Chronic TNF-α Neutralization Does Not Improve Insulin Resistance or Endothelial Function in "Healthy" Men with Metabolic Syndrome

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The possible contribution of tumor necrosis factor-α (TNF-α) to the development of obesity-associated insulin resistance in humans is still controversial. Our study investigated the effect of TNF-α neutralization on insulin resistance in healthy, obese and insulin resistant men. We performed a prospective, randomized, double-blind placebo-controlled trial in nine young, healthy obese male subjects with metabolic syndrome and insulin resistance. Volunteers received three infusions (wks 0, 2 and 6) of infliximab or placebo. Insulin resistance was measured at baseline and after 70 d by homeostatic model assessment (HOMA) index as well as by minimal model analysis of an intravenous glucose tolerance test. Endothelial function was accessed before and after intervention by flow mediated dilation. Infliximab improved the inflammatory status as indicated by reduced high sensitivity C-reactive protein (hsCRP) and fibrinogen levels (2.77 ± 0.6 to 1.8 ± 0.5 μg/L, and 3.42 ± 0.18 to 3.18 ± 0.28 g/L; (day 0 and day 70, $P = 0.020$ and 0.037 respectively), but did not improve insulin resistance (HOMA index and intravenous glucose-tolerance test (ivGGT)) or endothelial function. Despite improvements in inflammatory status, chronic TNF-α neutralization does not improve insulin resistance or endothelial function in seemingly healthy, but obese, insulin-resistant volunteers. This study severely questions the proposal that TNF-α is a causative link between adiposity and insulin resistance.

INTRODUCTION

Metabolically triggered inflammation has been proposed as a key step in the pathogenesis of obesity-induced insulin resistance and type 2 diabetes mellitus, and accelerated atherosclerosis and premature death (1,2).

The proinflammatory cytokine tumor necrosis factor-α (TNF-α) exerts detrimental effects on glucose homeostasis and lipid metabolism, and has been linked to β-cell apoptosis and endothelial dysfunction in type 1 and type 2 diabetes (3,4,5). In obese, insulin-resistant rodents, as well as humans, increased circulating levels of TNF-α are observed (4). TNF-α has thus been proposed to be causatively involved in the evolution of insulin resistance and type 2 diabetes, and its complications (6). This hypothesis is supported by animal studies showing that interference with TNF-α signaling protects against devolvement of the metabolic syndrome during development of obesity (7), and by human studies showing that TNF-α neutralization improves insulin sensitivity in patients with chronic inflammatory disease (8,9).

Alternatively, studies investigating TNF-α neutralization in type 2 diabetic patients have failed to demonstrate an effect of TNF-α neutralization on insulin sensitivity (10,11,12,13). The basis for this controversy is unclear but may relate to patient populations studied or to the time course of the experiments. Acute experiments (such as 12,13) potentially did not allow sufficient time for normalization of the metabolic derangements, while long lasting, poorly controlled diabetes (studied in 10–12) may reflect a condition that has become de-

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sensitized to the effects of TNF-α neutralization. Given the longstanding controversies on the role of TNF-α in the pathogenesis of insulin resistance/type 2 diabetes and the associated vascular dysfunction (14), we performed a prospective, randomized, double-blind trial of long-term TNF-α neutralization (Infliximab) in "healthy" overweight young men with a metabolic syndrome, but no overt diabetes.

METHODS

This trial was registered at www.clinicaltrials.gov (NCT00636142). The study was approved by the local ethics committee and conducted in accordance with good clinical practice (GCP) criteria. All participants gave written informed consent before entering the study.

Primary inclusion criteria were male gender, 20 to 50 years of age, BMI between 30 and 35 kg/m², fasting HOMA index > 2.5 and at least one of the following derangements: blood pressure > 135/85 mmHg or treated hypertension; triglycerides > 1.7 mmol/L or HDL cholesterol > 1.3 mmol/L. All subjects fulfilled the NCEP criteria for the metabolic syndrome (15). Major exclusion criteria were manifest diabetes mellitus, treatment with ACE inhibitors, angiotensin receptor blockers or statins; acute or chronic infection; history of tuberculosis; previous treatment with any drug targeting TNF-α, any contraindication to infliximab treatment. Randomization was performed within 14 d after screening.

This prospective study was performed in a randomized, double-blind, placebo controlled, parallel group design. Patients were randomized by external randomization to receive either infliximab (3 mg/kg bodyweight) or placebo as an intravenous infusion at $t = 0$, 2 and 6 wks. Efficacy parameters were evaluated at $t = 0$ (before the first treatment), after 3 d, 10 wks and 15 wks. A safety visit was scheduled at 32 wks.

Patients arrived in the research unit in the morning in a fasting state. Insulin resistance was estimated in fasting condi-

Table 1. Baseline characteristics of the study population (mean ± SEM)

	Infliximab	Placebo	P
N	5	4	
Age (years)	40 ± 2	38 ± 3	ns[a]
BMI (kg/m²)	31.9 ± 0.6	31.4 ± 1.1	ns
Waist (cm)	111 ± 4	107 ± 7	ns
BP syst. (mmHg)	142 ± 4	138 ± 5	ns
BP diast. (mmHg)	94 ± 4	87 ± 2	ns
FPG[b] (mmol/L)	5.7 ± 0.4	5.4 ± 0.2	ns
Fasting insulin (μU/L)	15.9 ± 3.5	15.2 ± 2.7	ns
HOMA index	4.2 ± 1.2	3.7 ± 0.8	ns
Cholesterol (mmol/L)	6.3 ± 0.8	6.9 ± 0.9	ns
LDL-C (mmol/L)	2.5 ± 0.4	3.4 ± 1	ns
HDL-C (mmol/L)	± 0.1	± 0.1	ns
Triglycerides (mmol/L)	5.9 ± 1.8	6.0 ± 1.6	ns

[a]ns: not significant.
[b]FPG: fasting plasma glucose.

tions by the HOMA index (16), which is considered to reflect mainly hepatic insulin resistance (16). Minimal model analysis of a frequently sampled ivGTT was performed to dynamically access insulin sensitivity of predominantly muscle and adipose tissue (17). Plasma cholesterol, triglycerides and C-reactive protein (CRP) levels were measured in a certified laboratory, and intercellular adhesion molecule 1 (ICAM-1), von Willebrand factor (vWF), plasminogen activator inhibitor-1 (PAI-1) and fibrinogen levels were measured by specific enzyme-linked immunosorbent assays (ELISAs) (18). Vascular endothelial function was quantified by flow-mediated dilation as well as nitroglycerine-mediated vasodilatation by using high resolution ultrasound (19).

Study size was based on the assumption that the effect of TNF-α neutralization is comparable to that of weight loss using data from a recently published study (18). On basis of this data, a sample size of 18 allows a 91% power to detect a similar difference with a significance level of 0.05 by use of a paired t test. An interim analysis after nine completed patients was specified to indicate a trend for a difference between groups with a $P < 0.2$ to justify continuation of the study. Data were analyzed by using paired nonparametric tests. All data are mean ± standard error of the mean.

RESULTS

Baseline characteristics of the patients are given in Table 1. Infliximab was well tolerated and there were no dropouts or serious adverse events. Interim analysis after nine completed patients did not indicate a trend for a difference between the two study groups. In accordance with the guidelines set in the protocol, the study was terminated.

Infliximab treatment reduced plasma levels of the acute phase proteins CRP and fibrinogen ($P = 0.020$ and 0.037 respectively, Table 2). Such an effect was not observed after placebo treatment.

The effect of TNF-α neutralization on insulin sensitivity was estimated by the HOMA index as well as by the insulin sensitivity index that was based on the ivGTT. Baseline values show that all subjects in the study were insulin resistant (Table 1). Infliximab did not influence the indices, indicating that chronic TNF-α neutralization did not improve insulin sensitivity (Table 3). In fact, data for the primary endpoint (day 70) even indicated a borderline significant reduction in insulin sensitivity ($P = 0.05$) in the infliximab group.

Endothelium-dependent, flow-mediated and endothelium-independent, nitroglycerine-mediated vasodilation, and circulating markers of endothelial cell activation were evaluated to test a possible effect of TNF-α neutralization

Table 2. The effect of infliximab or placebo on acute phase proteins and vessel wall parameters (mean ± SEM)

	Infliximab				Placebo			
	Day 0	Day 70	Δ day 70 – day 0	P^a	Day 0	Day 70	Δ day 70 – day 0	P^a
hsCRP (μg/L)	2.8 ± 0.6	1.8 ± 0.5	–1.0 ± 0.18	0.02	1.5 ± 0.9	2.0 ± 0.5	0.6 ± 0.4	ns[b]
Fibrinogen (g/L)	3.42 ± 0.18	3.18 ± 0.28	–0.26 ± 0.15	0.037	3.37 ± 0.26	3.83 ± 0.14	–0.47 ± 0.20	ns
FMD[c] (%)	6.4 ± 1.5	4.9 ± 0.4	–1.5 ± 1.8	ns	6.6 ± 0.5	4.9 ± 1.2	–1.7 ± 1.5	ns
NMD[d] (%)	13.6 ± 2.5	13.4 ± 1.5	–0.2 ± 1.6	ns	15.8 ± 3.4	16.4 ± 3.7	0.7 ± 3.3	ns
sICAM-1 (μg/L)	40 ± 3	41 ± 4	1 ± 1.3	ns	39 ± 7	30 ± 6	–3 ± 2	ns
vWF (%)	139 ± 30	134 ± 5	7 ± 21	ns	99 ± 9	66 ± 8	33 ± 11	ns
PAI-1 (μg/L)	54 ± 10	55 ± 14	1 ± 7	ns	48 ± 8	53 ± 16	4 ± 8	ns

[a]*P* is for the changes between d 70 to d 0.
[b]ns: not significant.
[c]FMD: flow-mediated vasodilatation.
[d]NMD: nitroglycerine-mediated vasodilatation.

therapy on endothelial dysfunction. As shown in Table 2, none of the parameters were influenced by infliximab treatment.

DISCUSSION

This study shows that chronic TNF-α neutralization through repeated administration of the chimeric anti–TNF-α antibody infliximab does *not* improve insulin resistance or vascular function in moderately obese men with metabolic syndrome.

The proinflammatory cytokine TNF-α has multiple adverse effects on tissue metabolism, including insulin signaling and lipid handling. Hotamisligil and colleagues 15 years ago observed enhanced TNF-α expression in adipocytes of obese individuals, and showed that TNF-α interferes with the insulin action in obese animals (20). This effect has been suggested to be related to interference of the TNF-α signaling pathways with phosphorylation of insulin receptor substrate-1 (IRS-1) (21). From this data, it has been proposed that enhanced adipocyte TNF-α expression constitutes the link between obesity and insulin resistance.

Although the TNF-α hypothesis has found broad acceptance, a causative role of TNF-α in the development of insulin resistance in obesity is unclear with preclinical and clinical data. There are a number of reports showing that endogenous (that is, in mice lacking TNF-α or TNF receptor) (22,23) or exogenous (TNF-α neutralization) interference with TNF-α signaling ameliorates insulin resistance during development of obesity (7,24), whereas other reports using similar strategies have failed to show an effect of TNF-α neutralization on insulin sensitivity (25,26). The reason for this discrepancy is unclear, but it may reflect dietary differences or differences in genetic backgrounds of the mice studied.

The relevance of the TNF-α hypothesis for the human situation is equally unclear. Although studies in patients with chronic inflammatory conditions such as rheumatoid arthritis clearly show that quenching TNF-α activity improves insulin sensitivity, (7,8) a putative role of TNF-α in the development of insulin resistance in human obesity is unclear. There is no evidence for TNF-α release from human subcutaneous (27) and visceral adipose tissue (28) *in vivo*; thus challenging an endocrine link between adipose tissue and whole body insulin resistance. The effect of TNF-α neutralization on insulin sensitivity in patients with type 2 diabetes has been studied in both open label studies (10,12,29) and randomized, placebo-controlled trials (10,13). None of these studies indicated an appreciable effect of TNF-α neutralization on insulin sensitivity (11,13). Yet, with the exception of the 2004 study by Di Rocco (29), all studies referenced have been performed in prediabetic or overt diabetic patients. It is conceivable that

Table 3. The effect of infliximab or placebo on glucose metabolism (mean ± SEM)

	Infliximab				Placebo			
	Day 0	Day 70	Δ day 70 – day 0	P^a	Day 0	Day 70	Δ day 70 – day 0	P^a
Fasting glucose (mmol/L)	5.7 ± 0.4	5.4 ± 0.3	–0.1 ± 0.1	ns[b]	5.4 ± 0.2	5.4 ± 0.2	–0 ± 0.1	ns
Fasting insulin (μU/L)	15.9 ± 3.5	15.9 ± 4.2	–0.9 ± 4	ns	15.2 ± 2.7	13.9 ± 3.3	0.4 ± 1.6	ns
HOMA index	4.2 ± 1.2	3.8 ± 1.0	–0.2 ± 0.9	ns	3.7 ± 0.8	3.4 ± 0.8	0.1 ± 0.4	ns
Insulin sensitivity index (10^{-4} $min^{-1}(μU/mL)^{-1}$)	2.2 ± 0.4	1.8 ± 0.4	–0.4 ± 0.1	0.049	1.8 ± 0.5	1.8 ± 0.5	0.1 ± 0.2	ns

[a]*P* is for the changes between d 70 to d 0.
[b]ns: not significant.

such a condition reflects a situation that has become desensitized to TNF-α neutralization. Although the volunteers in Di Rocco (29) had normal fasting glucose values, they were all morbidly obese. Another point is that almost all studies thus far evaluated the (semi)acute effects of TNF-α neutralization, thus potentially not allowing sufficient time for normalization. Additionally, the human studies have been performed with different classes of TNF-α antagonist. It has been pointed out that the anti-TNF monoclonal antibodies (mAbs) (adalimumab and infliximab), appear more efficient than the soluble TNF receptors, thus resulting in differences in TNF-α neutralization (30,31).

To confirm or reject a role for the TNF-α as a putative link between adipose tissue and insulin resistance/type 2 diabetes, we tested the effect of long-term TNF-α neutralization through the chimeric monoclonal antibody infliximab on insulin resistance and vascular function in moderately obese men with preclinical signs of the metabolic syndrome, but no overt diabetes. We found that infliximab reduced systemic inflammation as reflected by a reduction of the acute phase proteins CRP and fibrinogen, a finding that also has been reported in other studies of TNF-α neutralization in the metabolic syndrome (11,13). Yet, infliximab treatment did not improve insulin resistance as shown by an unchanged HOMA index. In fact, the results for the ivGTT even suggest a borderline deterioration of insulin sensitivity on day 70. As this effect was not seen on day 3 and week 15, we assume that this deterioration reflects a type I statistical error. Similar to the data for insulin sensitivity, no improvement in endothelial-dependent vasodilation or endothelial activation markers could be observed upon TNF-α neutralization, a finding that is in-line with previous investigations with etanercept (11).

We suggest that the main reason for the discrepant effects of infliximab on insulin resistance and endothelial function between patients with rheumatoid arthri-

tis and patients with metabolic syndrome is that TNF-α has a clinically significant impact on insulin signaling in high grade inflammatory diseases, whereas it does not contribute to insulin resistance and vascular dysfunction in the metabolically triggered inflammation (metaflammation) (32) in obesity or diabetes.

In summary, our investigation shows that long-term TNF-α blockade in patients with the metabolic syndrome without overt diabetes reduces systemic inflammation, but does not improve insulin sensitivity or endothelial function. This study, along with the other human reports on TNF-α neutralization (10–13,29) brings to question whether in fact TNF-α is a causative link between adiposity and insulin resistance.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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