Tumor Necrosis Factor-α Inhibits Insulin’s Stimulating Effect on Glucose Uptake and Endothelium-Dependent Vasodilation in Humans

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Background—Inflammatory mechanisms could be involved in the pathogenesis of both insulin resistance and atherosclerosis. Therefore, we aimed at examining whether the proinflammatory cytokine tumor necrosis factor (TNF-α) inhibits insulin-stimulated glucose uptake and insulin-stimulated endothelial function in humans.

Methods and Results—Healthy, lean male volunteers were studied. On each study day, 3 acetylcholine (ACh) or sodium nitroprusside (SNP) dose-response studies were performed by infusion into the brachial artery. Before and during the last 2 dose-response studies, insulin and/or TNF-α were coinfused. During infusion of insulin alone for 20 minutes, forearm glucose uptake increased by 220±44%. This increase was completely inhibited during coinfusion of TNF-α (started 10 min before insulin) with a more pronounced inhibition of glucose extraction than of blood flow. Furthermore, TNF-α inhibited the ACh forearm blood flow response (P<0.001), and this inhibition was larger during insulin infusion (P=0.01) but not further increased by N6-monomethyl-L-arginine acetate (P=0.2). Insulin potentiated the SNP response less than the ACh response and the effect of TNF-α was smaller (P<0.001); TNF-α had no effect on the SNP response without insulin infusion. Thus, TNF-α inhibition of the combined response to insulin and ACh was likely mediated through inhibition of NO production.

Conclusion—These results support the concept that TNF-α could play a role in the development of insulin resistance in humans, both in muscle and in vascular tissue. (Circulation. 2003;108:1815-1821.)

Key Words: tumor necrosis factor ■ insulin ■ acetylcholine ■ blood flow ■ endothelium-derived factors
Clinical Characteristics of Experimental Subjects

<table>
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<th>No.</th>
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<tr>
<td>Age, y</td>
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<tr>
<td>Body mass index, kg·m⁻²</td>
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<tr>
<td>Fasting plasma glucose, mmol·L⁻¹</td>
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<td>Fasting serum insulin, mU·L⁻¹</td>
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<td>Serum insulin OGTT (2 h), mU·L⁻¹</td>
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<td>Systolic blood pressure, mm Hg</td>
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<td>Plasma LDL cholesterol, mmol·L⁻¹</td>
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<td>Plasma HDL cholesterol, mmol·L⁻¹</td>
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<tr>
<td>Plasma triglyceride, mmol·L⁻¹</td>
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<td>0.71±0.11</td>
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OGTT (2 h) indicates oral glucose tolerance test, measured value 2 hours after ingestion of 75 g glucose.

Uptake and on vasodilation stimulated by an endothelium-dependent agonist.

Methods

Healthy subjects were included if they had no history of diabetes among first-degree relatives and if the parameters presented in the Table were normal. The study was approved by the Ethics Committee of Copenhagen County, and each subject gave written informed consent.

Each subject participated in up to 3 different vascular examinations on separate days, in random order, and at least 2 weeks apart. Each subject fasted from 8 hours before the examination started (at 8:00 AM) until its completion. An arterial cannula with an external diameter of 1 mm (Ohmeda) was placed in the brachial artery of the nondominant arm. A venous cannula was inserted in a cubital vein on both sides, retrogradely in the arm where the arterial cannula had been placed.

All drug solutions were prepared on the day of examination. Aliquots of human recombinant TNF-α (tasonermin) (Beromun; Boehringer-Ingelheim) had been stored at −80°C. Human albumin (Baxter Healthcare) 1% v/v in an isotonic NaCl solution was used as vehicle for TNF-α and insulin (Actrapid; Novo Nordisk). Doses described in Figure 1 for acetylcholine chloride (ACh) (Clinalfa), sodium nitroprusside dihydrate (SNP) (Nitropress; Roche), and N⁶-monomethyl-L-arginine acetate (L-NMMA) (Clinalfa) are for the compounds just listed, not for the active constituents.

During each examination, the forearm vasodilator response to 3 sequential dose-response studies with either ACh or SNP was measured. Infusion of each dose of ACh or SNP lasted 3 minutes, sufficient to obtain a steady state of vasodilation. Forearm blood flow was measured simultaneously in both arms by electrically calibrated strain gauge plethysmographs (D.E. Hokanson) with the subject resting in the supine position and with pneumatic wrist cuffs inflated to 200 mm Hg throughout each 9-minute dose-response study. Four readings on the plethysmographs were recorded during the last minute before the start of a dose-response study, or during the last minute of a 3-minute infusion of ACh or SNP at a certain dose. The slope of the plethysmographic recording was measured manually, and blood flow was calculated as the mean result from the 4 recordings.

Before and during the second and third dose-response studies, insulin, TNF-α, L-NMMA, or vehicle, alone or in combination, were infused into the brachial artery (Figure 1).

Blood samples were drawn simultaneously from the cannulas immediately before measurement of blood flow. Plasma glucose was measured by enzymatic colorimetry. Serum insulin was measured by microparticle enzyme immunoassay (AxSym Insulin B2D010; Abbott Diagnostics). Plasma TNF-α and IL-6 by enzyme-linked immunosorbent assay (Quantikine; R&D Systems).

Systemic blood pressure and heart rate were measured immediately after each recording on the plethysmographs by an electrical transducer connected to the arterial cannula and by a recording of an electrocardiogram, respectively.

Statistical Analysis

All variables, including blood flow before dose-response studies with ACh or SNP, were analyzed by paired or unpaired t test as appropriate. Correlations between glucose uptake, glucose extraction, and blood flow were tested with Pearson’s correlation analysis. Blood flow during dose-response studies were analyzed by mixed models, using the PROC MIXED procedure in the Statistical Analysis Software, version 8 (SAS Institute). The type of dose-response study and the interaction between a given dose-response study and dose of vasodilator entered the model as fixed effects. Study subject and the interaction between study subject and dose of vasodilator entered the model as random effects. When different protocols were compared, a single protocol (among the protocols described in Figure 1) was entered as a fixed effect together with interactions that protocol and other fixed effects. Furthermore, interaction between subject and protocol was added as a random effect. Blood flow was log-transformed to satisfy the assumptions of normal distribution of residuals and homogeneity of variance.

A two-sided P<0.05 was considered significant. Results, including geometric symbols with error bars in the figures, are presented as the mean±SEM.

Results

Insulin, Glucose, and TNF-α Levels

During the 5 insulin infusions in 3 protocols, plasma insulin measured in venous samples from the perfused arm (“local” plasma insulin) increased to between 80.1±9.9 and 115.5±10.6 mU·L⁻¹. During all insulin infusions in every protocol, there was a minor, but statistically significant, increase in systemic plasma insulin of 2.1±0.5 mU·L⁻¹ or less (P<0.05).

Systemic plasma glucose was not affected by insulin infusion, but local plasma glucose decreased 0.6±0.1 mmol·L⁻¹ (P<0.01) during the first insulin infusion in the ACh-Ins-Veh and ACh-Ins-Ins protocol.

Plasma TNF-α was measured in the first 7 consecutive subjects in the ACh-Ins-Ins and ACh-Ins-Veh protocols. During TNF-α infusion, plasma TNF-α increased from 1.1±0.2 to 252±29 ng·L⁻¹ in the perfused arm but only to 5.5±0.4 ng·L⁻¹ in the control arm. There was no difference between the TNF-α levels in the 2 protocols just mentioned (data not shown). Plasma concentrations of IL-6, which is induced by TNF-α, did not change (data also not shown).

Forearm Glucose Uptake

During 20 minutes of insulin infusion, forearm glucose uptake increased by 220±44% (P<0.001; Figure 2; pooled results from the first insulin infusion in the ACh-Ins-Ins and ACh-Ins-Veh protocols: the results from each protocol were almost identical, P=0.7). When TNF-α was infused before and during the repeated insulin infusion, this increase in glucose uptake was completely inhibited (ACh-Ins-Ins protocol, P=0.01) (Figure 2).

Glucose uptake was determined as the product between glucose extraction (the arteriovenous difference of plasma glu-
cose) and blood flow. Glucose extraction increased by 193±60% during insulin infusion but only by 43±31% during coinfusion of insulin and TNF-α. In contrast, blood flow increased by only 28±16% during insulin infusion and decreased by 16±4% during coinfusion with insulin and TNF-α.

Changes in glucose uptake were correlated to changes in glucose extraction during insulin infusion (P=0.02) as well as during coinfusion of insulin and TNF-α (P=0.02). However, changes in glucose uptake were not correlated to changes in blood flow in either case (P≥0.5), indicating that changes in glucose uptake

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**Figure 1.** Infusion protocols. The graph with stepwise increments shows dose and timing of dose-response studies of ACh or SNP; the first dose-response study was started at least 30 minutes after arterial cannulation. Black and white bars show the timing of the coinusions with insulin (0.05 mg · kg⁻¹ · min⁻¹), TNF-α (17 ng · min⁻¹), or vehicle (human serum albumin 1% v/v at an infusion rate of 0.33 mL · min⁻¹). After the first dose-response study, the vehicle infusion (represented by the first black bar) was delayed for 20 minutes or until forearm blood flow had returned to basal values. The time interval between the second and third dose-response studies was always 45 minutes. Arrows (v) indicate timing of blood sample aspiration for analysis of glucose uptake. The protocol names, listed in the first column of each table, describe the choice of ACh or SNP for the dose-response studies. They also describe the choice of insulin or vehicle before and during the second and third dose-response studies (represented in the figure by the 2 white bars A and B). A, The 3 protocols with ACh dose-response studies. Not shown is that the ACh-Ins-Ins protocol was repeated in 5 subjects during coinfusion of L-NMMA 2 mg · min⁻¹ together with TNF-α and insulin, with the L-NMMA infusion starting 10 minutes before the ACh dose-response study. B, The 2 protocols with SNP dose-response studies.
were dependent on changes in glucose extraction rather than changes in blood flow.

**Blood Flow During ACh or SNP Dose-Response Studies**

ACh increased forearm blood flow in a dose-dependent manner (Figure 3). During insulin infusion, the ACh forearm blood flow response increased by 133%, 44%, and 24% at each of the 3 increasing doses of ACh (pooled results from the first insulin infusion in the ACh-Ins-Ins and ACh-Ins-Veh protocols). When no insulin was infused during the second ACh dose-response study, thus serving as a control for the insulin stimulation just described, there was no significant difference in the blood flow response during the first and second ACh dose-response study (ACh-Veh-Veh protocol, data not shown).

Infusion of TNF-α without insulin immediately before the ACh dose-response study caused no change in blood flow (data not shown, \(P=0.2\)). TNF-α infusion inhibited the ACh response during insulin infusion to below the response of ACh during vehicle infusion (ACh-Ins-Ins protocol, \(P<0.001\)) (Figure 3). There was a similar inhibiting effect of TNF-α without coinfusion of insulin but with a prior insulin infusion stopped 45 minutes before the start of TNF-α infusion (ACh-Ins-Veh compared with ACh-Ins-Ins protocol, \(P=0.8\) (Figure 4)). Even though TNF-α inhibited the ACh response when insulin was not given at all (\(P=0.001\)), this inhibition was smaller than in the protocol in which insulin was coinfused with TNF-α or in the protocol in which a previous insulin infusion was stopped 45 minutes before the TNF-α infusion (ACh-Veh-Veh protocol compared with ACh-Ins-Ins or ACh-Ins-Veh protocol, \(P=0.01\)) (Figure 4). L-NMMA did not have an inhibitory effect additional to the effect of TNF-α, because the inhibitory effect of TNF-α with or without L-NMMA was not significantly different (ACh-Ins-Ins protocol compared with 5 subjects in whom the protocol was repeated with L-NMMA infusion, \(P=0.2\)) (Figure 3).

SNP increased the forearm blood flow in a dose-dependent manner and to a similar degree as ACh (SNP-Ins-Ins protocol, Figure 5). The SNP response increased during infusion of insulin (SNP-Veh-Veh protocol, \(P<0.001\)), but this effect of insulin was smaller than insulin’s potentiating effect on the ACh response (SNP-Ins-Ins protocol compared with the ACh-Ins-Ins protocol, \(P<0.001\)). TNF-α did inhibit the SNP response during insulin infusion (SNP-Veh-Veh protocol, \(P<0.001\)) (Figure 5), but this inhibitory effect of TNF-α was smaller than the pronounced effect of TNF-α on the insulin-potentiated ACh response (\(P<0.001\)) (SNP-Ins-Ins protocol compared with the ACh-Ins-Ins protocol). TNF-α had no effect on the SNP response when no insulin was infused before or during the SNP dose-response study (SNP-Veh-Veh protocol, \(P=0.6\)) (Figure 6).

The results were similar if blood flow was expressed as the ratio between the value for the perfused arm and control arm, respectively.

**Systemic Responses**

During the experiment, one subject developed a headache that improved after food and fluid intake at the planned termina-
tion of the study. No other subject felt malaise, nausea, headache, or other side effects to TNF-α during or after TNF-α infusion, and no one developed a fever.

Throughout the studies, there were no systematic changes in blood flow in the forearm contralateral to the perfused arm (Figures 3, 5, and 6, and data not shown), or changes in arterial blood pressure or heart rate (data not shown).

**Discussion**

In the present study, we show that a proinflammatory cytokine, TNF-α, has the ability to inhibit insulin-stimulated glucose uptake as well as endothelium-dependent vasodilation in humans, and that the inhibitory effect on vasodilator function is larger during local elevation of plasma insulin. TNF-α expression is increased in adipose tissue and skeletal muscle of obese people and in patients with type 2 diabetes, and TNF-α has been shown to interfere with insulin-stimulated glucose uptake in vivo and with insulin signaling in vitro in a receptor-mediated manner. Therefore, TNF-α can be a mediator of some of the proposed effects of chronic inflammation on insulin signaling in endothelium and other insulin-sensitive tissues. However, clarification of the role of TNF-α in human metabolism is warranted. For example, despite compelling evidence in animal models, the only published human study of the effect of TNF-α inhibition on insulin sensitivity in patients with type 2 diabetes showed no effect of a single injection of a neutralizing antibody towards TNF-α. The present study is the first to directly demonstrate that TNF-α inhibits insulin-stimulated glucose uptake in humans. Furthermore, it is the first human study to show an inhibitory effect of TNF-α on insulin-stimulated endothelial function.

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Changes in the ACh response caused by TNF-α in 3 different protocols. Each graph shows blood flow in the perfused forearm during the second ACh dose-response study subtracted from blood flow during the third dose-response study in a given protocol. The blood flow response to ACh (coinfused with vehicle) was inhibited by TNF-α (ACh-Ins-Veh protocol [open circles], P=0.001). The inhibitory effect of TNF-α was larger during or after insulin infusion, ie, during coinfusion of insulin (ACh-Ins-Ins protocol [closed circles]) or when insulin infusion was stopped 45 minutes before the TNF-α infusion (ACh-Ins-Veh protocol [closed triangles]) (P<0.001).

![Figure 5](http://circ.ahajournals.org/)

**Figure 5.** Effect of insulin and TNF-α on the SNP response. In the perfused forearm, the SNP blood flow response increased during insulin infusion (P<0.001). TNF-α inhibited the SNP response during insulin infusion (P<0.001). These effects were smaller than the situation in which ACh was infused in place of SNP (this figure compared with Figure 3, P<0.001).
We have previously shown that L-NMMA at the dose used in the present study inhibits a large portion of the insulin-potentiated ACh response. However, in the present study, L-NMMA had no additional effect on TNF-α/H9251’s inhibition of the insulin-potentiated ACh response. The inhibitory effect of TNF-α could not be explained as resulting in a decreased vascular sensitivity to NO, because TNF-α had no effect on the SNP response if insulin was not infused. Together, these observations suggest that TNF-α blocked NO production stimulated by insulin and ACh.

The effect of TNF-α on insulin-stimulated vasodilation could be independent of its effect on insulin-stimulated glucose uptake because TNF-α infusion affected glucose extraction more than blood flow. However, this does not preclude that vascular effects of TNF-α are responsible for the inhibition of glucose uptake because TNF-α could inhibit capillary recruitment, which can be independent of total blood flow.

Importantly, the inhibitory effect of TNF-α on the ACh response was larger during insulin stimulation, both when insulin was stopped 45 minutes before TNF-α infusion and when insulin was coinfused with TNF-α and ACh (Figure 4). Insulin exerts negative feedback on insulin signaling, and a recent study showed that in adipocytes, this negative feedback effect was greatly potentiated by TNF-α. If this is also true for vascular tissue, it could explain why the insulin-associated effects of TNF-α in the present study were observed during both immediate and delayed effects of insulin.

The serial design of insulin infusions would be expected to underestimate the effect of TNF-α on glucose uptake because TNF-α plasma levels are not. Therefore, it has been proposed that TNF-α could exert effects on insulin sensitivity in a paracrine or autocrine manner. Indeed, some effects of TNF-α have been shown to be dependent on site of production, not on plasma levels.

In conclusion, TNF-α inhibits insulin-stimulated glucose uptake as well as endothelium-dependent vasodilation. The vascular effect is more pronounced during insulin stimulation, in which it is likely mediated by inhibition of NO production. Finally, the effect operates after a short exposure time with local circulating concentrations of TNF-α in the

![Figure 6. Effect of TNF-α on the SNP response without insulin infusion. TNF-α had no effect on the SNP response (during coin infusion of vehicle) (P=0.6).](image-url)
high pathophysiological range. Future research should attempt to describe whether insulin resistance of endothelial function has a role in the development of atherosclerosis and to what extent inflammatory mediators are involved in this disease process.

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References
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