Targeted Disruption of the Tumor Necrosis Factor-α Gene

Metabolic Consequences in Obese and Nonobese Mice

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To address the hypothesis that tumor necrosis factor (TNF)-α has a role in obesity-associated insulin resistance or the regulation of in vivo lipid metabolism, mice with targeted disruption of the TNF-α gene were generated and studied. The absence of TNF-α protein in TNF-null (–/–) mice was confirmed. Lean or obese (gold-thioglucose [GTG]-injected) homozygous (–/–) mice were compared with lean or obese age- and sex-matched wild-type (+/+) mice derived from the same line at 13, 19, and 28 weeks of age. The following parameters were significantly affected in lean –/– versus +/+ mice: Body weight was not affected until week 28 (decreased by 14%); epididymal fat pad weight also decreased (25%) at this time, as did percentage body fat (16%), while percentage body protein was increased 13%. Fed plasma insulin levels decreased 47% (28 weeks), triglyceride levels decreased (all three ages; maximum 35% at 19 weeks), and fed plasma leptin decreased 33% (28 weeks). Fasting glucose was slightly (10%) reduced, but the glucose response to an oral glucose tolerance test (OGTT) was not affected. There was a trend (NS) toward increased total adipose tissue lipoprotein lipase in –/– versus +/+ mice. GTG-treatment resulted in obese –/– and +/+ mice with equal mean body weights (42 and 58% increased weight versus lean mice). The following parameters were significantly different in obese –/– mice: fasting plasma glucose decreased 13% (28 weeks), fed plasma insulin decreased 67% (28 weeks), and insulin response to OGTT was decreased by 50%. For both groups of obese mice, glucose levels during the OGTT were substantially increased compared with those in lean mice; however, mean stimulated glucose levels were 20% lower in obese –/– versus +/+ mice. We conclude *1*) that TNF-α functions to regulate plasma triglycerides and body adiposity and *2*) that although TNF-α contributes to reduced insulin sensitivity in older or obese mice, the absence of TNF-α is not sufficient to substantially protect against insulin resistance in the GTG hyperphagic model of rodent obesity. *Diabetes* 46:1526–1531, 1997

Tumor necrosis factor (TNF)-α is a catabolic proinflammatory cytokine that is also strongly implicated in the regulation of lipid metabolism, adipocyte differentiation, and in vivo insulin sensitivity (1,2). The role of TNF-α as a potential mediator of cachexia with associated hyperlipidemia has been studied extensively (1); however, the relative effects of TNF-α to stimulate hepatic lipogenesis versus to suppress lipoprotein lipase (LPL) as well as its potential effect to suppress appetite remain unresolved (2). Using in vitro experimental systems such as isolated adipocytes or cultured 3T3-L1 adipocytes, TNF-α has been shown to suppress potently the expression of several genes that normally regulate fatty acid uptake or lipogenesis. These include LPL, acetyl-CoA carboxylase, and glycerophosphate dehydrogenase (3,4). Moreover, TNF-α is a potent negative regulator of preadipocyte differentiation and may act to induce in vitro de-differentiation of 3T3-L1 adipocytes (3). These negative effects of TNF-α on adipogenesis correlate with marked suppression of other genes specifically expressed in adipocytes, such as adipocyte fatty acid binding protein (aP2), adipsin, and the insulin-responsive glucose transporter (GLUT4) (3,5). The mechanism that underlies the anti-adipogenic effects of TNF-α is incompletely understood but is likely to involve downregulation of two transcription factors implicated as positive regulators of adipocyte differentiation: CCAAT-enhancer binding protein (C/EBP)α and PPARγ (peroxisome proliferator–activated receptor-γ) (3,5). A potential role for TNF-α in modulating adiposity was further suggested by the finding of linkage between the TNF-α gene locus and obesity in Pima Indians (6).

In addition to being expressed in macrophages, TNF-α is expressed in fat. Importantly, the level of adipose tissue TNF-α is substantially increased in obesity in both rodents and humans, where levels correlate with hyperinsulinemia (2,7). This finding led to the hypothesis that adipocyte-derived TNF-α might cause or contribute to obesity-associated insulin resistance and type 2 diabetes (2). The most compelling data in favor of this hypothesis was provided by Hotamisligil et al. (8), who showed that in vivo neutralization of TNF-α ameliorated insulin resistance in Zucker fatty rats.

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aP2, adipocyte fatty acid binding protein; GTG, gold thioglucose; IRS-1, insulin receptor substrate-1; LPL, lipoprotein lipase; OGTT, oral glucose tolerance test; TNF, tumor necrosis factor.

In addition, infusion of TNF-α into animals and humans has been reported to result in reduced insulin sensitivity (9). More recent data also suggest that TNF-α may be overexpressed in skeletal muscle of human subjects with insulin resistance (10). The mechanism or mechanisms by which TNF-α may contribute to insulin resistance remain incompletely understood. In addition to downregulating GLUT4 expression in adipocytes (5), treatment of cultured adipocytes (9) or hepatoma cells (11) with TNF-α inhibited proximal steps in insulin signaling, including insulin receptor autophosphorylation and tyrosyl phosphorylation of insulin receptor substrate-1 (IRS-1). These effects may in part be due to increased serine phosphorylation of IRS-1 (12) and the activation of neutral sphingomyelinase (13), a pathway linked to TNF-α receptor–mediated cellular signaling.

In spite of the compelling evidence in favor of a role for TNF-α in the regulation of in vivo adiposity, lipid metabolism, or insulin sensitivity, no attempt has been made to directly address these issues through the use of mice that lack this important cytokine. We used a classic gene-targeting approach to generate mice deficient in TNF-α. By specifically disrupting the TNF-α gene without affecting the adjacent lymphotoxin-α locus, mice that lack only TNF-α were generated (14). Apart from the predicted resistance to endotoxin toxicity and increased sensitivity to experimental *Listeria monocytogenes* infection, homozygous TNF-α–null mice were found to be viable and fertile, with no overt phenotypic abnormalities (14). In the present study, we assessed several metabolic parameters in TNF-α–null (–/–) versus matched wild-type (+/+) control mice. In addition, at the time of weaning, mice were treated with gold thioglucose (GTG), which causes chemical ablation of the ventromedial hypothalamus, resulting in the induction of hyperphagic experimental obesity (15). Since both +/+ and –/– mice achieved substantial and equal degrees of obesity, we were able to study the impact of obesity on in vivo insulin and glucose homeostasis in both sets of mice.

RESEARCH DESIGN AND METHODS

Mouse breeding, initial characterization, and generation of experimental obesity. Homozygous F_1 wild-type (+/+) and TNF-α–null (–/–) mice were derived from intercrossed heterozygous (+/–) mice that were present on a C57Bl/6 × 129 hybrid strain background (14). Homozygous +/+ or –/– littermate mice were subsequently inbred for one generation in parallel to generate adequate numbers of mice for the present study. After weaning at 3 weeks of age, mice were housed one (males) or three (females) mice per cage in a sterile environment and were provided ad libitum access to food (breeder's diet) and water. The +/+ versus –/– genotype of mice used for further studies was verified by Southern blot analysis using genomic DNA, obtained from tail snips, which was digested with *Eco*RI and probed with an *Sca* I–*Eco*RI fragment of murine TNF-α as described previously (14). To confirm the absence of TNF-α protein in –/– mice, representative +/+ and –/– mice received a single intraperitoneal injection of 1 mg/kg lipopolysaccharide (LPS) (type 0111:B4; Sigma) or vehicle. Ninety minutes postinjection, the mice were anesthetized and blood was removed by cardiac puncture. Plasma was collected and assayed for the presence of murine TNF-α by enzyme-linked immunosorbent assay with the Cytoscreen Immunoassay Kit (BioSource International, Camarillo, CA). To induce experimental obesity, mice were given a single intraperitoneal injection of 0.5 mg/g body wt of GTG (Sigma) at 5 weeks of age (15).

Assessment of plasma parameters, oral glucose tolerance testing, body composition analysis. Blood was collected from snipped tail ends at 13 and 19 weeks (male) or 19 and 32–34 weeks (female) of age and via cardiac puncture at 28 weeks (male) for determination of plasma levels of glucose, total free fatty acids, triglycerides, insulin, and leptin. Plasma levels of glucose, triglycerides, and free fatty acids were measured with colorimetric assay kits using glucose oxidase (Sigma), glycerol kinase (Boehringer Mannheim Biochemica), and acyl-CoA oxidase (Boehringer Mannheim Biochemica), respectively. Insulin and leptin were measured with radioimmunoassay kits from Linco (St. Charles, MO) using rat insulin and mouse leptin as standards. Oral glucose tolerance tests (OGTTs) were performed in conscious mice at 19–23 weeks of age after a 6-h fast by administering 3 g glucose/kg body wt via oral gavage dosing. Blood samples were obtained via the tail vein before and 45 and 90 min after glucose dosing. Selected male mice were anesthetized by Nembutal injection (50 mg/kg i.p.) for cardiac blood collection, removal and weighing of epididymal fat pads, and complete carcass digestion by alcoholic potassium hydroxide hydrolysis for subsequent determination of total body triglycerides and protein as previously described (16). Total LPL activity was determined in homogenates of epididymal adipose tissue as previously described (17). Insulin-stimulated [^{14}C]glucose incorporation into glycogen was measured using primary cultured epididymal adipose tissue as previously described (18). Student's *t* test was used for statistical comparisons.

RESULTS

The absence of TNF-α in knockout mice was confirmed by measurement of plasma TNF-α protein after intraperitoneal administration of LPS. High levels (550–4,000 pg/ml) were readily detected in representative +/+ mice, and TNF-α was undetectable in LPS-treated –/– mice. TNF-α mRNA was also not detected in cells from –/– mice but was measurable in +/+ cells (14). Furthermore, TNF-α bioactivity was present in plasma or macrophages from +/+ mice, but could not be detected in –/– mice (14). Physical and metabolic parameters were primarily determined using male mice. Selected parameters were also measured using a smaller number (n = 10–13 for each group) of female mice. Southern blotting was used to confirm the genotype of +/+ versus –/– mice used for the present studies, as previously described (14).

Measurement of body weight using nonobese male mice suggested a trend (nonsignificant) toward lower mean weight in –/– mice at ages 13 and 19 weeks. At 28 weeks, –/– mice weighed 14% less than +/+ mice (Table 1). Mean epididymal fat pad weight in these –/– mice was 24% less than in +/+ mice. Although the ratio of fat pad weight to total body weight was not significantly different (not shown), total body triglycerides were modestly decreased (by 16%) in the 28-week-old –/– mice (Table 1). There was also a slight (13%) reciprocal increase in total body protein in –/– mice. Nonobese –/– female mice did not display reduced body weight at 19 weeks, but failed to gain substantial weight with advancing age (weighing 21% less than +/+ mice at 32–34 weeks; P = 0.03). GTG treatment at the time of weaning induced obesity in both +/+ and –/– male mice, with 41 and 58% increases, respectively, in mean body weight compared with nonobese mice. As shown in Table 1, obese –/– and +/+ mice had similar mean body and fat pad weights. At 19 and at 32–34 weeks, both groups of GTG-treated obese female mice weighed substantially (>40%) more than nonobese females; mean body weights of –/– and +/+ obese females were nearly identical (48.7 ± 4 vs. 48.4 ± 1 g, respectively, at 19 weeks).

At 28 weeks of age, nonobese male –/– mice displayed lower levels of insulin, triglyceride, and leptin compared with +/+ mice (Table 2). At this age, there was also a modest (10%) decrease in fasting plasma glucose in –/– mice. As shown in Fig. 1*A*, there was a trend toward lower plasma insulin levels in –/– mice at 13 and 19 weeks of age; mean levels were 46% lower than those in +/+ mice at 28 weeks. Plasma triglycerides were decreased in –/– mice at 13 weeks (16%) and 19 weeks (37%), as well as at 28 weeks (26%) (Fig. 2*A*). Given the differences in plasma triglycerides noted above, total LPL activity in epididymal adipose tissue was measured in nonobese male mice; a trend (NS) toward increased (18%)

TABLE 1
Physical characteristics of wild-type versus TNF-null mice

	Nonobese		GTG-obese	
	+/+	–/–	+/+	–/–
Body weight (g)	41.1 ± 1.3	35.3 ± 1.1†	58.4 ± 2.0	55.7 ± 1.4
Fat-pad weight (g)	1.60 ± 0.13	1.21 ± 0.11*	2.26 ± 0.11	2.49 ± 0.17
% body fat	28.5 ± 1.2	23.8 ± 1.9*	ND	ND
% body protein	10.7 ± 0.20	12.1 ± 0.36†	ND	ND

Physical parameters were assessed using 28-week-old male mice: nonobese wild-type (+/+, n = 26) or TNF-null (–/–, n = 22); GTG-obese +/+ (n = 14) or –/– (n = 15). Epididymal fat-pad weight was determined. Percentage body fat, percentage body protein (grams total triglyceride or protein content per gram body weight) were determined by carcass analysis. * $P < 0.05$ vs. +/+; † $P < 0.01$ vs. +/+. Similar effects on body weight were seen in female mice (see text).

mean enzyme levels was observed in –/– compared with +/+ mice. Plasma leptin values were 33% lower in –/– mice at 28 weeks of age. No differences in insulin or triglycerides were noted in nonobese –/– female mice at 19 weeks. However, at 32–34 weeks, significant reductions in mean fed insulin and triglyceride levels, similar to those observed in male mice, were noted.

Compared with nonobese male –/– and +/+ mice, corresponding groups of obese mice displayed only mildly increased fasting and fed plasma glucose levels (Table 2). However, mean fasting glucose was decreased by 14% in obese –/– mice versus obese +/+ mice at 28 weeks. GTG-induced obesity resulted in substantial increases in mean plasma insulin (19-fold and 11-fold, respectively) and leptin (2.4-fold and 4-fold, respectively) levels for 28-week-old +/+ and –/– mice (Table 2). Despite nearly identical mean body and fat pad weights, mean insulin levels in –/– obese mice were 67% lower than those in +/+ obese mice, a finding that was also apparent at 13 and 19 weeks (Table 2; Fig. 1*B*). To directly assess tissue insulin sensitivity, primary cultured epididymal adipose tissue from nonobese or obese +/+ and –/– mice was incubated with or without 10 and 100 mU/ml insulin, followed by determination of [^{14}C] glucose incorporation into glycogen. Insulin (10 mU/ml) stimulated glycogen synthesis by ~200% in both groups of nonobese mice (n = 3, each in triplicate). In contrast, adipose tissue from both +/+ and –/– obese mice (n = 6 mice for each group, each in triplicate) was substantially resistant to insulin (55 vs. 60% stimulation, respectively, at 10 mU/ml, NS; 78 vs. 100% stimulation, respectively, at 100 mU/ml, NS).

Plasma triglyceride levels were modestly increased in both groups of male obese mice compared with nonobese mice; however, mean levels were significantly decreased in –/– mice only at the age of 19 weeks (Fig. 2*B*). The absence of TNF-α did not decrease mean leptin levels in obese mice as it did in nonobese mice. In addition, no significant differences in mean free fatty acid values between –/– and +/+ were noted for either the nonobese or obese groups (Table 2). Plasma insulin levels in both groups of obese female mice were substantially (~15-fold) elevated relative to those in nonobese females. No differences in insulin levels between –/– and +/+ obese females were noted until 32–34 weeks, at which point mean values in –/– mice were 52% lower (P = 0.04).

OGTTs were performed using groups of conscious –/– and +/+ age-matched (19–23 weeks) nonobese and obese male mice. As shown in Fig. 3*A*, mean fasting and stimulated plasma glucose levels did not differ significantly between nonobese –/– and +/+ mice. Fasting glucose levels were significantly higher in obese +/+ mice than in obese –/– mice (P = 0.02); however, both groups of obese mice displayed substantially impaired glucose tolerance relative to lean mice. Stimulated (45-min) glucose values were modestly (20%) reduced in –/– versus +/+ obese mice (P = 0.03), and the mean area under the curve was decreased (15%) in –/– versus +/+ mice ($P < 0.05$). Plasma insulin levels were determined

TABLE 2
Measured plasma parameters

	Nonobese		GTG-obese	
	+/+	–/–	+/+	–/–
Fasting glucose (mg/dl)	171 ± 8.6	154 ± 5.9*	211 ± 10.3	183 ± 7.8*
Fed glucose (mg/dl)	217 ± 7.7	204 ± 7.4	225 ± 11	213 ± 8.4
Insulin (ng/ml)	3.77 ± 0.58	2.05 ± 0.49*	71.3 ± 18.9	23.3 ± 9.4*
Triglycerides (mg/dl)	123 ± 10	91 ± 10*	156 ± 17	130 ± 16
Free fatty acids (mmol/l)	0.70 ± 0.04	0.70 ± 0.07	0.53 ± 0.11	0.42 ± 0.08
Leptin (ng/ml)	33.1 ± 2.9	22.3 ± 3.4*	80.9 ± 3.3	89.8 ± 2.6

Plasma parameters were assessed using 28-week-old male +/+ or –/– mice that were either nonobese or obese as a consequence of GTG treatment. For fasting plasma glucose, n = 20 for each group; other parameters were measured during ad libitum feeding with n = 22–26 for non-obese mice, n = 14–15 for GTG obese mice. * $P < 0.05$ vs. +/+. Similar effects on insulin and triglyceride levels were seen in female mice (see text).

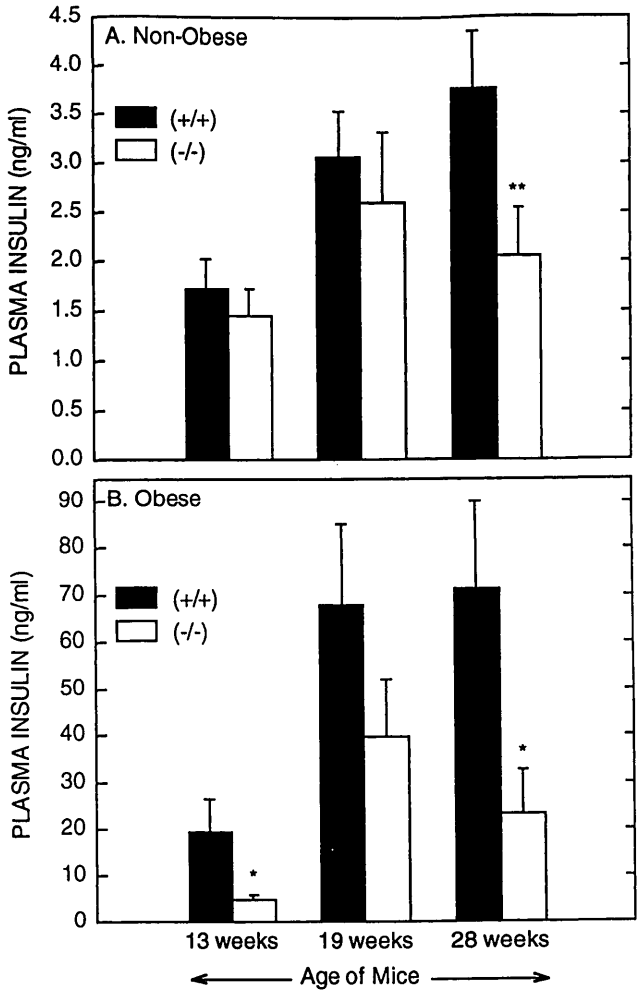

FIG. 1. Fed plasma insulin levels in +/+ (n = 26) and –/– (n = 22) nonobese male mice (A) and in +/+ (n = 19) and –/– (n = 17) GTG-obese male mice (B). *P < 0.05 compared with age-matched +/+ mice. **P < 0.01 compared with age-matched +/+ mice. Similar results were seen in older (32- to 34-week-old) female mice (see text).

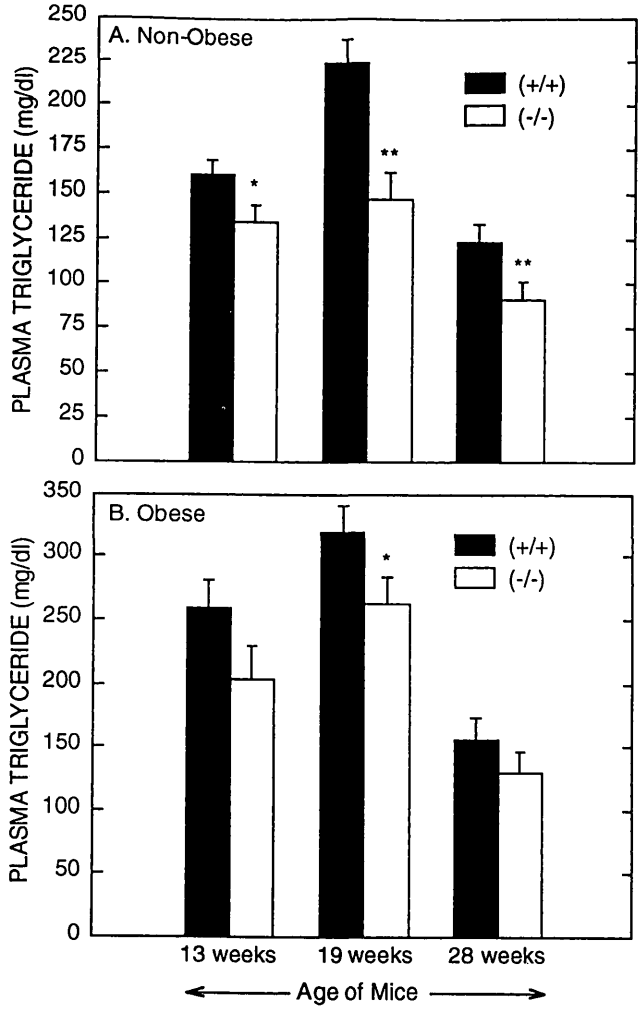

FIG. 2. Plasma triglyceride levels in +/+ (n = 26) and –/– (n = 22) nonobese male mice (A) and in +/+ (n = 19) and –/– (n = 17) GTG-obese male mice (B). *P < 0.05 compared with age-matched +/+ mice. **P < 0.01 compared with age-matched +/+ mice. Similar results were seen in older (32- to 34-week-old) female mice (see text).

using blood samples obtained from both groups of obese mice 45 min after oral glucose administration; mean stimulated insulin levels were reduced by 50% in –/– compared with +/+ mice (Fig. 3*B*).

DISCUSSION

To determine definitively the importance of TNF-α in the regulation of glucose and lipid metabolism in mice, the effects of targeted disruption of the TNF-α gene on body weight, body composition, and plasma nutrient and hormone levels were measured in nonobese and hyperphagic GTG-obese mice.

The absence of TNF-α in nonobese –/– mice resulted in significant changes in body composition relative to +/+ mice, as reflected by modest decreases in body weight, epididymal fat depot weight, and percentage body fat in older (28-week-old) mice along with a reciprocal increase in percentage body protein. These results suggest that a normal physiological role for TNF-α may involve direct or indirect effects that lead to increased adipose tissue mass over time. This conclusion is at odds with a large body of data indicating that TNF-α is associated with cachexia and that (at high concentrations) it is anti-adipogenic and causes delipidation of adipocytes in certain systems (19). In addition, the increased adipose tissue expression of TNF-α in obesity might be viewed as a compensatory mechanism designed to curtail further increases in adiposity (8,9). However, circulating TNF-α levels in cachexia, where extensive lipolysis also occurs, are elevated ~1,000-fold relative to those in obesity. Furthermore, tissue/cellular responses to TNF-α vary depending on the concentration of other hormones (insulin, glucocorticoids) and cytokines (9). Additionally, TNF-α could potentially augment in vivo adipogenesis, since it may function as a growth factor for pre-adipocytes or increase vascular permeability (9). In response to GTG injections, both +/+ and –/– mice achieved the same degree of obesity, as reflected by increased mean body weight, fat depot weight, and circulating leptin levels. Thus, in the face of overt hyperphagia, TNF-α does not appear to modulate the development of obesity.

In nonobese –/– mice, we observed that plasma triglyceride levels were substantially reduced. This may have resulted in part from the modest decrease in body adiposity. However, there was also a trend toward increased adipose tissue LPL in these mice and a trend toward relatively lower

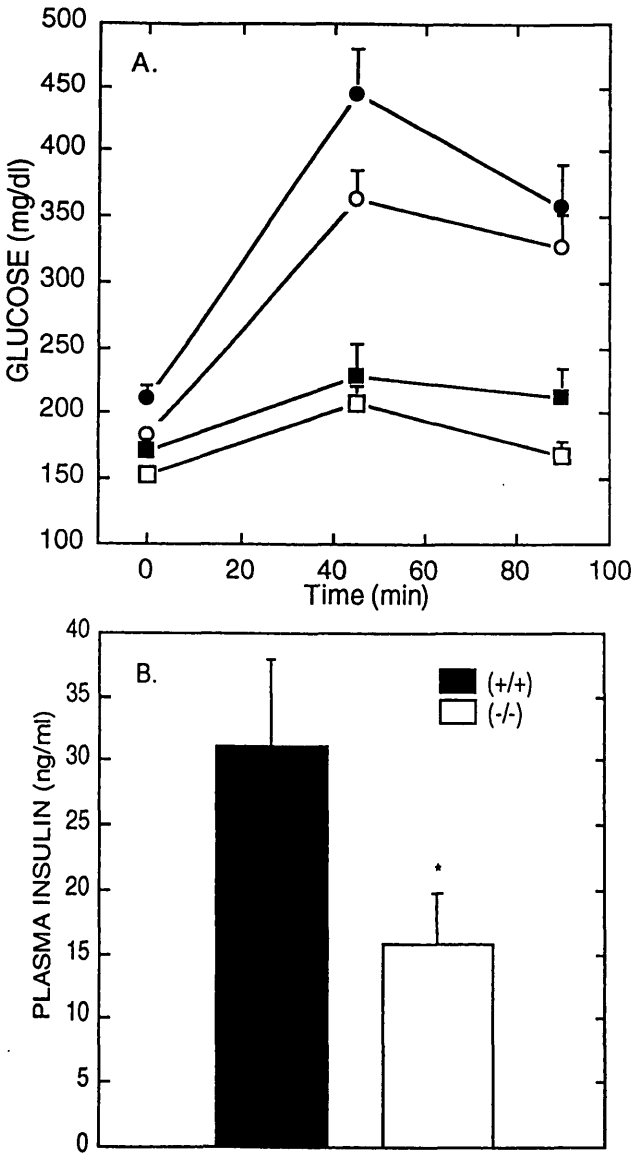

FIG. 3. *A:* **glucose response to an OGTT in conscious nonobese –/– (*n* = 22) (□) and +/+ (*n* = 26) (■) mice and GTG-obese –/– (*n* = 20) (○) and +/+ (*n* = 20) (●) mice. Male mice at 19–23 weeks of age were fasted for 6 h before a tail blood sample was obtained (time 0 min); 3 g glucose/kg body wt was administered by oral gavage, followed by repeat tail blood sampling at 45 and 90 min.** *B:* **plasma insulin levels in male GTG-obese –/– (*n* = 20) and +/+ (*n* = 20) mice at 45 min after 3 g/kg oral glucose administration in an OGTT. **P* < 0.05 compared with +/+ mice.**

triglyceride levels in weight-matched obese –/– versus obese +/+ mice. Although administration of TNF-α to rats and humans can clearly elicit increases in serum triglycerides (1), it is not known whether TNF-α has a normal physiological role in the regulation of triglyceride metabolism. Our findings offer further support for this hypothesis. Additional studies will be required to determine whether altered LPL expression or other potential mechanisms underlie the triglyceride reduction in –/– mice.

The absence of TNF-α in nonobese mice resulted in significantly lower plasma insulin levels. However, compared with their nonobese counterparts, obese –/– mice still developed hyperinsulinemia, although this effect was ameliorated relative to obese +/+ mice. Obese –/– mice were also characterized by improved fasting glucose levels, but they still developed impaired glucose tolerance, which was only partially improved relative to obese +/+ mice, along with glucose-stimulated insulin levels that were reduced by 50%. These results indicate that in this mouse strain and model of hyperphagic obesity, TNF-α contributes to hyperinsulinemia and glucose intolerance but is not a predominant mediator of these metabolic disturbances. Furthermore, assessment of insulin-stimulated glycogen synthesis in white adipose tissue of obese –/– mice ex vivo revealed persistent insulin resistance comparable to that observed in adipose tissue of obese +/+ mice.

The above conclusion differs from some of the published work suggesting a predominant role for TNF-α in obesity-associated insulin resistance and diabetes. Thus, infusion of soluble chimeric TNF-α receptor IgG protein into Zucker *fa/fa* rats resulted in an approximately twofold increase in peripheral insulin-stimulated glucose disposal (8). However, TNF-α neutralization did not affect insulin-mediated suppression of hepatic glucose output. Moreover, since no lean (*fa/+*) rats were studied in parallel, it is not clear whether the peripheral insulin resistance of *fa/fa* rats was partially or completely normalized (8). A recent study showed that mice with a null mutation in the gene encoding aP2 failed to express TNF-α in fat when rendered obese by high-fat feeding (20). Although fat-fed aP2-null mice achieved a similar degree of obesity as fat-fed controls, they did not develop hyperinsulinemia or hyperglycemia. There are two potential explanations for the apparent discrepancy between these reported results and our own observations: *1*) the extent of obesity and degree of hyperinsulinemia was more extreme in the case of GTG-treated mice in the present study; *2*) factors other than deficient adipose tissue expression of TNF-α could have contributed to the improved metabolic status in fat-fed aP2-null mice. Although published reports have demonstrated that TNF-α treatment of cultured cells impairs insulin receptor–mediated signaling and biological responses to insulin (21), the relevance of these findings to the potential role of TNF-α in in vivo insulin resistance could be questioned for several reasons. First, these studies generally used TNF-α concentrations likely to greatly exceed circulating levels or even locally elevated levels that might occur in the context of obesity. Second, although TNF-α suppression of insulin receptor and IRS-1 phosphorylation required 3 days of incubation with non–hepatocyte-derived cells, similar effects were seen in hepatoma cells after only 1-h incubation (21). Finally, one study (22) reported that short-term incubation of 3T3-L1 adipocytes with TNF-α actually promoted insulin-stimulated IRS-1 phosphorylation and the association of phosphatidylinositol 3-kinase with IRS-1.

A recently published study casts serious doubt on the relevance of TNF-α in human type 2 diabetes; long-term infusion of TNF-α–neutralizing antibody failed to change glucose levels or insulin sensitivity in a cohort of subjects with type 2 diabetes (23). However, the possibility that TNF-α acts in a paracrine rather than an endocrine manner might explain the ineffectiveness of TNF-α neutralizing antibody treatment in causing metabolic improvement. The characterization of TNF-α–null mice that we have performed clearly demonstrates that TNF-α does have a role in the in vivo regulation of triglyceride metabolism and glucose homeostasis. The absence of TNF-α substantially improved the marked degree

of hyperinsulinemia and modestly improved hyperglycemia in GTG-obese mice. However, mean insulin levels in these hyperphagic obese mice were still several-fold higher than the levels seen in lean mice, and impaired glucose tolerance was still evident. Further studies that examine phenotypes associated with the TNF-α–null mutation in other contexts (e.g., high-fat feeding, alternative inbred strains, cross-breeding with models of genetic obesity) will be required to characterize fully the extent to which TNF-α contributes to the in vivo regulation of insulin sensitivity in rodents under different physiological or pathophysiological conditions.

REFERENCES

1. Beutler B, Cerami A: Cachectin (tumor necrosis factor): a macrophage hormone governing cellular metabolism and inflammatory response. *Endocr Rev* 9:57–66, 1988
2. Spiegelman BM, Hotamisligil GS: Through thick and thin: wasting, obesity, and TNFα. *Cell* 73:625–627, 1993
3. Zhang B, Berger J, Hu E, Szalkowski D, White-Carrington S, Spiegelman BM, Moller DE: Negative regulation of peroxisome proliferator-activated receptor-γ gene expression contributes to the antiadipogenic effects of tumor necrosis factor-α. *Mol Endocrinol* 10:1457–1466, 1996
4. Torti FM, Dieckmann B, Beutler B, Cerami A, Ringold GM: A macrophage factor inhibits adipocyte gene expression: an *in vitro* model of cachexia. *Science* 229:867–869, 1985
5. Stephens JM, Pekala PH: Transcriptional repression of the GLUT4 and C/EBP genes in 3T3-L1 cells by tumor necrosis factor-alpha. *J Biol Chem* 266:21839–21845, 1991
6. Norman RA, Bogardus C, Ravussin E: Linkage between obesity and a marker near the tumor necrosis factor-α locus in Pima Indians. *J Clin Invest* 96:158–162, 1995
7. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM: Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. *J Clin Invest* 95:2409–2415, 1995
8. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. *Science* 259:87–91, 1993
9. Hotamisligil GS, Spiegelman BM: Tumor necrosis factor: a key component of the obesity-diabetes link. *Diabetes* 43:1271–1278, 1994
10. Saghizadeh M, Ong JM, Garvey WT, Henry RR, Kern PA: The expression of TNFα by human muscle: relationship to insulin resistance. *J Clin Invest* 97:1111–1116, 1996
11. Feinstein R, Kanety H, Papa MZ, Lunenfeld B, Karasik A: Tumor necrosis factor-α suppresses insulin-induced tyrosine phosphorylation of insulin receptor and its substrates. *J Biol Chem* 268:26055–26058, 1993
12. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM: IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF— and obesity-induced insulin resistance. *Science* 271:665–668, 1996
13. Kanety H, Hemi R, Papa MZ, Karasik A: Sphingomyelinase and ceramide suppress insulin-induced tyrosine phosphorylation of the insulin receptor substrate-1. *J Biol Chem* 271:9895–9897, 1996
14. Pasparakis M, Alexopoulou L, Episkopou V, Kollias G: Immune and inflammatory responses in TNFα-deficient mice: a critical requirement in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *J Exp Med* 184:1397–1441, 1996
15. Young JK, McKenzie JC, Brady LS, Herkenham M: Hypothalamic lesions increase levels of neuropeptide Y mRNA in the arcuate nucleus of mice. *Neurosci Lett* 165:13–17, 1994
16. Salmon DMV, Flatt JP: Effect of dietary fat content on the incidence of obesity among ad libitum fed mice. *Int J Obes* 9:443–449, 1985
17. Sadur CN, Eckel RH: Insulin stimulation of adipose tissue lipoprotein lipase. *J Clin Invest* 69:1119–1125, 1982
18. Doebber TW, Wu M, Ventre J: *In vitro* exposure of white adipose tissue to the insulin sensitizing thiazolidinediones enhances insulin stimulation of glycogen synthesis (Abstract). *Diabetes* 45 (Suppl. 2):92A, 1996
19. Petruschke TH, Hauner H: Tumor necrosis factor-α prevents the differentiation of human adipocyte precursor cells and causes delipidation of newly developed fat cells. *J Clin Endocr Metab* 76:742–747, 1993
20. Hotamisligil GS, Johnson RS, Distel RJ, Ellis R, Papaioannou VE, Spiegelman BM: Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. *Science* 274:1377–1379, 1996
21. Stephens JM, Lee J, Pilch PF: Tumor necrosis factor-α-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction. *J Biol Chem* 272:971–976, 1997
22. Guo D, Donner DB: Tumor necrosis factor promotes phosphorylation and binding of insulin receptor substrate-1 to phosphatidylinositol 3-kinase in 3T3-L1 adipocytes. *J Biol Chem* 271:615–618, 1996
23. Ofei F, Hurel S, Newkirk J, Sopwith M, Taylor R: Effects of an engineered human anti-TNF-α antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. *Diabetes* 45:881–885, 1996