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TNFα Decreases Gluconeogenesis in Hepatocytes Isolated from 10-Day-Old Rats

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ABSTRACT

Gluconeogenesis decreases during septic shock, but its mechanism is not well known. Tumor necrosis factor alpha (TNF-α), which is a key cytokine in septic shock, can increase GLUT1 gene expression and glucose uptake in muscles and fatty tissues. TNF-α does not alter the metabolism of hepatocytes in which GLUT2 is the predominant glucose transporter. However, GLUT1 is the predominant glucose transporter in hepatocytes of 10-d-old rats. Thus, we hypothesized that TNF-α might increase glucose uptake and glycolysis in those cells, and decrease gluconeogenesis. In the present study, hepatocytes isolated from 10-d-old rats were incubated with TNF-α at the concentrations of 0, 0.98, 9.8, 98, and 980 ng/mL to evaluate TNF-α effects on gluconeogenesis and glucose uptake. TNF-α increased glucose uptake (41.1 $\pm$ 8 to 114 $\pm$ 21.4 μmol/10^6 cells at the concentration of 980 ng/mL of TNF-α) in a dose-dependent manner, and decreased gluconeogenesis (98.2 $\pm$ 8.2 to 1.1 $\pm$ 3.2 μmol/10^6 cells at the concentration of 980 ng/mL of TNF-α) in a dose-dependent manner. The decrease of glucokinase mRNA and GLUT1 mRNA abundance correlated with glucose uptake (r = 0.988 and 0.997, respectively), and the decrease of phosphoenolpyruvate carboxykinase mRNA abundance correlated with the decrease of gluconeogenesis (r = 0.972). The decrease of gluconeogenesis by TNF-α correlated with the increase of glucose uptake (r = −0.988). We concluded that TNF-α reciprocally suppressed gluconeogenesis in hepatocytes isolated from 10-d-old rats. **(*Pediatr Res* 49: 552–557, 2001)**

Abbreviations

PEPCK, phosphoenolpyruvate carboxykinase
GK, glucokinase
TNF-α, tumor necrosis factor alpha
2DG, 2-deoxy-D-glucose

Gram-negative septic shock in the newborn remains a major medical problem because of its high incidence and mortality. Endotoxin (lipopolysaccharide: LPS), a component of outer cell membrane of Gram-negative bacteria, plays an important role in Gram-negative septic shock. Whereas the LD_{90} of *Salmonella enteritidis* LPS is 0.1 mg/kg in 10-d-old rats, it is 35 mg/kg in adult rats (1), indicating that the newborn is more sensitive to LPS than the adult. The high sensitivity to LPS in the newborn is ascribed to immature pituitary-adrenal function (2), but this alone cannot fully explain the mechanism of the high sensitivity.

Glucose dyshomeostasis such as hyperglycemia and hypoglycemia is a common sign in Gram-negative septic shock (1, 3–5). Because severe hypoglycemia may result in severe neurologic sequelae in the newborn, hypoglycemia during shock is a critical problem. During septic shock, glycolysis is increased and gluconeogenesis is decreased in the liver (4, 5). The activity and mRNA abundance of PEPCK, a key gluconeogenic enzyme, are decreased during septic shock (5–7). Because gluconeogenesis is necessary to maintain plasma glucose concentration in the newborn, even at the postprandial state (8), decrease of gluconeogenesis may easily lead to hypoglycemia. Because plasma concentration of insulin, which is a major glucoregulatory hormone and can inhibit gluconeogenesis, increases during septic shock in the adult (1, 3, 9), hyperinsulinemia is postulated to be responsible for the hypoglycemia in adults. In contrast to the adult, plasma insulin concentration does not increase in the newborn (1, 5). Thus, insulin does not seem to be responsible for the decrease of gluconeogenesis and plasma glucose concentration in the newborn. When hepatocytes are isolated from adult animals and incubated with LPS, metabolism such as protein synthesis is not altered (10). Our previous study showed that LPS infusion does not alter glucose production in livers isolated from 10-d-old rats when Kupffer cell function is decreased (11), suggesting that LPS by itself does not alter hepatocyte glucoregulation in the newborn.

TNF-α is thought to be a key mediator in septic shock. TNF-α increases in the plasma during septic shock (12), and

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TNF-α injection induces hypotension, metabolic alteration, and death in animals (13). TNF-α injection into adult mice decreases gluconeogenesis, PEPCK gene expression, and plasma glucose concentration (14, 15). TNF-α injection into 10-d-old rats also induces hypoglycemia, decreasing PEPCK gene expression (16). These results suggest that TNF-α decreases gluconeogenesis in both newborn and adult animals. In hepatocytes isolated from adult rats, however, gluconeogenesis is not altered after a short incubation with TNF-α (17). TNF-α effects on glucose metabolism in hepatocytes isolated from newborns are not well known.

Glucose transporters, a family of proteins, transport glucose across cell membranes. Glucose transporters are developmentally regulated (18). GLUT1, a ubiquitous glucose transporter, is predominant in the fetus and newborn, including livers of 10-d-old rats, and transports glucose primarily into cells (18, 19). GLUT2, the liver-specific glucose transporter, is predominant in adult hepatocytes (18) and transports glucose bidirectionally across cell membranes (18). Therefore, hepatocytes in the newborn are different from those in the adult.

TNF-α increases noninsulin-mediated glucose uptake and GLUT1 gene expression (20, 21). Because GLUT1 is an insulin-insensitive glucose transporter (18), GLUT1 seems to play an important role in TNF-α-induced glucose uptake. TNF-α injection increases tissue glucose uptake and GLUT1 gene expression in 10-d-old rats in which GLUT1 is predominant (16). TNF-α decreases gene expression of gluconeogenic enzymes in hepatoma cell lines in which GLUT1 is predominant (14). TNF-α decreases gluconeogenesis in hepatocytes isolated from adult rats when the hepatocytes are cultured for 5 d before TNFα treatment (22). Because prolonged cell culture or glucose deprivation induces GLUT1 predominance (23, 24), TNF-α-induced alteration of the hepatocyte glucoregulation may be a result of the increase of GLUT1. Thus, we hypothesize that, in hepatocytes isolated from the newborn, TNF-α increases glucose uptake and glycolysis and reciprocally suppresses gluconeogenesis.

In the present study, hepatocytes isolated from 10-d-old rats were used to evaluate whether LPS or TNF-α by itself decreases gluconeogenesis. We have used 10-d-old rats as an animal model of the newborn (1, 5). To compare TNF-α effects on hepatocyte gluconeogenesis between the newborn and the adult, hepatocytes isolated from adult rats were also used.

MATERIALS AND METHODS

Chemicals. Recombinant murine TNF-α was a gift from Genentech Inc. (San Francisco, CA, U.S.A.). [3-^{14}C]-lactate and [U-^{14}C]-2DG were purchased from Amersham Life Science (Arlington Heights, IL, U.S.A.). Collagenase was purchased from Worthington Biochemical Corp. (Freehold, NJ, U.S.A.), and CO_2-independent media and RPMI1640 were purchased from GIBCO BRL (Grand Island, NY, U.S.A.). Reagents were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), unless otherwise specified.

Animals. Pregnant Sprague Dawley rats (Harlan, Indianapolis, IN, U.S.A.) were purchased on gestational d 12. The rats were housed individually in our animal care facility with controlled room temperature, humidity, and light (0700–1700 h light on), and were fed water and rat chow (Allied Mills, Madison, WI, U.S.A.) *ad libitum*. Newborns were kept with their dams until experiments were performed. Ten-day-old rats and nonpregnant, nonlactating adult female rats were used in the experiments. The study was approved by the Institutional Review of Animal Care and Use Committee.

Hepatocyte isolation. Hepatocytes were isolated with a modified method of Harbrecht *et al.* (25) as follows: Portal vein cannulation was performed under pentobarbital (35 mg/kg i.p.; Butler Inc., Columbus, OH, U.S.A.) anesthesia. The liver was perfused *ex vivo* with PBS for 5 min at 15 cm H_2O, then digested with collagenase in CO_2-independent media at 15 cm H_2O. Collagenase was infused at the concentration of 0.01% for 5 min in the 10-d-old rats, and at 0.2% for 7 min in the adults. Liver cells in CO_2-independent media were dispersed by manual shaking, and filtered with 50-μm nylon mesh (Spectrum, Laguna Hills, CA, U.S.A.). The filtered cell suspension was centrifuged at 490 rpm (× 50 *g*) for 1 min, and the supernatant was discarded. The pellet was resuspended in CO_2-independent media, and the cell suspension was centrifuged at 490 rpm for 1 min. The supernatant was discarded. The cells in the pellet were considered hepatocytes. Hepatocytes were >80% in those cells and trypan blue exclusion test revealed that cell viability was >85% of hepatocytes.

Hepatocyte incubation. Hepatocyte incubation media was RPMI1640 supplemented with penicillin (100 unit/mL) and streptomycin (0.1 mg/mL). Hepatocytes (10^6 cells/mL) were incubated in 5% CO_2 at 37°C. As neither cell viability was altered nor RNA degradation was induced for a 3-h incubation, hepatocytes were incubated for 3 h.

Effects of endotoxin on glucose production of hepatocytes. Hepatocytes (10^6 cells/mL) from 10-d-old rats were incubated with 10 mM lactate, 1 mM pyruvate, and *Salmonella enteritidis* LPS (Difco, Detroit, MI, U.S.A.) at the dose of 0, 0.01, or 0.1 μg/mL in the incubation media for 3 h. Glucose concentrations in the incubation media before and after hepatocyte incubation were measured using a YSI glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH, U.S.A.). Glucose production was calculated from the difference in glucose concentrations of the incubation media before and after incubation. Data were obtained from 12 experiments.

Effects of TNF-α on gluconeogenesis. Hepatocytes isolated from 10-d-old rats were divided into five groups as follows: group 1 (noTNFα, $n = 12$): without TNF-α; group 2 (0.98TNF-α, $n = 12$): 0.98 ng/mL of TNF-α; group 3 (9.8TNF-α, $n = 10$): 9.8 ng/mL; group 4 (98TNF-α, $n = 12$): 98 ng/mL of TNF-α; and group 5 (980TNF-α, $n = 12$): 980 ng/mL of TNF-α. Hepatocytes were incubated in the incubation media with 10 mM of lactate, 1 mM of pyruvate, 10 μCi [3-^{14}C]-lactate, and recombinant murine TNF-α at the doses mentioned above for 3 h. Hepatocytes isolated from adult rats were also incubated in the incubation media with 10 mM of lactate, 1 mM of pyruvate, 10 μCi [3-^{14}C]-lactate, and recombinant murine TNF-α at the doses of 0, 9.8, or 980 ng/mL for 3 h. Eight experiments were performed on hepatocytes from adult rats. Glucose from the incubation media was isolated using AG1-X8 formate resin column (Bio-Rad Laboratories,

Hercules, CA, U.S.A.). Radioactivity of isolated glucose was counted using a liquid scintillation counter (Packard, Meriden, CT, U.S.A.). Lactate concentration was measured using a YSI lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH, U.S.A.). Gluconeogenesis was calculated from radioactivity of glucose in incubation media and lactate concentration. To determine abundance of mRNA of GLUT1, GLUT2, and PEPCK, hepatocytes were harvested after the incubation.

Glucose uptake. Glucose uptake in isolated hepatocytes was measured with a modification of the method of Chen *et al.* (26). Hepatocytes isolated from 10-d-old rats were divided into three groups as follows: group A (noTNFα, $n = 5$): without TNF-α; group B (9.8TNF-α, $n = 5$): 9.8 ng/mL of TNF-α; and group C (980TNF-α, $n = 5$): 980 ng/mL of TNF-α. Hepatocytes were incubated for 60 min. The incubation media contained 1 μCi [U-^{14}C]-2DG ($\approx$250 μCi/mM) and TNF-α at the doses mentioned above. Before incubation, 0.4 mL of cell suspension was taken to measure glucose concentration and to count radioactivity. Hepatocyte glucose uptake was measured as follows: The incubation media containing hepatocytes was centrifuged. The supernatant was used to measure glucose concentration and to count radioactivity. Hepatocytes were homogenized in 3 mL of 0.5N perchloric acid and centrifuged. Two milliliters of supernatant was neutralized with 5 M KOH, and centrifuged. Supernatant at the volume of 0.5 mL was used for the measurement of radioactivity of total 2DG (nonphosphorylated 2DG, phosphorylated glucose). Then, 0.5 mL of 5% $ZnSO_2$ was added to 1 mL of the supernatant, followed by 0.5 mL of 0.3 N $Ba(OH)_2$ to absorb phosphorylated 2DG. The mixture was centrifuged and the radioactivity in 1 mL of the supernatant was counted. Phosphorylated 2DG was calculated from total 2DG by subtracting nonphosphorylated 2DG. The incubation media was added to 300 μL of 4 M perchloric acid and neutralized with 300 K_2CO_3. After centrifugation, radioactivity of 30 μL of the supernatant was counted. Glucose concentration of the incubation media was also measured. Hepatocyte glucose uptake was calculated as follows:

$$\text{glucose uptake} = \text{phosphorylated 2DG uptake of cells}/\int_0^{60} (2DG_{supernatant}/G_{supernatant})dt$$

Furthermore, to determine mRNA abundance of GK, hepatocytes were incubated with TNF-α at the doses of 0, 9.8, or 980 ng/mL in the incubation media for 60 min.

The measurement of abundance of PEPCK mRNA, GK mRNA, GLUT1 mRNA, and GLUT2 mRNA. mRNA abundance of PEPCK ($n = 10$), GK ($n = 5$), GLUT1 ($n = 10$), and GLUT2 ($n = 10$) was measured using a Northern blot technique (27) as follows: Total RNA was isolated by a modified method of Chomczynski and Sacchi (28), then separated by electrophoresis on agarose-formaldehyde gel. The gel was blotted onto Nytran membrane (Schleicher & Schull, Keene, NH, U.S.A.) and hybridized to [^{32}P]-cDNA riboprobes for GK, PEPCK, GLUT1, and GLUT2. Hybridization was performed for 16–20 h at 65°C in modified Denhardt's solution. After hybridization, membranes were washed and exposed to Hyperfilm (Amersham, Arlington Heights, IL, U.S.A.) at −70°C with an intensifying screen for 2 h. The mRNA abundance of GK, GLUT1, GLUT2, and PEPCK in total RNA samples was measured by densitometry of autoradiographic bands using the AMBIS Imaging System (AMBIS, San Diego, CA, U.S.A.). Signals were normalized for loading based on 28S ribosomal RNA, and expressed as percentages of controls (27, 29). Riboprobes for PEPCK, GK, GLUT1, and GLUT2 were cGK-.ZIA (2.36-kb insert) in pBluescript, 846-bp fragment of PC116 in pGEM-3Z, phGT-2 (1.8-kb insert) in pGEM-4Z, and pLGT (0.9-kb insert) in pGEM-4Z, respectively.

RESULTS

Effects of LPS on glucose production in hepatocytes. Glucose production in hepatocytes isolated from 10-d-old rats was 96 ± 9 ($n = 12$), 105 ± 8 ($n = 12$), and 92 ± 7 ($n = 12$) μmol/10^6 cells after incubation with endotoxin at the dose of 0, 0.01, and 0.1 μg/mL, respectively. There was no significant difference in glucose production among the groups.

Effects of TNF-α on gluconeogenesis in hepatocytes. Gluconeogenesis in hepatocytes isolated from 10-d-old rats was 98.2 ± 8.2 ($n = 12$), 56.6 ± 21.1 ($n = 12$), 48.3 ± 6.6 ($n = 10$), 25.0 ± 7.8 ($n = 12$), and 1.1 ± 2.3 ($n = 12$) μmol/10^6 cells in groups 1 (noTNFα), 2 (0.98TNF-α), 3 (9.8TNF-α), 4 (98TNF-α), and 5 (980TNF-α), respectively (Fig. 1). Gluconeogenesis was less ($p < 0.05$) in group 3 (9.8TNF-α) than in group 1 (noTNFα), but was not different from that in group 2 (0.98TNF-α). Gluconeogenesis was less ($p < 0.01$) in group 4 (98TNF-α) than in group 1 (noTNFα), but was not different from that in either group 2 (0.98TNF-α) or group 3 (9.8TNF-α). Gluconeogenesis was less in group 5 (980TNF-α) than in both groups 2 (0.98TNF-α) and 3 (9.8TNF-α) ($p < 0.05$) but was not different from that in group 4 (98TNF-α). Therefore, TNF-α decreased gluconeogenesis in a dose-dependent manner.

Gluconeogenesis in hepatocytes isolated from adult rats was 226 ± 43 ($n = 8$), 185 ± 68 ($n = 8$), and 217 ± 44 ($n = 8$) μmol/10^{6}cells at the doses of 0, 9.8, and 980 ng/mL of TNF-α, respectively. There was no significant difference in gluconeogenesis among those three groups.

Glucose uptake. In hepatocytes isolated from 10-d-old rats, glucose uptake in group B (9.8TNF-α) was not different from that in group A (0TNF-α) (Fig. 2). Glucose uptake was greater

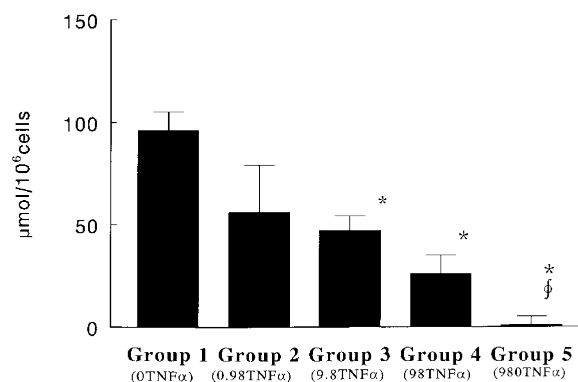

Figure 1. Gluconeogenesis in hepatocytes isolated from 10-d-old rats. Hepatocytes were incubated with TNF-α at different concentrations for 3 h. Each value expressed by mean ± SEM was obtained from 10 to 12 experiments. * $p < 0.05$ compared with group 1 (0TNF-α). § $p < 0.05$ compared with groups 1 (0TNF-α), 2 (0.98TNF-α), and 3 (9.8TNF-α).

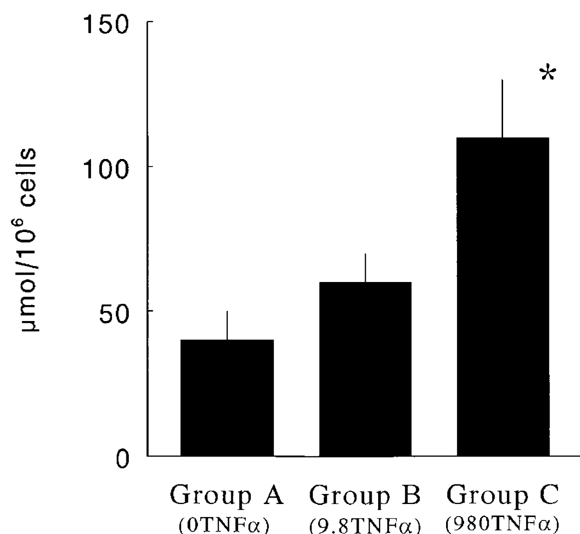

Figure 2. Glucose uptake in hepatocytes isolated from 10-d-old rats. Hepatocytes were incubated with TNF-α at different concentrations for 60 min. Each value expressed by mean ± SEM was obtained from five experiments. * $p < 0.05$ compared with group A (0TNF-α) and group B (9.8TNF-α)

($p < 0.05$) in group C (980TNF-α) than in both groups A (noTNFα) and B (9.8TNF-α). Thus, TNF-α seemed to increase the glucose uptake in a dose-dependent manner.

mRNA abundance in PEPCK, GK, GLUT1, and GLUT2. PEPCK mRNA abundance decreased in groups 3 (9.8TNF-α) ($p < 0.05$), 4 (98TNF-α) ($p < 0.05$), and 5 (980TNF-α) ($p < 0.01$), when compared with group 1 (noTNFα). The decrease of PEPCK mRNA abundance seemed to be in a dose-dependent manner (Fig. 3). However, PEPCK mRNA abundance was not significantly different among groups 2 (0.98TNF-α), 3 (9.8TNF-α), 4 (98TNF-α), and 5 (980TNF-α). The decreased PEPCK mRNA abundance was correlated to the decreased gluconeogenesis ($r = 0.972$) (Fig. 4). GK mRNA abundance seemed to be increased in a dose-dependent manner (Fig. 5). GK mRNA abundance was greater ($p < 0.05$) in group C (980TNF-α) than in group A (0TNF-α). GLUT1

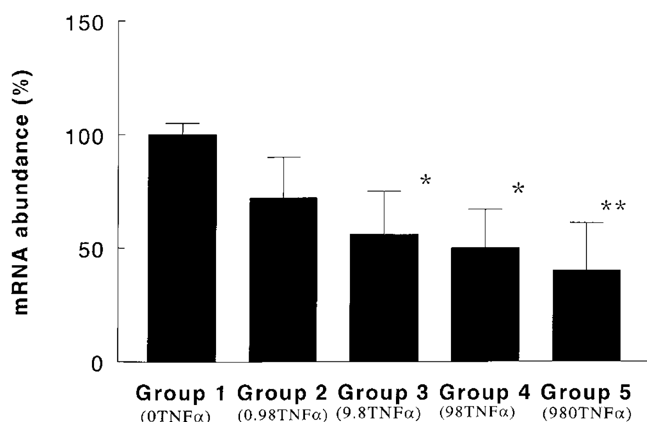

Figure 3. PEPCK mRNA abundance in hepatocytes after a 3-h incubation with TNF-α. Signals were normalized for loading based on 28S ribosomal RNA and expressed as percent of the mean value of group 1 (no TNF-α). Each value was obtained from 10 experiments. Mean ± SEM were calculated. * $p < 0.05$ compared with group 1 (0TNF-α). § $p < 0.01$ compared with group 1 (0TNF-α).

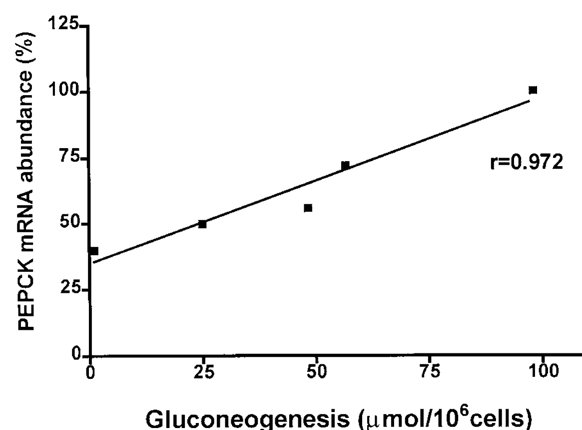

Figure 4. The correlation between the gluconeogenesis and PEPCK mRNA abundance. The decrease of mRNA abundance of PEPCK by TNF-α was correlated with the decrease of the gluconeogenesis ($r = 0.972$).

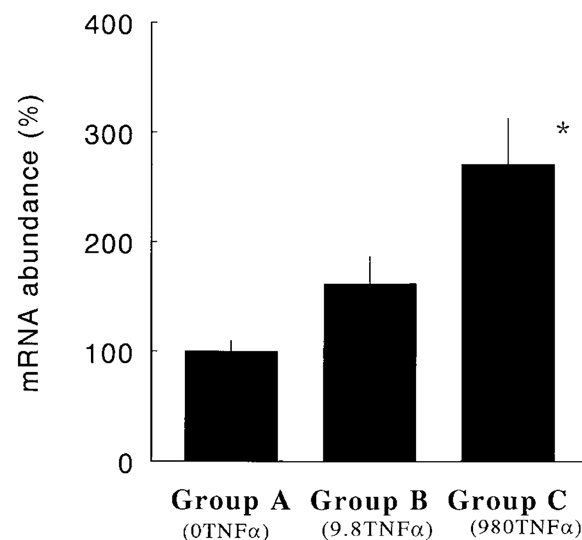

Figure 5. GK mRNA abundance in hepatocytes after a 3-h incubation with TNF-α. Signals were normalized for loading based on 28S ribosomal RNA and expressed as percent of the mean value of group 1 (noTNFα). Each value was obtained from five experiments. Mean ± SEM were calculated. * $p < 0.05$ compared with group A (0TNF-α).

mRNA abundance was also increased in a dose-dependent manner (Fig. 6). GLUT1 mRNA abundance was greater in groups 4 (98TNF-α) ($p < 0.05$) and 5 (980TNF-α) ($p < 0.01$) than in group 1 (noTNFα). GLUT1 mRNA abundance was greater ($p < 0.05$) in group 5 (980TNF-α) than in groups 2 (0.98TNF-α) and 3 (9.8TNF-α). The increased glucose uptake was correlated with both the increased GK mRNA abundance ($r = 0.988$) and the increased GLUT1 mRNA abundance ($r = 0.997$) (Fig. 7). GLUT2 mRNA abundance was decreased to 30–35% ($p < 0.05$) in groups 2 (0.98TNF-α), 3 (9.8TNF-α), 4 (98TNF-α), and 5 (980TNF-α) when compared with group 1 (noTNFα) (Fig. 8). There was no significant difference in GLUT2 mRNA abundance among groups 2 (0.98TNF-α), 3 (9.8TNF-α), 4 (98TNF-α), and 5 (980TNF-α).

DISCUSSION

The present study demonstrated that LPS did not alter glucose production in hepatocytes isolated from 10-d-old rats

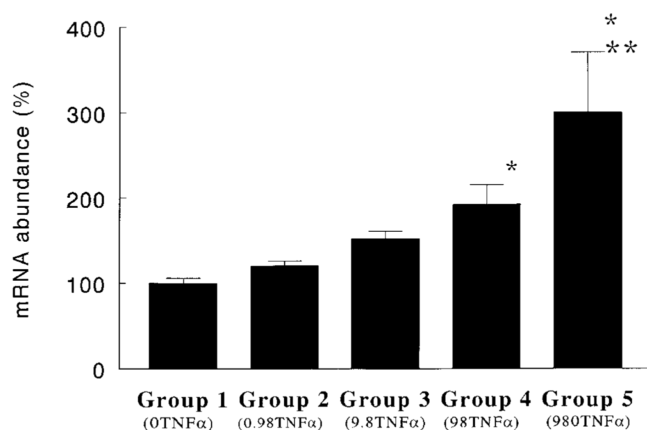

Figure 6. GLUT1 mRNA abundance in hepatocytes after a 3-h incubation with TNF-α. Signals were normalized for loading based on 28S ribosomal RNA and expressed as percent of the mean value of group 1 (noTNFα). Each value was obtained from 10 experiments. Mean ± SEM were calculated. * $p < 0.01$ compared with group 1 (0TNF-α). § $p < 0.05$ compared with groups 2 (0.98TNF-α) and 3 (9.8TNF-α).

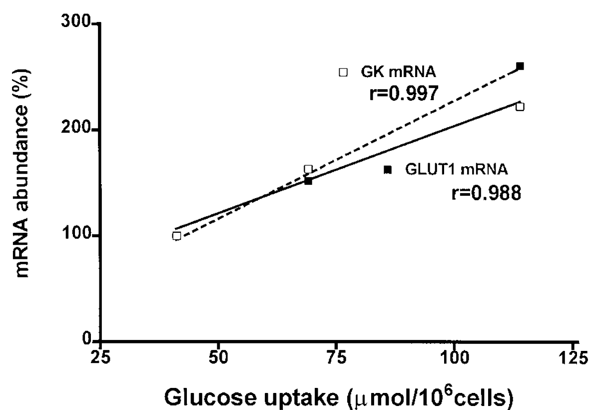

Figure 7. The correlation between glucose uptake and mRNA abundance of GK and GLUT1. The increase of mRNA abundance of GK and GLUT1 correlated with the increase of the glucose uptake (r = 0.988 and 0.997, respectively).

but TNF-α decreased gluconeogenesis in a dose-dependent manner. The lack of LPS effects on glucose production confirmed previous observations by other investigators (10) and ourselves that LPS by itself might not alter hepatocyte glucoregulation (11). In the present study, abundance of mRNA of PEPCK, a rate-limiting enzyme in gluconeogenesis, was decreased by TNF-α in a dose-dependent manner, and the decrease in gluconeogenesis was closely related to the decrease in PEPCK mRNA abundance (r = 0.972). Our previous study shows that the decrease in gluconeogenesis in 10-d-old rats is associated with the decrease in activity and mRNA abundance of PEPCK during endotoxic shock (5). The decreased PEPCK gene expression confirmed the decrease of gluconeogenesis. In our present study, TNF-α decreased GLUT2 mRNA abundance. Because GLUT1 transports glucose primarily into cells and GLUT2 transports glucose bidirectionally across cell membranes (18), GLUT2 plays an important role in glucose release from hepatocytes. Therefore, a decrease in GLUT2 mRNA abundance might be expected to correlate with the decrease in gluconeogenesis. In the present study, however, the

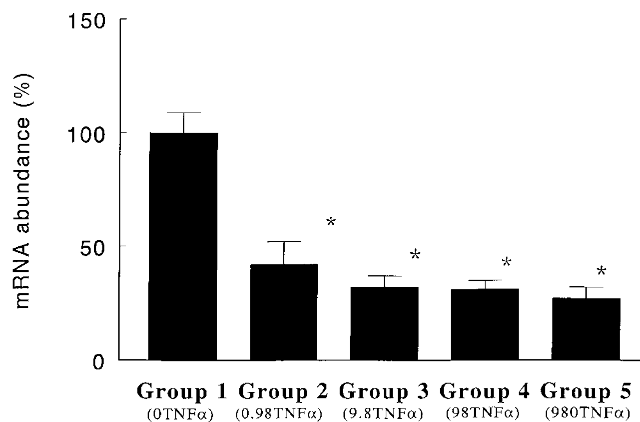

Figure 8. GLUT2 mRNA abundance in hepatocytes after a 3-h incubation with TNF-α. Signals were normalized for loading based on 28S ribosomal RNA and expressed as percent of the mean value of group 1 (noTNFα). Each value was obtained from 10 experiments. Mean ± SEM were calculated. * $p < 0.05$ compared with group 1.

decrease in GLUT2 mRNA abundance did not correlate with the decrease in gluconeogenesis. These results suggested that gluconeogenesis might not correlate with GLUT2 concentration, but might correlate with altered GLUT2 function (K_m).

TNF-α increased glucose uptake and GLUT1 mRNA abundance of the isolated hepatocytes in a dose-dependent manner. There was a good correlation (r = 0.997) between the increase of glucose uptake and the increase of GLUT1 mRNA abundance. Other investigators have shown that increase of noninsulin-mediated glucose uptake is associated with increase of GLUT1 gene expression in muscles during septic shock (20). Our previous study also showed that TNF-α increases glucose uptake and GLUT1 gene expression in the liver and fatty tissues of 10-d-old rats (16). Furthermore, TNF-α increases glucose uptake and GLUT1 gene expression in 3T3 cells (30). Therefore, the good correlation (r = 0.997) between the increase of glucose uptake and the increase of GLUT1 mRNA abundance suggested that GLUT might play an important role in the increase of glucose uptake. Because glucose uptake is assessed by the amount of phosphorylated 2-deoxy-glucose (26), the increased glucose uptake indicates the increased glucose phosphorylation, the first step of glycolysis. In the present study, abundance of mRNA of glucokinase, one of hexokinases, was increased in a dose-dependent manner, which correlated with the increase of glucose uptake (r = 0.997). These results suggested that TNF-α increased glycolysis in isolated hepatocytes. In the present study, there was a close relationship (r = −0.988) between the increase of glucose uptake and the decrease of gluconeogenesis (Fig. 9). Glycolysis and gluconeogenesis are shown to be reciprocally regulated until a futile cycle is developed (31).

The present study showed that TNF-α did not alter gluconeogenesis in hepatocytes isolated from adult rats, confirming the findings of other investigators (17). Hill and McCallum (6) concluded from their *in vivo* studies that TNF-α was a transcriptional regulator of PEPCK and suppressed PEPCK function. However, the present study suggested that decreased PEPCK gene expression might not be caused by direct effects

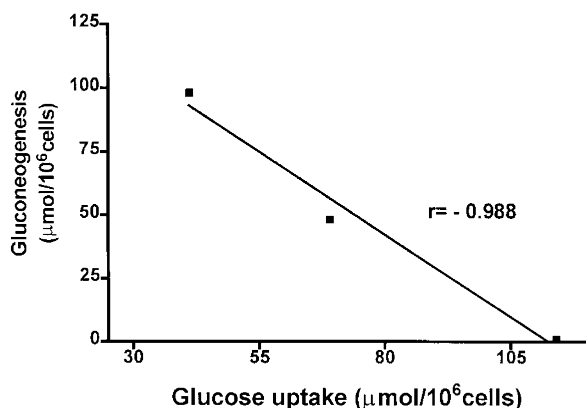

Figure 9. The correlation between the gluconeogenesis and the glucose uptake. The decrease of the gluconeogenesis by TNF-α was correlated with the increase of the glucose uptake ($n = -0.988$).

of TNF-α. From the present study, we speculated the mechanism of the lack of TNF-α effects on gluconeogenesis in hepatocytes isolated from adult rats as follows: GLUT2 is the predominant glucose transporter in the liver of adult rats (18, 19). Therefore, although TNF-α increased GLUT1-mediated glucose uptake and decreased GLUT2 gene expression, the existing GLUT2 continued to shuttle enough glucose. Thus, TNF-α did not alter gluconeogenesis. However, neither glucose transporter proteins nor their functions (K_m) were measured in the present study. The role of glucose transporters in TNF-α effects on gluconeogenesis needs further investigations.

The newborn often develop hypoglycemia without hyperinsulinemia during septic shock (32, 33). The present study showed that TNF-α by itself inhibited hepatocyte gluconeogenesis in the newborn rats but not the adult rats. Our results indicate that the propensity to hypoglycemia during septic shock in the newborn may be related to TNF-α-induced decrease in gluconeogenesis. Blood glucose concentration can be maintained by dextrose infusion. However, dextrose may suppress gluconeogenesis. Because TNF-α effects on gluconeogenesis seem to be related to GLUT1 predominance, a method to attenuate GLUT1-mediated glucose transport may be useful for the treatment of hypoglycemia during septic shock in the newborn.

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