Insulin-Like Growth Factor-I Improves Glucose Utilization in Tumor Necrosis Factor–Treated Rats Under Hyperinsulinemic-Euglycemic Conditions

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The purpose of this study was to determine the effects of insulin-like growth factor-I (IGF-I) on glucose metabolism in normal and tumor necrosis factor (TNF)-treated rats under euglycemic and hyperinsulinemic conditions. During a hyperinsulinemic clamp (10 mU/kg · min), rats further received either saline or IGF-I (3.33 μg/kg · min) infusion for 2 hours. Glucose kinetics were determined with [^{3}H-3]-glucose. Glucose utilization in peripheral tissues was examined by glucose uptake using [^{14}C-2]-deoxyglucose (^{14}C-DG) and by glycogen content in select tissues. The results showed that TNF infusion significantly decreased the rate of glucose infusion required to maintain euglycemia. TNF decreased glycogen content significantly in liver and marginally in abdominis muscle. TNF also decreased glucose uptake in muscle, although the decrease was only statistically significant compared with IGF-I infusion. In addition, TNF significantly reduced plasma IGF-I concentration. However, during hyperinsulinemic and euglycemic conditions, exogenous IGF-I significantly increased glucose uptake in muscle and glycogen storage in the liver and abdominis muscle in both saline- and TNF-treated groups. IGF-I normalized each of the effects of TNF in the rats, including those on plasma IGF-I, glucose uptake in muscle, and glycogen content in liver and abdominis muscle. These data suggest that under hyperinsulinemic and euglycemic conditions, TNF-treated rats, although resistant to insulin, have a normal response to IGF-I, indicating that the TNF-induced defect in the insulin pathway may not be a step in the IGF-I pathway.

INSULIN-LIKE GROWTH FACTOR-I (IGF-I) has structural homology with insulin. IGF-I also shares a wide range of functions with insulin, including stimulation of cell growth and differentiation and participation in cellular transport and metabolism of glucose and protein.[1-4]

In skeletal muscle in vitro, IGF-I has major insulin-like stimulatory effects on glucose uptake, glycolysis, and glycogen synthesis.[5-7] In normal rats, IGF-I infusion causes hypoglycemia,[8-11] primarily by stimulating peripheral glucose uptake. Under catabolic conditions produced by a variety of stimuli including corticosteroids,[6] fasting,[10,11] dietary protein restriction,[12,13] and tumor necrosis factor (TNF) infusion,[14] IGF-I administration has had positive effects on protein synthesis and nitrogen balance, as well as on the rate of glucose clearance, suggesting that the anabolic effects of IGF-I are well preserved in the "stress state." In human studies, the effects of IGF-I infusion in normal volunteers and catabolic or diabetic subjects are comparable to the effects seen in rats.[8,15-18] Since catabolic stress and diabetes are associated with insulin resistance, these findings suggest that IGF-I may have a distinct and significant role in the regulation of metabolism.

The present study was undertaken to compare the effects of IGF-I infusion on glucose metabolism in normal and TNF-treated rats. Previous studies have demonstrated that TNF infusion can induce insulin resistance both in hepatic glucose production and in glucose utilization in peripheral tissues.[19-21] Therefore, the same animal model was used in this study. The present study was conducted under conditions of hyperinsulinemia and euglycemia. Using the hyperinsulinemic-euglycemic clamp technique, we were able to determine the in vivo effects of IGF-I on glucose metabolism without any influence from the changes in plasma insulin and glucose concentrations.

MATERIALS AND METHODS

Animal Preparation

Male Sprague-Dawley rats (weight, 45 to 50 g; Taconic Farms, Germantown, NY) were acclimatized in individual cages in a light-controlled room (12 hours on/12 hours off) at a temperature of 22° to 24°C for 4 days. During this period, animals were fed a regular rat chow diet and had access to tap water ad libitum. Animals were then switched to a modified AIN-76 diet with 30% of nonprotein calories from fat and water ad libitum for 6 weeks. The final body weight was about 350 g. On the day before the experiment, the animals underwent a surgical procedure under ether inhalation for catheter placement. One catheter (Polyethylene Tubing, PE 50, ID 0.58 mm, OD 0.965 mm; Becton Dickinson, Parsippany, NJ) was inserted into the left carotid artery for sampling blood; two other silastic catheters (Silicone tubing, ID 0.025 in, OD 0.047 in; Baxter Healthcare, Deerfield, IL) were placed in both jugular veins for administration of tracers and hormones. The animals were then allowed to recover in individual cages. There were no noticeable changes in eating pattern and activity levels, and there was no obvious infection.

Experimental Design

After overnight fasting, the animals were randomly divided into two groups. One group received saline infusion as a control (saline), and the other group (TNF) received 20 μg/kg recombinant murine TNF containing less than 200 pg endotoxin/ng protein (Genentech, San Francisco, CA). Half of the dose (10 μg/kg) of TNF was administered by intravenous bolus, and the remainder (10 μg/kg) was constantly infused over 3 hours. Within each group (saline or TNF), animals were further divided into two groups according to treatment: saline or 3.33 μg/kg · min IGF-I. Therefore, a total of four groups were included in this study: (1) saline, saline (S/S, n = 9); (2) saline, IGF-I (S/IGF, n = 11); (3) TNF, saline (TNF/S, n = 9); and (4) TNF, IGF-I (TNF/IGF, n = 10). Saline, TNF, and IGF-I solutions were freshly prepared on the day of the experiment by mixing with 0.1% human albumin.

Basal arterial blood samples were drawn for determination of blood

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glucose, insulin, and IGF-I before the infusions. By the end of the first hour of infusion of saline or TNF, blood was drawn again for determination of plasma glucose, insulin, and IGF-I (60 minutes); then, a modification of the hyperinsulinemic-euglycemic clamp technique was used. Insulin was continuously infused at 10 mU/min · kg beginning 1 hour after the initial infusions, followed within 1 minute by 20% dextrose (Astra Pharmaceutical Products, Westborough, MA) infused at a variable rate through another jugular vein with a variable syringe infusion pump (Harvard Apparatus, South Natick, MA). Arterial blood was sampled every 10 minutes for measurement of glucose concentration. The rate of glucose infusion was adjusted empirically after each arterial plasma glucose determination to maintain a basal euglycemic condition. All animals received a 5-μCi (Du Pont, Wilmington, DE) bolus injection of purified [^{3}H-3]-glucose. This was followed by a constant infusion of 5 μCi/h through the same jugular vein for 2 hours for determination of plasma glucose appearance and endogenous glucose production. At 138 minutes of the initial infusion, a bolus of 5 μCi [^{14}C-2]-deoxyglucose (^{14}C-DG) was injected intravenously; then, 400-μL arterial blood samples were drawn at 140, 150, 160, 170, and 180 minutes for measurement of ^{14}C-DG levels in plasma. Blood samples, 100 μL each, were drawn at 140, 160, and 180 minutes for measurement of specific activity of plasma glucose. After each sampling, 0.5 mL saline was injected to replace the blood volume. Isotopic steady state was achieved during the last hour of the clamp period as demonstrated by constant plasma glucose specific activity (data not shown).

At the end of the infusions (180 minutes of total infusion time or 120 minutes during the hyperinsulinemic-euglycemic clamp), a blood sample was collected, and then 5 mg pentobarbital sodium was intravenously injected to euthanize the animal. The rectus abdominis muscle, gastrocnemius muscle, and liver were taken by an in situ freeze-clamping technique for determination of glycogen content. Other pieces of liver and muscle were taken for measurement of ^{14}C-DG counts. The abdominal mesenteric adipose tissue was also removed and weighed for ^{14}C-DG counts. All samples were stored at −20°C until analysis.

The experiment was approved by the Animal Care Committee of the Beth Israel Deaconess Medical Center, which follows guidelines established by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

Analytical Procedures

Plasma glucose concentration was determined by the glucose oxidase method using a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma insulin was determined using a standard radioimmunoassay with a porcine insulin standard (Binax, South Portland, ME). Plasma total IGF-I was determined by a radioimmunoassay kit using an acid-ethanol extraction method to separate binding proteins (Nichols Institute, San Juan Capistrano, CA).

Glucose specific activities in plasma were determined as previously described.[22,23] Briefly, plasma samples were first deproteinized with equal volumes of $Ba(OH)_2$ and $ZnSO_4$ and immediately centrifuged. The supernatant was then passed through a 1.5 × 1.5-cm column of analytical-grade anion (200 to 400 mesh; Bio-Rad Laboratories, Richmond, CA) resin and a 1.5 × 1.5-cm column of cation (200 to 400 mesh; Bio-Rad) resin. This procedure resulted in adherence of ^{3}H-labeled organic acids to the resin. Each column was then washed with 3 mL distilled water to elute the labeled glucose. The eluate was collected and evaporated to dryness in an oven at 60°C, and the residue was dissolved in 1 mL distilled water. One aliquot was counted for ^{3}H-glucose content using Beckman Ready Gel scintillation fluid (Beckman Instruments) and external standard for efficiency determination. Another aliquot was used for determination of glucose content.

Plasma ^{14}C-DG level and accumulation of ^{14}C-DG in the tissues were determined using a method described elsewhere.[24,25] In brief, blood samples were deproteinized with $Ba(OH)_2$-$ZnSO_4$ and immediately centrifuged. An aliquot (100 μL) of the supernatant was used for counting ^{14}C-DG radioactivity (Beckman Instruments). Tissue samples were placed in 0.5 or 1 mL 1-mol/L NaOH (according to the size), digested at 60°C for 1 hour, and then neutralized with 1 mol/L HCl. Separate 200-μl aliquots of digested tissues were treated with either 1 mL $HClO_4$ (4% wt/vol) or 1 mL $Ba(OH)_2$-$ZnSO_4$ and centrifuged. The supernatants were counted for ^{14}C-DG plus ^{14}C-6-phosphate ($HClO_4$-treated fractions) and ^{14}C-DG only ($Ba(OH)_2$-$ZnSO_4$–treated fractions).

Glycogen content in the tissues was determined using the method described by Carr and Neff[26] with modifications. Briefly, 200 mg frozen tissue sample was homogenized in 3 mL 100-mmol/L sodium citrate (pH 5.0). The samples were then placed in a boiling water bath for 5 minutes, following which the sample was rehomogenized. One aliquot was incubated with 0.5% amyloglucosidase (Sigma Chemical, St Louis, MO) at 25 μL/mL sample in 100 mmol/L sodium citrate buffer overnight. The other was incubated only with sodium citrate buffer. Rabbit liver glycogen (Sigma; Type III) in replicate was used as the standard and treated in the same manner as the samples, ie, incubated with and without 0.5% amyloglucosidase in 100 mmol/L sodium citrate buffer overnight. After incubation, the standards and samples were centrifuged at 16,000 rpm for 30 minutes. A Beckman glucose analyzer was used to assay the glucose content of the supernatants.

Calculations

The rate of glucose appearance (Flux) was calculated using the infusion rate of ^{3}H-labeled tracer (I) and steady-state plasma ^{3}H-3-glucose specific activity, as follows: Ra = I/plasma ^{3}H-3-glucose specific activity. The rate of endogenous glucose production was calculated from the difference between the determined total rate of glucose appearance and the exogenous glucose infusion rate during the clamp.

The apparent rate of glucose uptake in tissue was calculated based on accumulation of 2-DG-P in the tissue and the integrated 2-DG to glucose ratio in plasma during the 42 minutes after ^{14}C-DG injection, as described previously.[21-23] The lumped constant in different tissues was assumed to be 1.0.

The following formula was used to convert the glucose values of enzyme-treated and untreated samples back to the tissue glycogen concentration[26]:

$$G = \frac{(T - U) \times (Se/S_0) \times V}{100 \times W}.$$

G is the glycogen concentration (mg/g); T, glucose reading of enzyme-treated aliquot (mg/dL); U, glucose reading of untreated sample (mg/dL); Se, expected glucose reading for the glycogen standard (eg, 111 mg/dL for 0.1% glycogen standard); S_0, observed glucose reading for the glycogen standard; V, volume of extracting solution in which the tissue sample was homogenized (mL); and W, weight of the tissue sample (g).

Statistical Analysis

Data are presented as the mean ± SEM. Group means were compared by two-way ANOVA using the SYSTAT statistical software package (SYSTAT, Evanston, IL). Significance was defined as a P value less than .05. Comparisons among groups were made by Fisher's least-significant difference (LSD) test for the changes in plasma glucose, IGF-I, glucose kinetics, insulin-stimulated glucose uptake, and glycogen content in tissues when ANOVA was found to be significant at the 95% confidence level.

RESULTS

Blood glucose concentrations are summarized in Table 1. Basal plasma glucose level was 109 to 112 mg/dL, and there were no significant differences among groups. Infusion of TNF for 1 hour did not change plasma glucose concentration. However, IGF-I infusion at the rate of 3.33 μg/kg · min for the first hour significantly decreased plasma glucose levels in both saline- and TNF-treated rats, indicating a stimulatory action of IGF-I on glucose utilization. During the clamp period, the plateau for plasma glucose was reached after 1 hour of infusion of insulin (10 mU/kg · min) (data not shown), and was well maintained at the basal level between 103 and 106 mg/dL in all groups thereafter.

Basal insulin concentrations were 54 to 56 μU/mL (Table 2). Similar to the changes in plasma glucose concentrations, 1 hour of TNF infusion also did not change insulin levels, and IGF-I infusion for the first hour resulted in significant decreases in insulin concentrations. There was a 45% decrease in the saline group and a 37% decrease in the TNF group, respectively. During the clamp with insulin infusion at 10 mU/kg · min, plasma insulin significantly increased to the range 312 to 400 μU/mL. There were no significant differences among groups.

Figure 1 shows changes in plasma IGF-I concentrations in all groups. With TNF infusion alone (TNF/S), basal plasma IGF-I was significantly decreased from 647 ng/mL to 378 ng/mL at 60 minutes, and further to 201 ng/mL at 180 minutes. With saline infusion alone (S/S), plasma IGF-I was not changed at the first hour, but significantly decreased from the basal level to 457 ng/mL at 180 minutes. Therefore, the plasma level of IGF-I was significantly lower in the TNF group at 60 minutes ($P < .005$ by LSD) and 180 minutes ($P < .005$ by LSD) compared with all other groups. IGF-I administration significantly increased plasma IGF-I levels in both saline and TNF groups, but there were no significant differences between saline- and TNF-treated animals at either 60 or 180 minutes.

Table 3 lists the exogenous glucose infusion rate achieved in the last hour of the clamp, as well as the rates of glucose appearance (Flux) and hepatic glucose production. The glucose infusion rate required to maintain similar glucose levels was significantly lower in the TNF/S group compared with the other groups. Glucose flux measured by isotope dilution was not different among groups. However, the rate of hepatic glucose production, calculated by subtracting the rate of infusion of exogenous glucose from the tracer-determined total rate of plasma glucose flux, was higher in the TNF/S group ($P < .05$ by interaction), although in no group was this value significantly different from zero. IGF-I administration in the saline group did not require an increased rate of exogenous glucose infusion, nor did it change the rate of glucose appearance. In contrast, IGF-I infusion in the TNF group restored the rate of glucose infusion to the control level.

Table 1. Changes in Plasma Glucose (mg/dL)

Group	Treatment	No.	Basal	60 min	180 min
Saline	Saline (S/S)	9	109 ± 4	100 ± 2	103 ± 2
	IGF-I (S/IGF)	11	112 ± 12	95 ± 5*	103 ± 2
TNF	Saline (TNF/S)	9	108 ± 7	103 ± 4	104 ± 2
	IGF-I (TNF/IGF)	10	106 ± 2	91 ± 3*	106 ± 3

NOTE. Results are the mean ± SE.
*$P < .05$, S *v* IGF by 2-way ANOVA.

Table 2. Changes in Plasma Insulin (μU/mL)

Group	Treatment	No.	Basal	60 min	180 min
Saline	Saline (S/S)	9	56 ± 5	52 ± 2	312 ± 44
	IGF-I (S/IGF)	11	54 ± 3	28 ± 4*	349 ± 46
TNF	Saline (TNF/S)	9	56 ± 6	53 ± 6	400 ± 68
	IGF-I (TNF/IGF)	10	55 ± 4	33 ± 3*	309 ± 46

NOTE. Results are the mean ± SE.
*$P < .001$, S *v* IGF by 2-way ANOVA.

Figure 2 shows the apparent rates of insulin-stimulated glucose uptake by individual tissues. TNF tended to increase glucose uptake in the liver as compared with saline, but not significantly. On the other hand, TNF infusion (TNF/S) significantly decreased the rate of insulin-stimulated glucose uptake in both rectus abdominis and gastrocnemius muscles compared with both IGF treatments (S/IGF and TNF/IGF), although the decreases did not reach statistical significance compared with saline infusion (S/S). IGF-I infusion significantly enhanced the rate of glucose uptake in these two muscles in both TNF and saline groups ($P < .05$ by treatment, two-way ANOVA). Such effects of IGF-I to increase glucose uptake in muscle tissue were more effective in the TNF group as compared with the saline group. IGF-I infusion did not change glucose uptake in liver in either saline or TNF groups. No significant differences were observed in fat tissue either between saline and TNF or between saline and IGF-I infusion.

Figure 3 shows glycogen content in the tissues. TNF treatment decreased glycogen content significantly in the liver and marginally in rectus abdominis muscle. IGF-I administration significantly enhanced glycogen content in the liver and abdominis muscle ($P < .05$ by two-way ANOVA), with significant reversal of the TNF effect in both ($P < .05$ by LSD). In the gastrocnemius muscle, the same trend was observed with TNF infusion, but it did not reach statistical significance ($P = .09$ by two-way ANOVA).

DISCUSSION

We have previously shown that infusion of TNF 20 μg/kg for 3 hours leads to inhibition of insulin action in rats.[21] The present results confirm the insulin resistance as evidenced by less glucose being required to maintain euglycemia and by the lower rate of insulin-stimulated glucose uptake in different skeletal muscles in rats receiving TNF only. Consistent with previous findings,[27] infusion of TNF also decreased glycogen content in the liver and one of the two muscles examined. Under normal conditions, insulin accelerates glycogen formation in the liver, in keeping with its role as an anabolic hormone. In this study, during euglycemia, infusion of insulin 10 mU/kg · min resulted in 102.4 ± 12.4 mg glycogen in 1-g wet liver tissue in the TNF (TNF/S) group, which was 25% less than in the saline (S/S) group. Moreover, despite normal and similar plasma glucose levels between saline and TNF groups, a trend to less glycogen was also observed in the abdominis muscle in the TNF group. These findings provide further support to the idea that TNF administration directly results in resistance to insulin action on glucose utilization.

The present results also showed that infusion of TNF 20

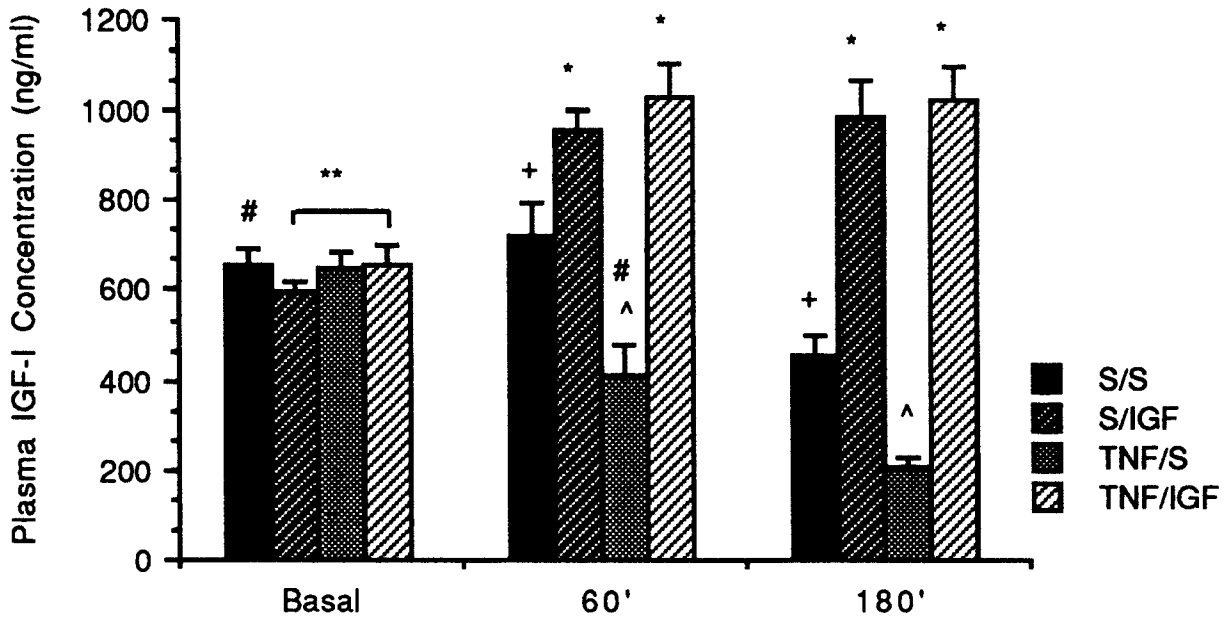

Fig 1. Changes in plasma IGF-I concentrations (mean ± SEM). **$P < .005$ *v* 60′ and 180′ by 1-way ANOVA; *$P < .001$, S *v* IGF by 2-way ANOVA; #$P < .005$ *v* 180′ and †$P < .001$ and ^$P < .001$ *v* all at 60′ and 180′ by LSD.

μg/kg significantly decreased plasma IGF-I from 647 ± 36 to 378 ± 48 ng/mL after the first hour of infusion, which further decreased to 203 ± 27 ng/mL after 3 hours of infusion ($P < .005$ by LSD). In the saline-only group, a decrease in plasma IGF-I was also observed at the end of the study, which may be a consequence of the stress from blood sampling. However, plasma IGF-I was still significantly higher in saline groups compared with TNF groups. Since all animals underwent the same experimental procedure, the higher plasma IGF-I concentration in the saline groups suggested a direct effect of TNF on the decrease of plasma IGF-I, which was also significantly lower in the TNF-only group compared with all other groups. Fan et al[28] have reported that infusion of TNF at 0.1, 1, or 5 μg/kg · h overnight significantly reduces plasma IGF-I by 57% to 63%, and the decreases were independent of the dose of TNF used. Our data, together with their findings, suggest that the reduction of plasma IGF-I by TNF is also not time-dependent. However, these results are different from our previous findings. In that study,[23] when TNF 20 μg/kg was infused into the rats in the same fashion as in this study, it did not change plasma IGF-I concentrations. The major difference that might account for this discrepancy is the nutritional status. Our previous study was performed in the fed state. Before infusion of TNF, animals were intravenously fed by an adequate nutritional regimen supplying 250 kcal/kg · d, 2 g nitrogen/kg · d, and 30% of nonprotein calories from fat for 20 hours to achieve a fed steady state. Animals were also continuously receiving nutritional support during TNF infusion. In the current study, animals were fasted overnight and then received glucose infusion only sufficient to maintain euglycemia during TNF infusion. Therefore, the nutritional status of the host may have contributed to the responses of plasma IGF-I to TNF administration in vivo.

The present results also showed that increasing plasma IGF-I levels up to 1.5-fold over the short term (after the first hour of infusion of 3.33 μg/kg · min IGF-I) acutely depressed plasma insulin, in agreement with previous findings. This is probably due to the effect of IGF-I to directly suppress endogenous insulin secretion.[29,30] High-affinity IGF-I receptors have been identified on rat pancreatic α and β cells.[3,31] This dose of IGF-I also significantly decreased plasma glucose over the same period, indicating a direct insulin-like effect of IGF-I on glucose uptake, which would also decrease insulin secretion. However, when insulin 10 mU/kg · min was constantly infused with IGF-I for 2 hours, a hyperinsulinemic condition was reached with euglycemia by infusion of glucose. Then, IGF-I significantly increased the rate of ^{14}C-DG uptake in rectus abdominis and gastrocnemius muscle in animals infused with either normal saline or TNF compared with animals not receiving IGF-I infusion. IGF-I also stimulated greater glycogen deposition in

Table 3. Effects of Exogenous IGF-I on Glucose Kinetics (mmol/kg · h) in TNF-Treated Rats

Group	Treatment	No.	Body Weight (g)	Glucose Infusion†	Flux	Hepatic Glucose Production†
Saline	Saline (S/S)	9	353.8 ± 3.0	7.33 ± 0.52	5.62 ± 0.75	−1.75 ± 0.93
	IGF-I (S/IGF)	11	352.1 ± 2.6	7.01 ± 0.51	8.70 ± 0.88	1.29 ± 0.92
TNF	Saline (TNF/S)	9	354.0 ± 2.3	4.35 ± 0.58*‡	6.74 ± 1.35	2.38 ± 1.37
	IGF-I (TNF/IGF)	10	351.9 ± 2.0	6.78 ± 0.70*	5.88 ± 0.69	−0.60 ± 0.65

NOTE. Results are the mean ± SE.

*$P < .01$, saline *v* TNF, †$P < .05$ by interaction: 2-way ANOVA.

‡$P < .05$ *v* all by LSD.

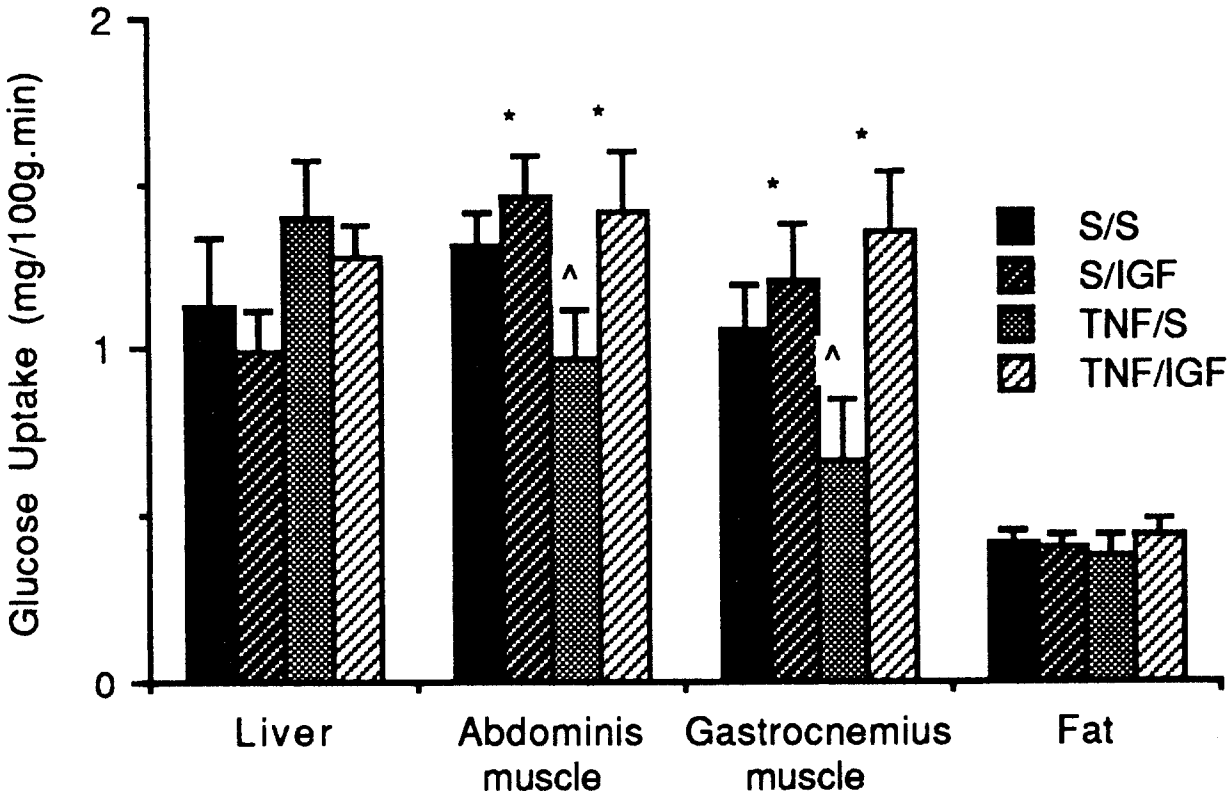

Fig 2. Rate of glucose uptake in different tissues (mean ± SEM). *$P < .05$, S *v* IGF by 2-way ANOVA; ^$P < .05$ *v* S/IGF and TNF/IGF by LSD.

the liver and abdominis muscle in both the saline and TNF groups. Both the euglycemic-hyperinsulinemic condition and administration of pharmacological doses of IGF-I resulted in glycogen values much higher than previously reported,[32] which may be related to the high physiologic or pharmacologic levels, respectively, of the two potent glycogenic hormones. Moreover, IGF-I normalized all the measured effects of TNF in the rats. For instance, IGF-I significantly increased mean plasma IGF-I in the TNF group to levels similar to those in the saline group. Secondly, the effects of TNF on glucose uptake and glycogen

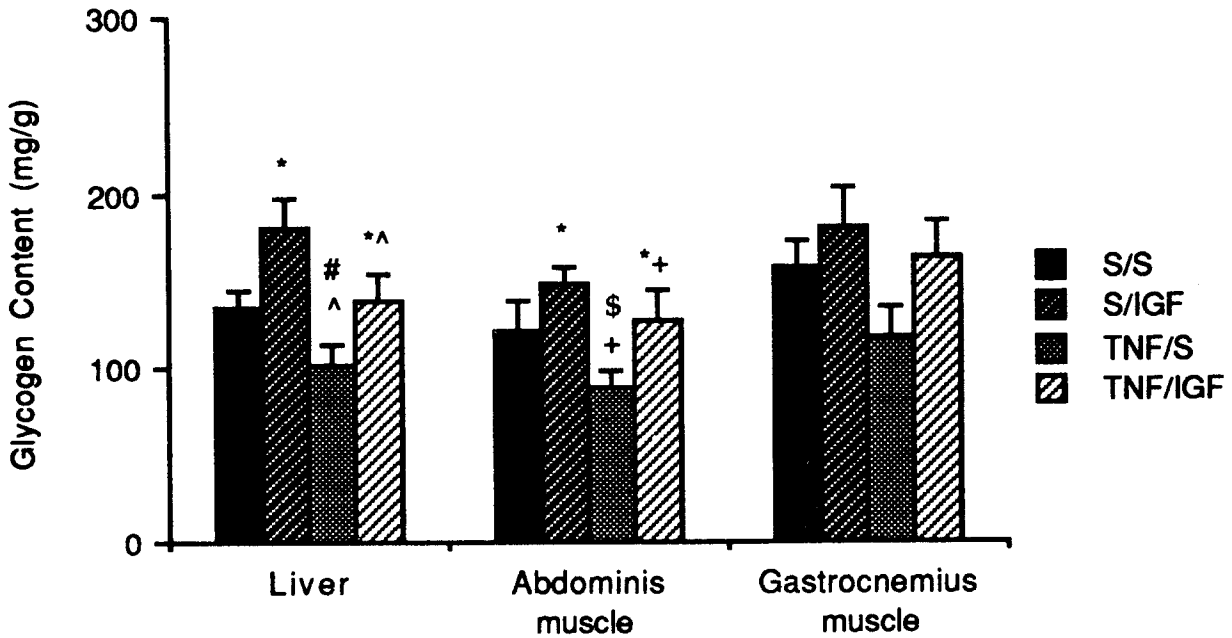

Fig 3. Glycogen content in different tissues (mean ± SEM). *$P < .05$, S *v* IGF, ^$P < .05$, S *v* TNF, †$P = .06$, S *v* TNF by 2-way ANOVA; #$P < .05$ *v* all and $P < .01$ *v* S/IGF by LSD.

stores in peripheral tissues were also overcome by the simultaneous infusion of IGF-I. Thus, TNF-treated rats, although resistant to insulin, seem to have a normal response to exogenous IGF-I. Such phenomena have also been observed in septic rats.[27,33] Although the exact mechanisms for insulin resistance during infection are not known, recent evidence strongly suggests that sepsis-induced insulin resistance is not due to a downregulation of insulin receptor in muscle,[34] but is related to alterations of various postreceptor events. TNF has been shown to have inhibitory effects on tyrosine phosphorylation signaling pathways.[35,36] In cultured adipocytes or hepatocytes, TNF results in a decrease in insulin receptor autophosphorylation, tyrosine phosphorylation of endogenous IRS-1, and kinase activity toward exogenous substrates without altering insulin binding.[35,36] Since insulin and IGF-I have a similar pattern of biological effects after binding to their respective receptors, our data suggest that under a hyperinsulinemic-euglycemic condition, the TNF-induced defect in the insulin signaling pathway might not be a step in the IGF-I pathway, although a firm conclusion cannot be drawn from the present study.

At physiological levels, IGF-I induces metabolic actions through its own receptors. However, at higher concentrations, IGF-I is able to bind to and activate the insulin receptor.[37] Therefore, it is possible that the excess IGF-I induced by pharmacological amounts of IGF-I infusion in the present study activated insulin receptors, because the signal to final metabolic action, ie, the increase in glucose utilization, has been postulated to be mediated mostly by insulin receptors. However, our data[21] and Lang's[27] have demonstrated that the TNF-treated rat is resistant to maximal physiologic levels of insulin. This appears to suggest that at certain levels, plasma IGF-I may be able to bypass the insulin receptor or some postreceptor defect in insulin action, such as tyrosine phosphorylation or glucose transporter activity, to overcome insulin resistance. Furthermore, it is unlikely that a further increase in insulin levels would achieve the same effects as IGF-I in this study. First, the present study was conducted in a hyperinsulinemic condition. Second, insulin binds to the IGF-I receptor with low affinity.

In conclusion, exogenous IGF-I can reverse the effect of TNF to decrease plasma IGF-I, as well as the TNF impairment of insulin action on glucose utilization. These results suggest that during a hyperinsulinemic-euglycemic condition, TNF-treated rats, although resistant to insulin, have a normal response to exogenous IGF-I. These findings indicate that the TNF-induced defect in the insulin pathway may not be a step in the IGF-I pathway. However, a threshold level of IGF-I may be required to reverse insulin resistance in different catabolic stress conditions.

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