Effects of an Engineered Human Anti–TNF-α Antibody (CDP571) on Insulin Sensitivity and Glycemic Control in Patients With NIDDM

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Inhibition of tumor necrosis factor (TNF)-α action has recently been shown to reverse insulin resistance dramatically and to improve glycemic control in obese rodents. This double-blind study was designed to assess the effects of a recombinant-engineered human TNF-α–neutralizing antibody (CDP571) on glucose homeostasis in obese NIDDM patients. Glycemic control and insulin sensitivity were monitored in 21 NIDDM subjects for a 2-week run-in and then for 6 weeks after treatment in a randomized fashion with a single intravenous dose of either CDP571 (5 mg/kg) or an equivalent volume of normal saline. The prolonged half-life of the antibody ensured adequate plasma levels as measured throughout the study. Concentrations of fasting glucose (CDP571: 10.0 ± 0.8, 10.1 ± 0.8, 10.0 ± 1.0; placebo: 8.5 ± 0.6, 8.1 ± 0.5, 8.7 ± 0.8 mmol/l at baseline, day 1, and week 4, respectively), fasting serum insulin (CDP571: 21.2 ± 2.8, 21.0 ± 2.8, 24.8 ± 3.3; placebo: 19.0 ± 2.8, 20.8 ± 2.9, 17.5 ± 2.2 pmol/l, respectively), and C-peptide remained unaffected by the type of treatment throughout the study. The percentage rate of glucose clearance per minute (K_{ITT}) during intravenous insulin sensitivity tests was identical in the CDP571 and placebo groups at baseline and also at 1 and 4 weeks after treatment (mean ± SE; CDP571: 1.33 ± 0.21, 1.44 ± 0.25, 1.26 ± 0.18; placebo: 1.38 ± 0.15, 1.47 ± 0.20, 1.52 ± 0.20; P = 0.85, 0.93, and 0.36, respectively). TNF-α neutralization over a period of 4 weeks had no effect on insulin sensitivity in obese NIDDM subjects. ***Diabetes*** **45:881–885, 1996**

Although the etiology of NIDDM is still unclear (1), the pivotal role played by insulin resistance is well established (2). The precise cause of the abnormal response to insulin has escaped identification so far despite intensive research. However, recent studies into the molecular processes that mediate altered glucose homeostasis in obesity-diabetes syndromes have provided convincing evidence implicating tumor necrosis factor (TNF)-α, a polypeptide cytokine produced by activated immune cells in insulin-resistant states.

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CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; FBG, fasting blood glucose; FFA, free fatty acid; K_{ITT}, percentage rate of glucose clearance per minute; OHA, oral hypoglycemic agent; TNF, tumor necrosis factor.

Adipose tissue TNF-α protein production has been reported to be significantly higher in NIDDM patients than in IDDM patients and normal volunteers (3). Moderate and sustained systemic (circulating) levels of TNF-α have been shown to correlate with insulin resistance in patients with cancer and chronic sepsis (4,5). TNF-α mRNA is overexpressed in adipose tissues, relative to other tissues, of multiple genetic animal models of obesity, diabetes, and insulin resistance but not in their lean littermates or in other animal models of chemically induced diabetes or obesity, where insulin resistance is not a characteristic feature (6,7). In these experimental animal models, the amounts of local and circulating TNF-α protein have also been found to correlate with the mass of adipose tissue (6).

It has been suggested from other studies in experimental animals that TNF-α induces insulin resistance by interference with the phosphorylation cascades of the insulin receptor β-subunit and insulin receptor substrate–1, thereby altering the transmembrane signaling that is essential for insulin action in various insulin-sensitive tissues (8,9,10). Other studies have demonstrated either impaired function or depletion of GLUT4, the insulin-sensitive glucose transporter, in adipocyte cultures and human muscle biopsies after chronic exposure to TNF-α (6,11,12), suggesting a further mechanism by which TNF-α may induce insulin resistance.

In two studies by Hotamisligil and colleagues (6,10), in one rodent model of obesity, hyperinsulinemia and insulin resistance bring into focus the therapeutic potential for TNF-α neutralization in human diabetes. These studies demonstrated improvement in insulin sensitivity (6) and dramatic reductions in plasma insulin, glucose, and nonesterified fatty acid levels (10) in obese, as compared with lean, rats after intravenous administration of a recombinant TNF-α receptor antibody. However, interspecies differences can occur, and it remains to be shown that similar results can be obtained in human trials. Nonetheless, a number of studies leave no doubt that there are good reasons to anticipate therapeutic possibilities for the modulation of TNF-α status (3,4,13).

Our purpose in carrying out this study therefore was to assess the effects of an engineered human TNF-α antibody (CDP571) on glucose homeostasis in obese individuals with NIDDM. CDP571 has previously been given to animals, healthy volunteers, and patients with endotoxic shock, rheumatoid arthritis, and inflammatory bowel disease with no significant adverse events (14,15). We report the conclusions of a double-blind evaluation of the effects of antibody-mediated TNF-α neutralization on insulin sensitivity and glucose control in obese subjects with NIDDM.

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TABLE 1
Summary of patient characteristics

	CDP571	Placebo
No. of patients	10	11
Age (years)	61.9 ± 1.8	59.2 ± 2.2
Duration of NIDDM (years)	8.9 ± 1.2	7.6 ± 2.0
Sex (M:F)	7:3	6:5
Weight (kg)	93.6 ± 4.1	89.9 ± 2.8
BMI (kg/m^2)	32.1 ± 0.7	32.4 ± 1.2

Data are means ± SE.

RESEARCH DESIGN AND METHODS

The study was approved by the Joint Ethics Committee of the Newcastle and North Tyneside Health Authorities and the University of Newcastle and was conducted at the Royal Victoria Infirmary, Newcastle upon Tyne, U.K.

CDP571 was provided as a clear solution in vials of 5.1 mg/ml (Celltech Therapeutics, Slough, U.K.). CDP571 was generated from a mouse monoclonal antibody raised against recombinant TNF-α. The complementarity determining regions, together with additional framework residues required to maintain conformational integrity, were transferred into the framework of a human antibody with human γ4 constant and κ light chains, as described previously (14). The engineered antibody retains the full-binding activity for TNF-α, as assessed by competition enzyme-linked immunosorbent assay (ELISA) and neutralizes TNF-α in vitro and in vivo. The plasma half-life in humans is over 1 week.

The patients with NIDDM (n = 21) gave written consent to participate in the study, which was a double-blind placebo-controlled trial with a parallel design. Patients selected for entry had diabetes of >2 years duration and were treated with diet only or with the addition of oral hypoglycemic agents (OHAs). Where an OHA was taken, the dose was unchanged for at least 2 months before entry into the run-in phase and throughout the entire study. No patients had intercurrent infection, renal or liver dysfunction, a history of asthma, or clinically important allergies. Previous organ transplantation, malignancy, and pregnancy or intended pregnancy during the study were exclusion factors. Prohibited concurrent or recently used medications were insulin, glucocorticoids, and pentoxifylline (13) in view of their known effects on glucose homeostasis and on TNF-α expression. Subjects randomized to the CDP571 and placebo treatment groups were suitably matched with respect to age, body weight, BMI, and duration of NIDDM (Table 1).

The study was made up of a 2-week run-in phase and a 6-week treatment phase (Fig. 1). Patients were assessed after an overnight fast and abstained from medication on the morning of tests on eight separate occasions. Study visit assessments for each patient included a standard physical examination at the screening and final visit, routine urinalysis, and blood samples for hematological and biochemical parameters.

Fasting blood glucose (FBG) was measured on venous whole blood at weekly intervals during the run-in phase. Only patients with stable FBG values (not varying by >25%) on three consecutive occasions and who continued to fulfill the entry criteria were randomized to receive either CDP571 or placebo and entered into the 6-week double-blind phase. Randomization was carried out after stratification of patients into two groups according to BMI (group A, BMI = 27–35 kg/m^2 and group B, BMI = 35.1–42 kg/m^2) to limit any marked imbalance in patient weights between treatment groups. On entering the treatment phase, each subject received a single dose (5 mg/kg body weight) of either 5.1 mg/ml undiluted CDP571 (equivalent to 0.98 ml/kg body weight) or an equivalent volume of 0.9% (wt/vol) saline through an antecubital vein by slow infusion over ~1 h. Both subject and investigator were blinded to the nature of the infusion. Vital signs were monitored regularly during and for 2 h after the infusion.

Each patient underwent a short insulin sensitivity test immediately before the CDP571 infusion and 1 and 4 weeks after the infusion (16). Data from short insulin sensitivity tests correlate highly with euglycemic-hyperinsulinemic clamp studies (16). A dose of 0.1 U/kg body weight of human Actrapid insulin (Novo-Nordisk, Denmark) was given intravenously as a bolus in a volume of 2 ml and flushed through the cannula with 5 ml of saline. All blood samples taken during the insulin sensitivity test were arterialized samples taken from a wrist vein, the hand being maintained at 50°C in a heated box (17). Blood samples for glucose determinations were taken basally and every minute for 15 min after the intravenous injection of insulin, and those for serum insulin, free fatty acids (FFAs), and intermediary metabolites were taken basally and at 5-min intervals. Samples for C-peptide, catecholamines, and glucagon were taken basally and at 15 min.

Blood samples were taken at all study visits for fasting glucose, insulin, C-peptide, catecholamines, glucagon, and metabolites. Blood samples for measurement of CDP571 concentrations and anti-CDP571 antibody levels were taken just before and after the administration of the study medication and thereafter at each study visit. All plasma samples were kept frozen at −20°C (−40°C for C-peptide) until assayed.

Blood glucose concentrations were measured on whole blood by the glucose oxidase method using a Yellow Springs glucose analyzer (Yellow Springs, Yellow Springs, OH; interstudy coefficient of variation [CV], 3.5%). Blood for intermediary metabolites was collected into tubes containing perchloric acid precooled to 0°C and subsequently analyzed using a Cobas Bio centrifugal analyzer fitted with a fluorimetric attachment (interassay CV, 6.0%) (18). Serum insulin concentration was determined by ELISA (interassay CV, 5.0%) (19) and C-peptide (3-h human C-peptide radioimmunoassay kit, antiserum K6, Novo Nordisk A/S, Denmark) (20) and glucagon (21) by radioimmunoassay (interassay CV, 6.2% and 9.5%, respectively). Epinephrine and norepinephrine were measured by high pressure liquid chromatography (interassay CV, 12.4% and 7.6%, respectively) (22) and FFA by centrifugal enzymatic analysis (interassay CV, 3.7%) (23). CDP571 levels were measured in an ELISA using TNF-α coated microtiter plates and an anti-human IgG4 second antibody. Anti-CDP571 antibodies were measured using a double antigen sandwich ELISA (14). Total TNF-α was measured using an ELISA assay (Medgenix Diagnostics, Fleurous, Belgium). To determine CDP571 activity in vivo, plasma samples containing CDP571 were titrated into the L929 cytotoxic bioassay in the presence of a fixed (200 pg/ml) concentration of TNF-α, and data was expressed as the concentration of CDP571 able to inhibit 90% of TNF-α cytotoxic activity. Because p55 TNF-α soluble receptors in the pretreatment plasma samples were within the normal range (1–5 ng/ml) and a previous study has shown no change in p55 receptor levels after CDP571 infusion (data on file), soluble receptor levels in plasma were not measured in postinfusion samples.

The slope of regression lines for blood glucose concentrations during the insulin sensitivity tests were calculated for each subject and are expressed as a percentage (K_{ITT}). Numeric data are presented as means ± SE. The significance of differences between the treatment groups was assessed by Student's t test.

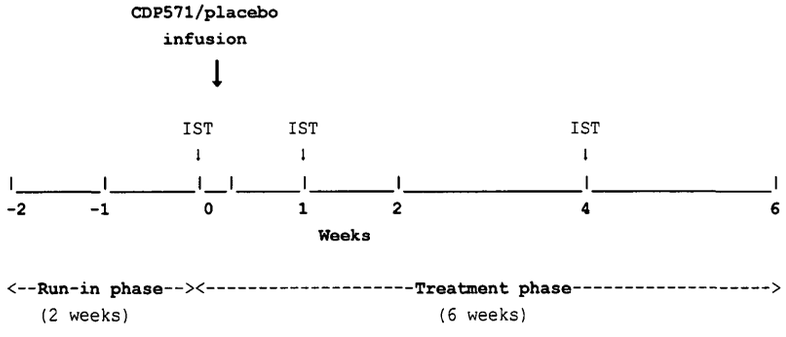

FIG. 1. Study protocol.

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TABLE 2
Insulin sensitivity measured by short intravenous sensitivity test and fasting plasma insulin immediately before the TNF neutralizing antibody infusion (week 0), 1 week later, and 4 weeks later

	Week 0	Week 1	Week 4
K_{ITT} (mmol · l^{-1} · min^{-1})			
CDP571	1.33 ± 0.21	1.44 ± 0.25	1.26 ± 0.18
Placebo	1.38 ± 0.15	1.47 ± 0.20	1.52 ± 0.20
Fasting plasma insulin (μU/l)			
CDP571	21.2 ± 2.8	21.0 ± 2.8	24.8 ± 3.3
Placebo	19.0 ± 2.8	20.8 ± 2.9	17.5 ± 2.2

Data are means ± SE.

RESULTS

All patients who entered the run-in phase of the study completed the trial. No significant adverse events related or unrelated to CDP571 were reported.

FBG did not change during the study in either the CDP571 or placebo group, and at no time was there a significant difference between the groups (Fig. 1). Similarly, fasting plasma insulin did not change during the study and did not differ between the groups (Fig. 1). Fasting C-peptide data confirmed the lack of difference in insulin secretion rates (1.03 ± 0.09 vs. 0.98 ± 0.13, 1.02 ± 0.08 vs. 1.02 ± 0.14, and 1.07 ± 0.10 vs. 1.01 ± 0.13 for CDP571 and placebo at day 0, day 1, and week 4, respectively). The percentage rate of glucose clearance per minute (K_{ITT}) during intravenous insulin sensitivity tests was identical in the CDP571 and placebo groups at baseline and also at 1 and 4 weeks after treatment (Table 2).

As an index of adipose tissue insulin sensitivity, fasting plasma FFA levels were assessed. No change was observed in either group during the study and there were no significant differences between groups at any point (Fig. 3). Additionally, the rate of suppression of FFA after intravenous insulin was almost identical between the two groups before CDP571 or placebo administration and also 1 week and 4 weeks later (Fig. 2). Plasma catecholamines and glucagon levels were not different during these tests (Table 3).

Plasma levels of CDP571 antibody were maximal immediately after the infusion (122 μg/ml), and the antibody was eliminated with a half-life of ~1 week (Table 4). Anti-idiotype antibodies became detectable 2 weeks after the infusion but did not increase the clearance rate of CDP571. Total immunoreactive plasma TNF-α levels did not change in the placebo group before and after infusion (16.4 ± 1.7 vs. 13.1 ± 1.8 pg/ml; $P > 0.05$), whereas in the CDP571 infusion group, plasma TNF-α levels fell from 22.0 ± 4.9 to below the limit of detection of the assay (2 pg/ml) in 8 of the 10 subjects. In the two subjects whose plasma levels remained detectable, concentrations fell from 38.8 to 11.1 and 20.3 to 4.0 pg/ml. To determine whether the anti–TNF-α antibody remained biologically active in vivo, plasma samples were titrated into the cytotoxic bioassay in the presence of a fixed concentration of TNF-α. The concentration of CDP571 required to inhibit 90% of the TNF effect was 65 and 70 ng/ml in separate assays on a CDP571 standard, 65.2 ± 4.2 ng/ml in the plasma samples taken 1 h after the infusion, and 68.1 ± 8.5 ng/ml in the plasma samples taken 7 days later. The antibody thus retained full bioactivity in vivo.

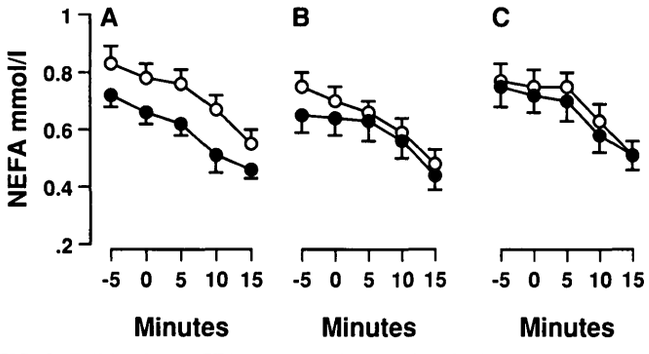

FIG. 2. Fall in plasma FFA concentrations during the intravenous insulin sensitivity tests before CDP571 (●) or placebo (○) (*A*), 1 week later (*B*), and 4 weeks later (*C*). Insulin (0.1 U/kg) was injected at time 0.

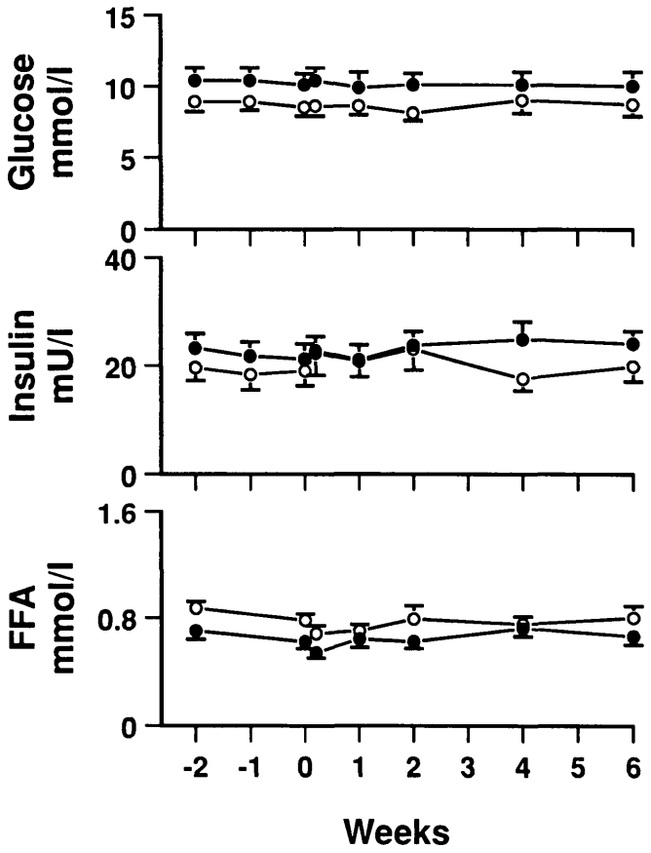

FIG. 3. Sequential measurements of FBG, fasting plasma insulin, and fasting plasma FFA during the study for the CDP571 (●) and placebo (○) groups.

Serum total cholesterol and triglycerides remained unchanged over the 6-week treatment phase in both groups of patients. No abnormal results were obtained throughout the study from urinalysis and routine hematology and biochemistry tests.

DISCUSSION

This study is the first to assess the effects of TNF-α neutralization on glucose homeostasis in humans. CDP571 given parenterally as an infusion of 5 mg/kg body weight did not change insulin sensitivity of either glucose or fat metabolism. The NIDDM subjects were insulin resistant, the K_{ITT} being ~30% of a previously reported normal group (16). The study was designed to measure change in insulin sensitivity over a period of 1–4 weeks, during which antibody levels were known to remain elevated. It is unlikely that shorter term

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TABLE 3
Catecholamine and glucagon levels during the insulin sensitivity tests in the CDP571 and placebo groups

	Test 1			Test 2			Test 3		
	−15	0	+15	−15	0	+15	−15	0	+15
Epinephrine (nmol/l)									
CDP571	0.23 ± 0.03	0.20 ± 0.02	0.27 ± 0.04	0.24 ± 0.03	0.26 ± 0.05	0.26 ± 0.02	0.26 ± 0.03	0.23 ± 0.03	0.20 ± 0.03
Placebo	0.22 ± 0.02	0.24 ± 0.04	0.25 ± 0.03	0.24 ± 0.02	0.27 ± 0.04	0.24 ± 0.04	0.19 ± 0.02	0.20 ± 0.02	0.22 ± 0.03
Norepinephrine (nmol/l)									
CDP571	1.76 ± 0.16	1.67 ± 0.18	1.89 ± 0.25	1.45 ± 0.14	1.44 ± 0.15	1.80 ± 0.17	1.61 ± 0.17	1.56 ± 0.18	1.73 ± 0.19
Placebo	1.51 ± 0.18	1.54 ± 0.20	1.87 ± 0.23	1.54 ± 0.16	1.63 ± 0.24	1.85 ± 0.23	1.72 ± 0.22	1.60 ± 0.20	1.85 ± 0.30
Glucagon (pg/ml)									
CDP571	115 ± 10	120 ± 11	100 ± 7	133 ± 19	121 ± 16	107 ± 13	137 ± 20	129 ± 19	124 ± 12
Placebo	96 ± 11	100 ± 13	86 ± 12	105 ± 17	103 ± 16	82 ± 12	123 ± 16	109 ± 13	100 ± 8

Data are means ± SE.

effects were missed because the plasma glucose and insulin levels on the day after CDP571 administration were unchanged. These data were unexpected in view of the convincing data from studies on animals, and three major questions must be addressed. First, was the dose of neutralizing antibody sufficient? Second, was TNF-α neutralization in the appropriate site achieved? Third, is there a major species difference in the role of TNF-α in modulating insulin sensitivity?

Measurement of plasma CDP571 levels confirmed that the half-life was as previously reported (14), and the dose administered achieved adequate plasma levels in the context of the study design. The affinity of CDP571 for TNF-α would ensure that plasma TNF-α concentration was effectively zero. The biological effects of in vivo neutralization of TNF-α by CDP571 have been demonstrated on rheumatoid arthritis disease activity in humans (15). In the latter study, a significant beneficial effect was noted after single intravenous doses as low as 1.0 mg/kg, five times less than in the present study. It is relevant to record that in the present study, one patient with osteoarthritis reported marked reduction in bilateral knee pain after placebo or CDP571 infusion and was able to walk without a stick for several weeks. He was subsequently found to have received CDP571. However, this improvement in symptoms was not objectively assessed and cannot be commented on further. The lack of any relevant effects in our study patients makes it unlikely that a doubling or even quadrupling of the dose of CDP571 administered would produce useful effects on insulin sensitivity.

To prevent TNF-α action, delivery of the cytokine to its receptor site must be interrupted. It is clear that effective plasma neutralizing antibody concentrations were achieved in the present study and that the IgG antibody used would diffuse readily into the extracellular fluid compartment. However, if the TNF-α was being produced in close proximity to its presumed site of action in muscle tissue, antibody circulating in plasma and extracellular fluid may not be fully effective in this microenvironment. If this was so, it is clear that TNF-α could not be acting as an endocrine factor produced by adipose tissue and targeted at muscle. Alternatively, if TNF-α interacted with the insulin-signaling pathway in a relatively inaccessible microenvironment, or even intracellularly, the present data would not be incompatible with a role for TNF-α in the regulation of insulin sensitivity in humans. The beneficial effect on carbohydrate metabolism of pentoxifylline, a drug known to act through inhibition of TNF-α production (24), rather than by interaction after release of the cytokine from production sites would lend support to this hypothesis.

Hotamisligil et al. (6) observed peripheral glucose utilization to increase in obese rats after daily administration for 3 days of a recombinant soluble TNF-α receptor immunoglobulin G chimeric protein. The measurements of insulin sensitivity indicated improvement in muscle insulin sensitivity. Not all muscle-like cell lines respond to TNF-α. L6 myoblasts have been reported to exhibit no change in insulin sensitivity in culture during TNF-α exposure, whereas in the same study, 3T3-L1 adipocytes were observed to have lower rates of glucose transport after incubation with TNF-α (25). TNF-α mRNA overexpression in adipose tissue has previously been reported from animal experiments (6). A recent study in human subjects also suggests similar findings (26). However, the extent to which this overexpression occurs differs in obese animals and humans, being five- to tenfold in animals and only 2.5-fold in humans. Furthermore, in the rodent models, circulating levels of TNF-α protein are measurable, albeit at low concentrations, and correlate with adipose tissue mass and TNF-α mRNA expression, whereas in human subjects, systemic levels of TNF-α protein are reported to be extremely low (26), a finding confirmed in the present study.

TABLE 4
Plasma levels of CDP571 and endogenous anti-CDP571 antibody

	Week 0 before infusion	Week 0 after infusion	Week 1	Week 2	Week 4	Week 6
Plasma CDP571						
CDP571	0.05 ± 0.04	125.1 ± 9.2	27.9 ± 4.0	17.8 ± 1.5	4.6 ± 1.2	0.8 ± 0.4
Placebo	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Plasma anti-CDP571						
CDP571	0.17 ± 0.05	—	0.10 ± 0.01	0.89 ± 0.32	0.71 ± 0.13	1.32 ± 0.42
Placebo	0.15 ± 0.05	—	0.14 ± 0.04	0.15 ± 0.05	0.14 ± 0.04	0.14 ± 0.04

Data are means ± SE.

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This lack of complete comparability between animal and human studies may be an indicator of subtle species differences, which are nonetheless important and could account for the absence of an effect of TNF-α neutralization in NIDDM subjects.

In summary, antibody-mediated neutralization of TNF-α over a period of 4 weeks has no effect on insulin sensitivity in obese NIDDM subjects. There may be important species differences in the role of TNF-α in modulating insulin sensitivity. Further detailed work on TNF-α effects in tissues from NIDDM subjects is required before the relevance of the experimental animal to humans becomes clear. Direct neutralization of plasma TNF-α in humans causes no change in insulin sensitivity.

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