Tumor Necrosis Factor-α Antagonism Improves Vasodilation During Hyperinsulinemia in Metabolic Syndrome

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OBJECTIVE — Obesity is associated with chronic inflammation due to overproduction of proinflammatory cytokines, including tumor necrosis factor (TNF)-α. We assessed the effects of TNF-α neutralization by infliximab on vascular reactivity during hyperinsulinemia in obesity-related metabolic syndrome.

RESEARCH DESIGN AND METHODS — Vascular responses to intra-arterial infusion of acetylcholine (ACh) and sodium nitroprusside (SNP) were assessed in patients with metabolic syndrome, before and after administration of infliximab.

RESULTS — Patients had blunted vasodilator responses to ACh and SNP during hyperinsulinemia compared with control subjects; a potentiation of the responsiveness to both ACh and SNP, however, was observed in patients following infliximab. The antioxidant vitamin C improved the vasodilator response to ACh in patients with metabolic syndrome, but its effect was not further enhanced by concurrent administration of infliximab.

CONCLUSIONS — TNF-α neutralization ameliorates vascular reactivity in metabolic syndrome during hyperinsulinemia, likely in relation to decreased oxidative stress, thereby suggesting an involvement of inflammatory cytokines in vascular dysfunction of these patients.

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Central obesity is associated with low-grade, chronic inflammation, which might affect insulin action and thus contribute to both insulin resistance and vascular dysfunction characteristic of metabolic syndrome. Among various inflammatory cytokines, tumor necrosis factor (TNF)-α seems to play an important role in the pathophysiology of insulin resistance. However, no clear link has been established between the vascular pathology of metabolic syndrome and a particular inflammatory cytokine in humans. This study, therefore, assessed the effects of TNF-α neutralization by the monoclonal antibody infliximab on vascular reactivity during hyperinsulinemia in metabolic syndrome.

RESEARCH DESIGN AND METHODS

— A total of 16 patients with metabolic syndrome (National Cholesterol Education Program Adult Treatment Panel [NCEP ATP] III criteria) and 13 healthy control subjects, approximately matched for sex and age, were recruited for this study. In all patients, waist circumference was >102 cm in male subjects and >88 cm in female subjects, thus indicating central obesity. Studies consisted of infusion of drugs into the brachial artery and measurement of forearm blood flow responses by means of strain-gauge plethysmography. In Study 1, control subjects and 10 patients with metabolic syndrome received infusion of regular insulin (0.2 mU · kg^{-1} · min^{-1}); after 45 min of insulin infusion, dose-response curves to graded doses of acetylcholine (ACh) (release of endogenous NO) and sodium nitroprusside (SNP) (exogenous NO donor) were obtained. Thereafter, while keeping insulin infusion constant, patients with metabolic syndrome received infusion of infliximab (200 μg/min for 45 min) and dose-response curves to ACh and SNP were repeated. In Study 2, to assess whether the effect of infliximab on vascular response to ACh might relate to reduction of oxidative stress, six additional patients with metabolic syndrome underwent a first dose-response curve to ACh during hyperinsulinemia alone. Vitamin C was then given at 25 mg/min for 45 min and a second dose-response curve to ACh was obtained. Finally, infliximab infusion was superimposed and a third dose-response curve to ACh was obtained during concurrent administration of vitamin C and infliximab. Statistical analyses were performed by *t* test and ANOVA, as appropriate. Data are reported as mean ± SEM and $P < 0.05$ was considered statistically significant.

RESULTS

— Patients had higher blood pressure ($P < 0.001$), plasma cholesterol ($P < 0.05$), triglycerides ($P < 0.05$), and glucose ($P < 0.01$) than control subjects. Baseline insulin was lower in control subjects than in patients (6.2 ± 1.5 vs. 11.3 ± 1.7 μU/ml, respectively; $P = 0.03$); following insulin administration, venous insulin concentration in the perfused forearm rose to 171 ± 37 in control subjects versus to 224 ± 32 μU/ml in patients ($P = 0.44$).

The vasodilator response to ACh was blunted in patients compared with control subjects (12.7 ± 1.4 vs. 26.5 ± 3.1 ml · min^{-1} · dl^{-1} at the highest dose of ACh, respectively; $P < 0.001$). Similarly, the vasodilator response to SNP was lower in patients than in control subjects

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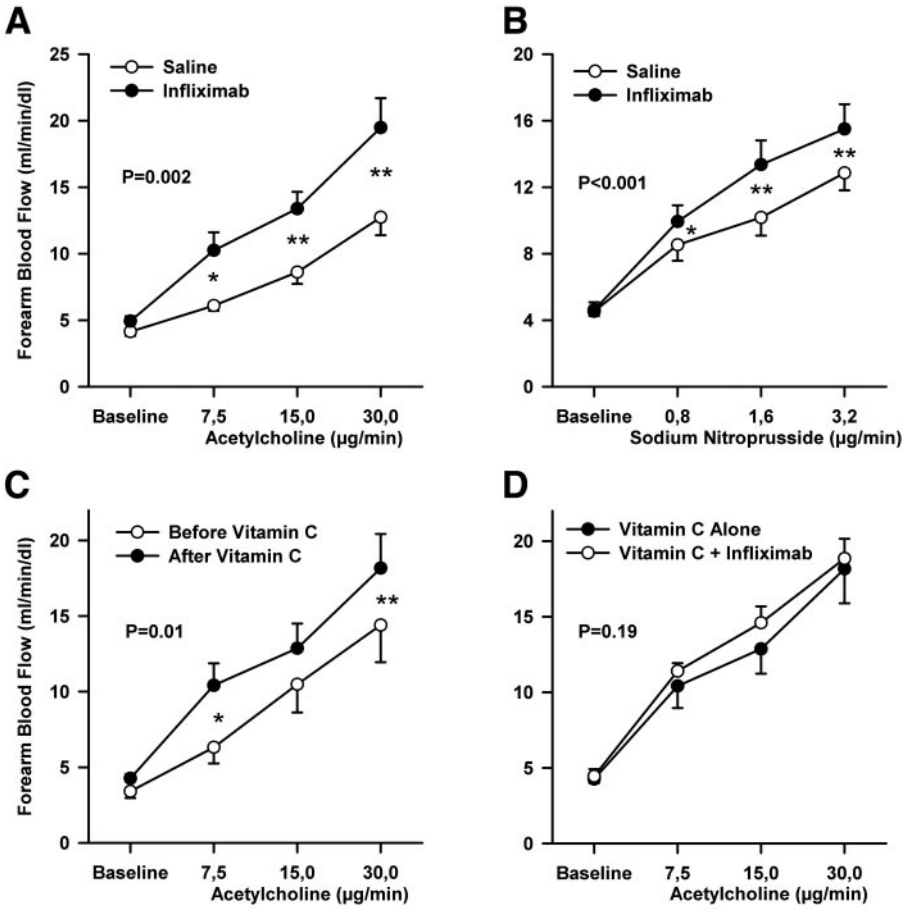

Figure 1—P *values refer to the comparison between treatments by two-way ANOVA for repeated measures.* *P > *0.05;* †P < *0.01 at post hoc pairwise comparisons by Bonferroni* t *test.*

(12.9 ± 1.1 vs. 16.3 ± 0.6 ml · min^{-1} · dl^{-1}, respectively; $P < 0.001$). In patients with metabolic syndrome participating in Study 1, infliximab improved the vasodilator response to both ACh (Fig. 1*A*) and SNP (Fig. 1*B*). In patients participating in Study 2, vascular response to ACh was significantly enhanced by administration of vitamin C compared with hyperinsulinemia alone (Fig. 1*C*); under those conditions, however, infusion of infliximab in conjunction with vitamin C did not modify vascular responsiveness to ACh versus vitamin C alone (Fig.1*D*).

CONCLUSIONS — This study provides the novel finding that TNF-α neutralization improves NO-dependent vasodilation during hyperinsulinemia, thereby suggesting that TNF-α activation is involved in vascular dysfunction of metabolic syndrome.

Overexpression of TNF-α has previously been reported not only in obese adipose tissue (1) and in the skeletal muscle of insulin-resistant animals and humans (2), but also in vascular smooth muscle cells (VSMCs) (3). The decreased responsiveness to both endothelium-dependent and -independent stimuli seen during hyperinsulinemia in our patients, taken in conjunction with the favorable effect of infliximab on responses to both ACh and SNP, suggests that TNF-α activation in metabolic syndrome affects NO-dependent vasodilation through mechanisms other than endothelial dysfunction. This phenomenon might be determined by impaired insulin signaling within VSMCs, thus affecting the facilitatory action physiologically exerted by insulin on vasorelaxation. This hypothesis stems from studies showing that insulin may impact VSMCs' vasodilator capacity through multiple mechanisms. In particular, impairment of insulin inactivation of the small GTPase RhoA and its target ρ-kinase, leading to decreased vasodilation, has been reported in VSMCs from insulin-resistant animals (4). Because this defect localizes at the same level of PI3-kinase activation (5) at which TNF-α upregulation affects insulin signaling (6), the vascular effect of TNF-α antagonism observed in our patients is consonant with restoration of the facilitatory role of insulin on VSMCs relaxation.

Recent experimental evidence indicates that TNF-α overexpression increases oxidative stress (7) and that TNF-α–induced reactive oxygen species play a causal role in insulin resistance (8). We tested the possibility that increased oxidative stress could also be involved in human TNF-α vasculopathy by use of vitamin C. This antioxidant improved the vasodilator response to ACh during hyperinsulinemia in metabolic syndrome, whereas infliximab infusion on top of vitamin C did not exert additive effects. This suggests that increased oxidative stress is indeed involved in mediating the effects of TNF-α on vasodilation during hyperinsulinemia, a view supported by previous results showing that reactive oxygen species could affect vascular reactivity through changes in receptor function or activity of signaling pathways (9,10).

The beneficial action of infliximab demonstrated in our study suggests a novel mechanism by which TNF-α activation might be involved, via increased oxidative stress, in vascular dysfunction of patients with obesity-related metabolic syndrome; it remains to be elucidated whether interventions targeting cytokines may become an effective strategy for prevention in these patients.

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