-α stimulates lipolysis is still unclear and is probably complex. It has been reported that perilipin downregulation is a key event in TNF-α stimulation of lipolysis in 3T3-L1 cells (21,22). In human preadipocytes, downregulation of phosphodiesterase 3B and subsequent increase in cAMP concentrations may be important (24). We have reported that downregulation of the inhibitory G proteins collectively known as G_i are essential for TNF-α stimulation of lipolysis in primary rat adipocytes (18,19). This G_i downregulation seems to lead to release of inhibition of lipolysis by endogenous agents such as adenosine in the primary rat adipocyte model. However, in unpublished findings, we have been unable to detect inhibition of lipolysis through any G_i-mediated process in 3T3-L1 cells; indeed, we have been unable to detect G_i in these cells, and so this mechanism is unlikely to be involved in stimulation of lipolysis in this model. These findings underscore the observations of Rydén et al. (23) that the mechanism by which TNF-α stimulates lipolysis may vary between cell type.

An alternative interpretation of the findings presented here is that stimulation of lipolysis in these cells has more to do with glucose metabolism than with a specific effect of TNF-α. It has been reported that TNF-α increases the rate of glucose uptake and metabolism in a variety of cells, including 3T3-L1 adipocytes (38–42). This is confirmed by our observation that TNF-α increased the rate of lactate production from glucose. Furthermore, it has been reported that human adipose tissue explants show a higher rate of lipolysis after prolonged treatment with insulin (43), and similar findings have been reported in rat adipocytes (44). Similarly, we have reported that prolonged treatment of primary rat adipocytes with a combination of insulin and glucose increases the rate of lipolysis (28). Perhaps the higher rate of lipolysis in these studies is a consequence of increased glucose metabolism, rather than a direct effect of either TNF-α or insulin on lipolysis.

In conclusion, we have found that the lipolytic effect of TNF-α in 3T3-L1 adipocytes is markedly influenced by the concentration of glucose to which the cells are exposed. Given that adipocytes in vivo are likely continuously exposed to endogenous TNF-α and other cytokines (especially in obese subjects), it is likely that the rate of lipolysis

is regulated on a long-term basis by blood glucose concentration. Therefore, mild hyperglycemia may result in long-term increases in adipocyte lipolysis, which would be expected to increase FFA concentrations and contribute further to development of insulin resistance.

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