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Diet-genotype interactions in the early development of obesity and insulin resistance in mice with a genetic deficiency in tumor necrosis factor–α

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Abstract

The onset of insulin resistance, the sites of action, and the mechanisms through which tumor necrosis factor–α (TNF-α) exacerbates the increase in adiposity and the development of insulin resistance in mice fed high-fat (HF) diet remain unclear. Here we investigated the effect of TNF-α deficiency on adiposity and insulin resistance during the initial 1 to 4 weeks of HF feeding. We examined body weight; the distribution of white adipose tissue (WAT); homeostasis model assessment; and levels of leptin, resistin, and adiponectin in the initial 4 weeks of HF feeding in TNF-α knockout (KO) mice and wild-type (WT) controls. Through 4 weeks of HF feeding, KO mice, unlike WT mice, maintained normal insulin sensitivity. Although WT-HF and KO-HF mice had similar levels of WAT at this time, KO-HF mice had more subcutaneous and less epididymal fat than WT-HF mice. The KO-HF mice also had less liver fat than the WT-HF mice. Finally, KO-HF mice had lower plasma levels of resistin than WT-HF mice. These data demonstrate that genetic lack of TNF-α protects insulin sensitivity during the early phase of HF feeding in the absence of altered total WAT. The data also suggest that the mechanism maintaining insulin sensitivity in the absence of TNF-α may involve redirection of the fat deposition to the metabolically more inert subcutaneous depot or decreases in circulating resistin and resultant decrease in liver fat deposition. The efficacy of therapeutic measures designed to counteract the effects of TNF-α may be increased during the early stages of obesity and insulin resistance.

1. Introduction

It is now recognized that changes in innate immune function contribute substantially to the endocrine and metabolic disorders of obesity, including insulin resistance and type 2 diabetes mellitus (reviewed by Ferrante [1], de Luca and Olefsky [2], and Wellen and Hotamisligil [3]). The proinflammatory cytokine tumor necrosis factor–α (TNF-α) appears to play a key role in this pathogenic contribution of the immune system in some rodent obesity models, although in human patients its role is still controversial. For example, in a now classic series of studies [4-10], it was demonstrated that (1) both expression of TNF-α messenger RNA (mRNA) in adipose tissue, most likely in resident immune cells, and circulating levels of TNF-α were elevated in 4 monogenic rodent obesity models (*db/db*, *ob/ob*, *tub/tub*, and *fa/fa*); (2) passive immunization against TNF-α improved insulin sensitivity during a hyperinsulinemic-euglycemic clamp in obese, insulin-resistant wild-type (WT) rats; and (3) mice with transgenic deletions of TNF-α (knockout [KO]) or TNF-α receptors developed less adiposity and insulin resistance and had improved endocrine profiles when fed high-fat (HF) diets for 12 weeks or more.

Despite these impressive results, the physiologic events that stimulate immune responses in enlarged fat depots, including both the local inflammatory reactions in adipose tissue and the changes in concentrations of immune signaling molecules and adipokine hormones such as leptin, resistin, and adiponectin in the systemic circulation, as well as the mechanisms through which these responses in turn initiate or exacerbate the development of insulin resistance, are poorly understood [2]. Because insulin resistance begins to develop in rodents as soon as 1 week after onset of HF feeding [11], chronic obesity models, such as those reviewed above, do not reveal when and on which aspect of the control of insulin sensitivity TNF-α may act. Here we addressed these issues in

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the TNF-α KO mouse model. Specifically, to better identify the potential initial contribution of TNF-α in the development of HF feeding–induced insulin resistance, we examined whether TNF-α KO affects the development of adiposity; insulin resistance; or circulating levels of the adipokine hormones leptin, resistin, or adiponectin in the initial 4 weeks of HF feeding.

2. Method

2.1. Subjects

Male c57BL/6 background mice (n = 22) homozygous for a targeted null mutation at the TNF-α locus were bred on site from homozygous pairs generously donated by Dr M Freudenberg (Max Planck Institute, Freiburg, Germany). Controls were male WT c57BL/6 mice (n = 21) of the same age (LTK, Fuellinsdorf, Switzerland). At 6 weeks of age, mice were singly housed in solid-bottom plastic cages with pine bedding in a temperature-controlled room (22°C ± 1°C) with a 12:12-hour light-dark cycle (lights off at 12:00 PM). Standard rodent chow (CH; containing 4.5%, 41%, and 18.5% weight from fat, carbohydrates, and protein, respectively; estimated metabolizable energy content, 13.1 kJ/g; #3436, Provimi Kliba, Kaiseraugst, Switzerland) and tap water were available ad libitum. Animals were treated in accordance with the *Principles of Laboratory Animal Care* (National Institutes of Health publication no. 85-23, revised 1985; http://grants1.nih.gov/grants/olaw/references/phspol.htm). All procedures were approved by the Veterinary Office of the Canton of Zurich.

2.2. HF feeding

Starting at 8 weeks of age, food was removed about 1 hour before onset of darkness, mice were weighed (body weight [BW], ±0.1 g), and fresh food was offered. One week later (experimental day 0), half the WT and half the KO mice were switched to HF diet (35.5%, 15.5%, and 24% fat (lard), carbohydrate, and protein, respectively; estimated metabolizable energy content, 22 kJ/g; #2127, Provimi Kliba); and the rest continued on CH. Groups are designated *WT-HF*, *KO-HF*, *WT-CH*, and *KO-CH*.

2.3. Metabolic measurements

Fed blood samples were obtained from awake ad libitum–fed mice 3 hours into the dark phase on days 8 and 27. Nocturnal food intake during the 3 hours before sampling was measured (±0.01 g). Fasting blood samples were obtained from awake mice 8 hours into the light phase after 12 hours of food deprivation on days 10 and 29. Blood (~100 μL) was collected in heparinized capillary tubes (Sarstedt, Nümbrecht, Germany) from unanesthetized mice by puncturing the saphenous vein with a 24-gauge needle (Braun, Melsungen, Germany) and was centrifuged at 9000*g* and 4°C for 10 minutes; and plasma was stored at −20° C. Blood glucose concentration was measured at the time of sampling with a glucometer (AccuCheck Aviva; Roche, Mannheim, Germany). Plasma insulin was measured by radioimmunoassay (Linco Research, St Charles, MO; lower detection limit = 0.1 ng/mL), and plasma resistin, leptin, and adiponectin by multiplex assay (Linco, lower detection limit = 50 pg/mL), in duplicate. Sample duplicates that varied greater than 10% from each other and duplicate means outside the 20 to 80 percentile range of the logit standard curve were not accepted for further statistical analyses.

2.4. Adiposity and liver fat measurements

On day 35, the mice were euthanized with pentobarbital (15 mg/mouse; Kantonsapotheke, Zurich, Switzerland); and the liver, mesenteric (MES) fat, and epididymal (EPI), retroperitoneal (RP), and subcutaneous (SC) fat pads from the leg, back, and neck region were removed and weighed. Livers were fixed in 4% paraformaldehyde and dehydrated in ascending concentrations of sucrose; and 30-μm sections were cut, mounted on gelatinized slides, and stained with oil red O (Sigma, Buchs, Switzerland). Fat content was quantified by optical density of stain using Image Pro Software (Media Cybernetics, Gaithersburg, MD).

2.5. Data analysis

Data collected on days 8 and 10 are referred to as week 1; and data collected on days 27 and 29, as week 4. Fasting glucose and insulin levels were used to calculate the homeostatic model of assessment of insulin resistance (HOMA; glucose [in millimoles per liter] × insulin [in microunits per milliliter]/22.5 [12]). Total white adipose tissue (WAT) mass was estimated as the sum of the weights of the MES, SC, RP, and EPI fat pads. Individual fat pad weights were expressed as percentage total WAT for analysis. To increase statistical power, data in each group were converted to standard scores using the median absolute deviate method; and standard scores with absolute values greater than 1.96 (ie, $P < .05$) were excluded as outliers. Data were analyzed by planned comparisons using the Bonferroni-Holm method [13] and analysis of variance to generate mean square errors. Because TNF-α KO affected several dependent variables in CH-fed mice as well as HF-fed mice, the crucial comparison to substantiate an effect of TNF-α on the response to HF feeding vs CH feeding was the difference between the gene effects in HF-fed and in CH-fed mice, that is, (KO-HF minus WT-HF) vs the (KO-CH minus WT-CH). In addition, progressive effects of TNF-α KO on longitudinally measured parameters were assessed by contrasts between values at different time points. Data are presented as mean ± SEM. Planned comparisons with an experiment-wide $P < .05$ were considered significant. For such differences, the Bonferroni-Holm t values, the critical P (P_C) required to achieve the experiment-wide criterion, and the standard errors of the difference (SED) based on the

mean square errors used for the planned comparisons are reported.

3. Results

3.1. Body weight

The WT-HF mice accrued substantial amounts of excess BW during the third and fourth weeks of HF feeding. The TNF-α KO completely prevented this (Fig. 1A). The KO-HF mice weighed significantly less than the WT-HF mice after 3 and 4 weeks HF feeding, and 4-wk BW gain was less in KO-HF than WT-HF mice (Fig. 1A; $t[48] = 3.38$, 5.41, and 3.29; $P_C < .013$, .01, and .017; SED = 0.3 g; for the 3 comparisons, respectively). No significant BW differences were detected between KO-CH and WT-CH mice, with the result that the difference in BW gain between HF and CH mice was significantly greater in WT than in KO mice ($t[21] = 3.59$, $P_C < .05$, SED = 0.6 g). That is, TNF-α significantly affected the BW response to HF feeding vs CH feeding.

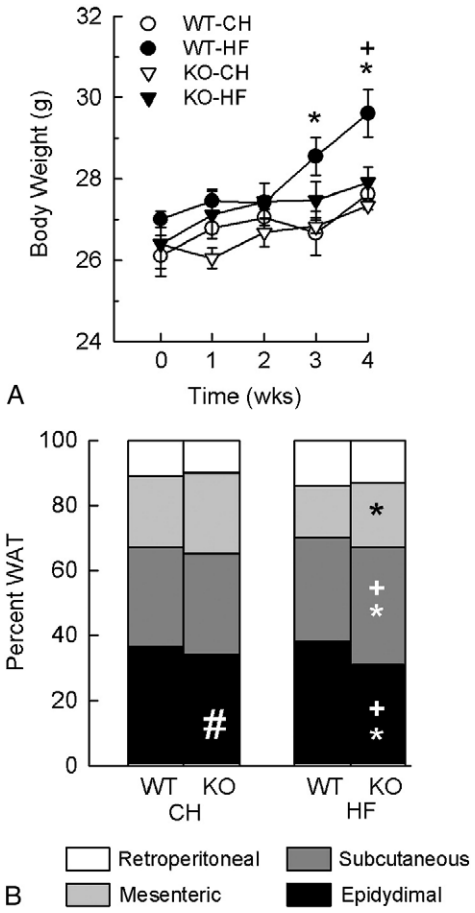

Fig. 1. Genetic deficiency in TNF-α affected changes in BW and regional fat distribution in response to HF feeding. A, After 4 weeks of HF feeding, WT-HF mice gained excess BW in comparison to WT-CH mice; but KO-HF mice did not. Data are means ± SEM. B, KO-CH mice had less EPI fat than WT-CH mice; but after 4 weeks of HF feeding, KO-HF mice had more SC and MES fat than WT-HF mice. Data are mean percentage total WAT; SED range = 1.0% to 1.8%. *KO-HF different from WT-HF; $P < .05$ ($t[25] = 4.90$, 3.40, and 2.70; $P_C < .017$, < .025, < .017; SED = 1.3%, 1.8%, and 1.7 %; for EPI, SC, and MES fat, respectively). #KO-CH different from WT-CH; $P < .05$($t[25] = 2.04$, $P_C < .05$, SED = 1.3 %). +(KO-HF minus WT-HF) different from (KO-CH minus WT-CH); $P < .05$ ($t[25] = 2.87$ and 3.48, $P_C < .025$ and < .017; SED = 1.3% and 1.8%; for EPI and SC fat, respectively).

3.2. Adiposity and liver fat

Total WAT increased similarly in KO-HF and WT-HF vs their CH-fed controls (2.48 ± 0.10 g and 2.51 ± 0.04 g vs 1.44 ± 0.03 g and 1.50 ± 0.02 g, respectively; $t[25] = 4.07$, 4.19, and 0.03; $P_C < .017$, < .025 and > .05; SED = 0.25 g; for KO, WT, and the difference, respectively). Relative deposition of fat in the different depots, however, was affected by diet and genotypes (Fig. 1B). That is, expressed as percentage total WAT, there tended to be more EPI than SC fat in WT-CH, KO-CH, and WT-HF mice; but there was significantly less EPI than SC fat in KO-HF mice. The crucial comparison, that is, the difference in KO-HF and KO-CH mice vs the difference between WT-HF and WT-CH mice, was significant for both EPI and SC fat.

Tumor necrosis factor–α KO completely prevented the increase in liver fat induced by 4-week HF feeding in WT mice (Fig. 2). That is, liver fat contents were similarly low in

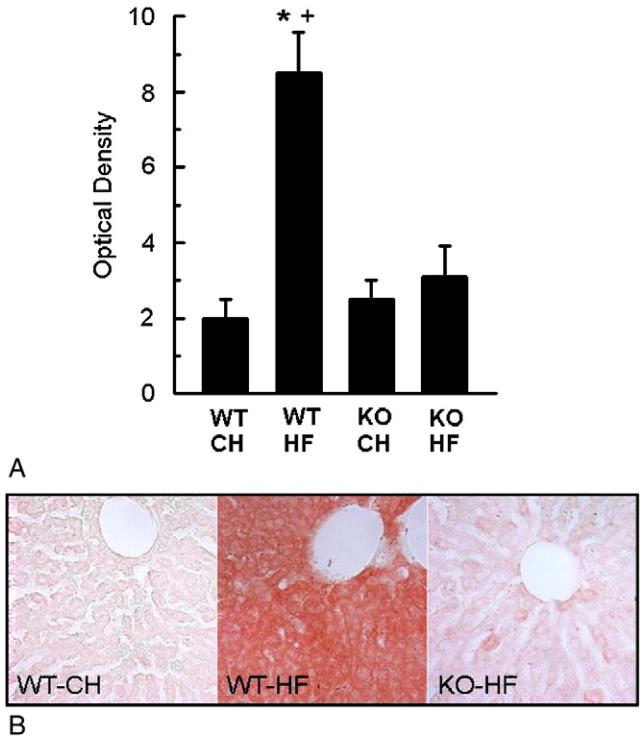

Fig. 2. Genetic deficiency in TNF-α prevented the increase in liver fat induced by 4 weeks of HF feeding in WT mice. A, After 4 weeks of HF feeding, WT-HF mice accrued excess liver fat in comparison to WT-CH mice; but KO-HF mice did not. Data are arbitrary optical density units; means ± SEM. B, Representative photomicrographs of oil red O–stained liver sections. +(KO-HF minus WT-HF) different from (KO-CH minus WT-CH); $P < .05$ ($t[25] = 3.85$, $P_C < .017$, SED = 1.5 units).

KO-CH, WT-CH, and KO-HF mice, but markedly elevated in WT-HF mice. Liver weights did not vary significantly either between genotypes or between diets (WT-CH = 1.39 ± 0.02 and WT-HF = 1.43 ± 0.04; KO-CH = 1.36 ± 0.04 and KO-HF = 1.33 ± 0.04; $t[26] = 0.78$, 0.54, and 0.24; $P_C > .05$; SED = 0.05 g; for KO, WT, and the difference, respectively).

3.3. Blood glucose and plasma insulin levels

Fasting blood glucose and fasting plasma insulin were lower in KO-HF than WT-HF mice at both 1 and 4 weeks and were lower in KO-CH mice than WT-CH mice at 1 week (Fig. 3A, B). Nevertheless, the crucial comparisons, that is, differential gene effects in HF- vs CH-fed mice, for fasting blood glucose and fasting plasma insulin did not achieve significance. The HOMA insulin resistance index values were also generally lower in TNF-α KO mice; and at 4 weeks, a differential gene effect in HF- vs CH-fed mice was detected (Fig. 3C). Indeed, at 4 weeks, HOMA in KO-HF mice was nearly identical to that in KO-CH or WT-CH mice.

Unfortunately, our measures of insulin and glucose in fed mice were confounded by significant differences in energy intakes during the 3 hours before sampling. For example, at week 1, WT-CH mice ate more than KO-CH mice (18.6 ± 0.5 vs 16.3 ± 0.7 kJ, $t[11] = 3.22$, $P_C < .017$, SED = 9.6 kJ); and WT-HF mice ate less than KO-HF mice (20.9 ± 2.5 vs 28.4 ± 2.0 g, $t[11] = 3.13$, $P_C < .025$, SED = 2.9 kJ). We therefore regard the differences in glucose and insulin levels in fed mice uninterpretable and do not report them here.

3.4. Plasma adipokines

3.4.1. Leptin

High-fat feeding increased, and fasting and TNF-α KO decreased, plasma leptin levels (Fig. 4). The KO-HF mice had significantly lower fasting and fed plasma leptin levels than the WT-HF mice at both week 1 and week 4. The KO-CH mice also had significantly lower fed leptin levels at both times. Although the effects of TNF-α KO to reduce plasma leptin appeared larger in HF- than CH-fed mice, the crucial comparisons were however not statistically significant.

3.4.2. Resistin

In fasted mice, resistin levels increased in WT-CH between weeks 1 and 4, with the result that they were higher than in KO-CH mice at week 4 (Fig. 5A); but no such effect of TNF-α KO was observed in HF-fed mice. In contrast, in fed mice, TNF-α KO clearly influenced plasma resistin levels in HF-fed mice (Fig. 5B). That is, fed resistin levels were significantly lower in KO-HF mice at both weeks 1 and 4; and the crucial contrast between the effects of TNF-α KO in CH- and HF-fed mice was significant at week 4.

3.4.3. Adiponectin

Fasting plasma adiponectin levels were less in KO-HF mice than WT-HF mice at week 4, but not week 1, a significant increase in this gene effect between weeks 1 and 4 (Table 1). No significant effects were detected in week 4 in fed adiponectin levels. Week 1 fed adiponectin levels,

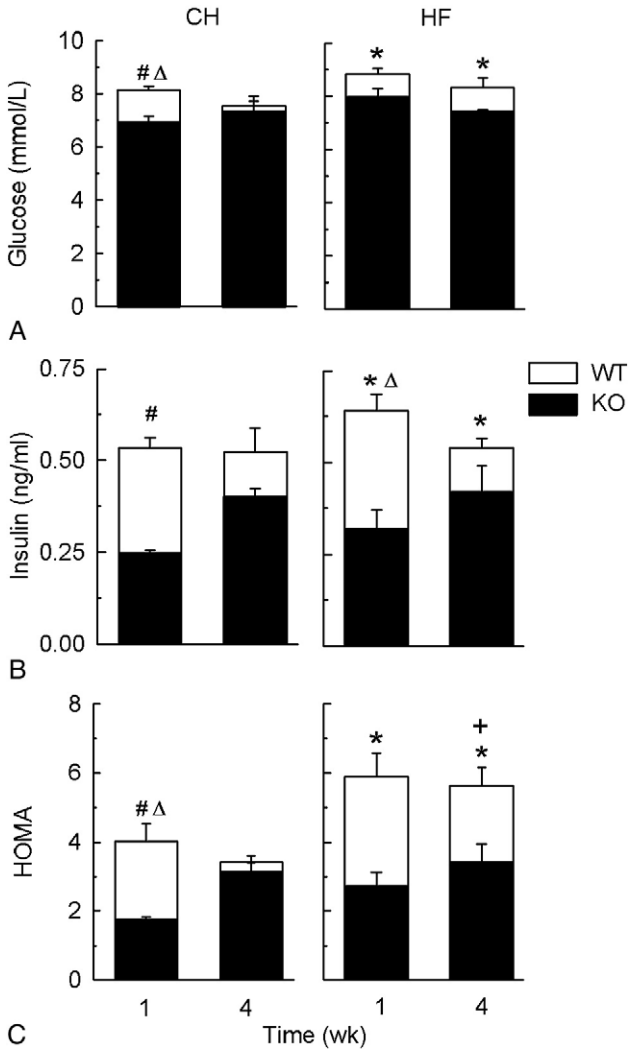

Fig. 3. Genetic deficiency in TNF-α increased insulin sensitivity in CH-fed mice and protected against the development of insulin resistance in response to HF feeding. Left panels: KO-CH mice had lower fasting glucose (A), insulin (B), and HOMA (C) than WT-CH mice at week 1, but not at week 4. Right panels: KO-HF mice had lower fasting glucose (A), insulin (B), and HOMA (C) compared with WT-HF mice at both weeks 1 and 4. The opposite seems to be the case at 4 weeks, when the interaction between diet and genotype produced an effect that was 10-fold higher in WT vs KO mice. Because of greater variability in the CH- vs HF-fed mice, however, this effect was not statistically significant. Data are means ± SEM. *WT-HF different from KO-HF; $P < .05$ (glucose: $t[12] = 2.96$ and 3.10, $P_C < .025$ and .017, for weeks 1 and 4, respectively, SED = 0.29 mmol/L; insulin: $t[10] = 6.48$ and 2.56, $P_C < .017$ and .05, for weeks 1 and 4, respectively, SED = 0.05 ng/mL; HOMA: $t[12] = 6.03$ and 4.19, $P_C < .017$ and .025, for weeks 1 and 4, respectively, SED = 0.52). #KO-CH different from WT-CH; $P < .05$ (glucose: $t[11] = 3.05$, $P_C < .017$, SED = 0.39 mmol/L; insulin: $t[7] = 3.75$, $P_C < .017$, SED = 0.08 ng/mL; HOMA: $t[7] = 5.17$, $P_C < .017$, SED = 0.45) Δ(WT week 1 minus KO week 1) different from (WT week 4 minus KO week 4), within the same diet; $P < .05$ (CH glucose: $t[11] = 2.61$, $P_C < .025$, SED = 0.39 mmol/L; HF insulin: $t[10] = 3.91$, $P_C < .025$, SED = 0.05 ng/mL; CH HOMA: $t[7] = 4.52$, $P_C < .017$, SED = 0.45). +(KO-HF minus WT-HF) different from (KO-CH minus WT-CH); $P < .05$ ($t[25] = 2.08$, $P_C < .05$, SED = 0.68).

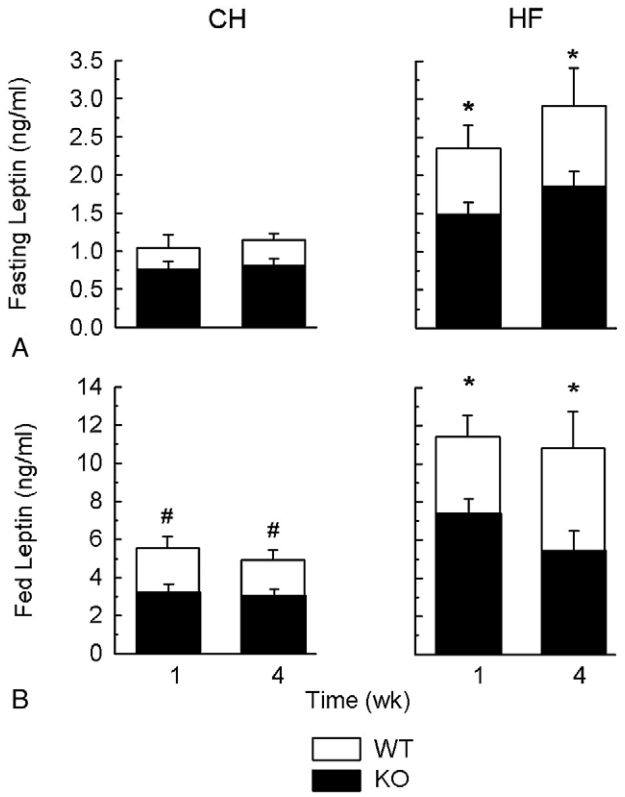

Fig. 4. Genetic deficiency in TNF-α decreased plasma leptin levels. A, Fasted mice. B, Fed mice. Data are means ± SEM. *WT-HF different from KO-HF; $P < .05$ ($t[12] = 2.55$ and 3.13, $P_C < .025$ and .017, SED = 0.34 for fasted mice at 1 and 4 weeks, respectively; and $t[10] = 2.82$ and 3.78, $P_C < .025$ and .017, SED = 1.42 ng/mL, for fed mice at 1 and 4 weeks, respectively). #KO-CH different from WT-CH; $P < .05$ ($t[12] = 5.23$ and 4.26, $P_C < .017$ and .025, SED = 0.44 ng/mL, for fed, respectively).

however, increased, but not decreased, in KO-HF mice in comparison with WT-HF mice.

4. Discussion

Previous studies in long-term obesity models amply demonstrate that genetic or pharmacologic removal of TNF-α protects from insulin resistance [7,9,10,14]. The role of TNF-α in the initial stages of obesity pathophysiology, however, has not been determined. Therefore, the goals of this study were to determine, first, whether TNF-α affects incipient insulin resistance at the onset of obesity or simply enhances the ultimate degree of pathology after obesity or diabetes is already established and, second, if TNF-α does have such early effects, whether a staggered onset of endocrine or other changes might suggest the mediating mechanisms.

With respect to the first goal, we determined that TNF-α influenced insulin sensitivity after 4 weeks, but not after 1 week, of HF feeding, as indicated by a significant difference in HOMA (WT-HF and WT-CH mice) vs HOMA (KO-HF and KO-CH mice). We conclude, therefore, that TNF-α contributes to the deterioration of insulin sensitivity between 1 and 4 weeks of HF feeding under our conditions. This is a markedly earlier contribution of TNF-α than previous studies have documented. These data, therefore, establish a time frame to enable further investigations at the systemic, tissue, and molecular levels to identify how, when, and where the absence of TNF-α is protective [15-18].

With respect to the second goal, the rapid TNF-α–mediated decrease in insulin sensitivity was associated with a number of physiologic changes that have been implicated in the control of insulin sensitivity in mouse models and that parallel the metabolic syndrome in humans that is prodromal to type 2 diabetes mellitus. Perhaps the most interesting were, first, the reduced effect of HF feeding on circulating resistin level and liver fat content in TNF-α KO mice and, second, the alteration of regional fat distribution in KO-HF mice, which had more SC and less EPI fat. We consider these changes to be candidate components of the mechanism through which TNF-α affects insulin sensitivity.

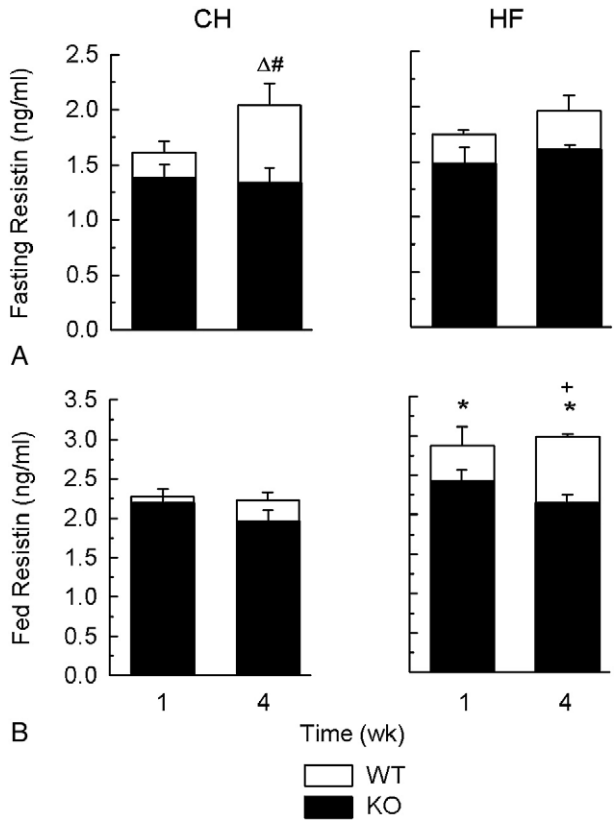

Fig. 5. Genetic deficiency in TNF-α decreased fed plasma resistin in HF-fed mice more than HC-fed mice. A, Fasted mice. B, Fed mice. Data are means ± SEM. *WT-HF different from KO-HF; $P < .05$ ($t[7] = 2.77$ and 5.09, $P_C < .025$ and .017, SED = 0.17 ng/mL). #KO-CH different from WT-CH; $P < .05$ ($t[12] = 4.33$, $P_C < .017$, SED = 0.16 ng/mL). Δ(WT week 1 minus KO week 1) different from (WT week 4 minus KO week 4), within the same diet; $P < .05$ ($t[12] = 2.92$, $P_C < .025$, SED = 0.16 ng/mL). +(KO-HF minus WT-HF) different from (KO-CH minus WT-CH); $P < .05$ ($t[22] = 3.84$, $P_C < .05$, SED = 0.16 ng/mL).

Table 1
Plasma adiponectin levels after CH or HF feeding in WT and TNF-α KO mice

	Week 1	Week 4
Fasted state		
WT-CH	14.30 ± 1.71	21.18 ± 3.14
KO-CH	15.15 ± 8.31	13.00 ± 2.70
WT-HF	17.12 ± 2.57	31.12 ± 5.24[a,b]
KO-HF	14.80 ± 2.09	13.12 ± 0.91
Fed state		
WT-CH	30.11 ± 6.39	24.08 ± 1.99
KO-CH	17.27 ± 3.40	26.12 ± 5.82
WT-HF	12.25 ± 3.92	22.68 ± 4.31
KO-HF	20.51 ± 3.23[c]	26.82 ± 4.41

Data are mean ± SEM (in nanograms per milliliter); significance level, $P < .05$. Fed plasma adiponectin levels were similar in KO and WT mice whether they were fed CH or HF diet ($t[10] = 2.03$ and $t[9] = 2.65$, all not significant; SED = 4 and 5 μg/mL; for HF and CH diet, respectively), but the differential effect (ie, [WT-CH minus WT-HF] vs [KO-CH minus KO-HF]) was significant ($t[24] = 2.50$, $P_C < .05$, SED = 6 μg/mL).

[a] WT-HF vs KO-HF, within time.

[b] WT vs KO, within diet, between time.

[c] (KO-HF minus KO-CH) vs (WT-HF minus WT-CH), within time.

To our knowledge, this is the shortest period of HF feeding in which TNF-α KO has produced a reliable BW effect. Lee et al [19] reported a nonsignificant decrease in BW gain of TNF-α KO mice (5.2 ± 0.6 vs 8.2 ± 1.9 g in WT) after 5 weeks of HF feeding. Any of several procedural differences, such as the age of the mice, the particular diets used, etc, may account for our similar, but more reliable effect. We did not measure food intake or energy expenditure, so it is not clear whether TNF-α KO affected energy intake or energy expenditure to influence BW gain. In previous tests with these diets, however, WT and KO ate similar amounts (unpublished observations). Therefore, the increased BW gain in WT-HF relative to KO-HF mice is likely to be due to decreased energy expenditure rather than increased energy intake.

This is the first report of the effects of TNF-α KO on total WAT, regional fat distribution, and liver fat deposition after HF feeding. In one previous study, EPI weights, but not other fat pads, were measured after 12 weeks of HF feeding [9]. As here, EPI weight was significantly less in KO-HF mice than in WT-HF mice, although no significant difference was detected in CH-fed mice. The relative decreases in EPI fat and liver fat in KO-HF mice in the absence of a decrease in total WAT suggest that decreases in EPI fat and liver fat may be involved in the protective effect of TNF-α KO on insulin sensitivity, as described below.

It is unclear why total WAT was similar in WT-HF and KO-HF mice even though WT-HF mice weighed more. Because excess lipid can accumulate ectopically in the liver, muscle, pancreas, and other organs during positive energy balance [20], the BW difference between WT-HF and KO-HF mice may reflect, in part, a stimulatory effect of TNF-α on ectopic fat deposition. The markedly increased liver lipid content of WT-HF mice supports this hypothesis. Our data would suggest that the largest relative increases in WT-HF WAT would occur in the SC and MES pads, not the EPI pad.

The demonstration that TNF-α KO prevented the development of HF feeding–induced insulin resistance, as measured by HOMA, in fasting mice as early as 4 weeks extends the report of Uysal et al [9] of a decrease in HOMA after 12 weeks of HF feeding. It is important to note, however, that unlike Uysal et al [9], we took into account the background effect of TNF-α KO on HOMA in WT mice. The importance of this is highlighted by our fasting-insulin data. Fasting insulin was significantly decreased in KO-HF mice compared with WT-HF mice at both time points, suggesting a protective effect on HF feeding–induced insulin resistance. Inspection of the CH data, however, indicates that this protective effect is equally clear in CH-fed mice and therefore is not specifically related to the obesifying HF diet. Finally, of course, the fact that we detected gene-diet interactions in HOMA, but not fasting blood glucose or plasma insulin, suggests that HOMA is a more sensitive measure of insulin sensitivity in TNF-α KO mice.

The reduced plasma leptin levels in TNF-α KO mice here are consistent with both in vivo and in vitro evidence attesting to the close association between leptin and TNF-α in obesity and insulin resistance [21,22]. For example, Kirchgessner et al [22] reported that, after 12 weeks of HF feeding, KO-HF mice were more insulin sensitive and had lower plasma leptin levels than WT-HF mice. Interestingly, because our KO-HF mice had reduced BW in comparison with WT-HF mice, but not reduced total WAT, it appears that, at least early in HF feeding, leptin levels may depend more on regional fat distribution than total WAT, at least in TNF-α KO mice. The reduction in leptin levels in these mice may be related to the decrease in EPI fat because on a per-gram basis, EPI, rather than SC fat, appears to be the major source of leptin secretion in mice [23]. Reductions in EPI fat weight and plasma leptin levels in KO-CH compared with WT-CH mice were also associated in a previous study [24]. Nevertheless, whether such associations reflect a mechanistic link between leptin and TNF-α in the control of insulin sensitivity in HF feeding–induced obesity remains to be established. In this regard, it is important to note that in none of the previous studies has the background effect of TNF-α KO in control CH-fed mice been considered; and in here, we were not able to statistically distinguish leptin's effects in KO-HF mice from this background.

Increased levels of circulating resistin have often been associated with decreased insulin sensitivity [25-27]; and in 2 studies, Scherer [28] and his colleagues [29] and Singhal et al [30] observed increases in plasma resistin, glucose, and insulin levels after as little as 3 weeks of HF feeding. Ours is the first study we know of, however, to suggest the possibility that TNF-α may play a role in the effects of obesity on resistin secretion and insulin resistance. In one report [19], no differences in resistin mRNA or protein levels were detected in the WAT (which fat depot was not specified) of TNF-α KO vs WT HF-fed mice.

Because we failed to observe any effect of TNF-α KO on fasting plasma resistin levels in HF-fed mice, our data appear

not to suggest a relationship between fasting plasma resistin and HOMA insulin sensitivity. However, given that resistin is more associated with hepatic rather than peripheral insulin sensitivity [26,29] and that our mice were ad libitum fed except for 2 nights, it may be that TNF-α KO chronically inhibited resistin secretion, as seen in our fed resistin levels, thereby preserving hepatic insulin sensitivity, and that this tonic effect translated into improved HOMA even in the absence of a simultaneous increase in fasting resistin level. Finally, whether the effects of TNF-α on regional fat distribution—in particular, the reduced EPI fat—that we observed influenced resistin secretion also deserves further study.

There is evidence that the effect of resistin increasing hepatic insulin resistance plays a key role in HF feeding–induced systemic insulin resistance in mice. To our knowledge, however, alterations in resistin levels in TNF-α KO mice have not previously been reported. Muse et al [29] demonstrated that administration of an antisense oligodeoxynucleotide against resistin mRNA reduced both hepatic insulin resistance, as indexed by hepatic glucose production, and systemic insulin resistance, as measured in an insulin clamp. More recently, the addition of a transgenic knockout of resistin to *ob/ob* leptin-deficient mice was shown to increase obesity, but to decrease hepatic fat content and systemic glucose and insulin levels [30]. In light of these data, our finding that TNF-α in part controls resistin levels in HF feeding suggests that an important initial component of the HF feeding insulin resistance syndrome in mice is an effect of adipose tissue TNF-α to stimulate resistin secretion, leading to an accelerated accumulation of fat in the liver and, consequently, to accelerated development of hepatic and systemic insulin resistance. Finally, studies in human subjects also support a strong link between hepatic fat content and the onset of insulin resistance, independent of obesity [31,32]. The role of resistin in this effect, especially in nonobese individuals, however, remains controversial [31,32].

Increased adiponectin expression in the adipose tissue is associated with an increase in insulin sensitivity [28]. Increased insulin resistance in mice with a genetic deficiency in adiponectin is also associated with increased levels of TNF-α in the adipose tissue [33], in part because adiponectin decreases the activation of nuclear factor–κB pathway [34] and thus leads to a decrease in production of proinflammatory cytokines, such as TNF-α. In humans, adiponectin decreases circulating levels of TNF-α by facilitating its removal via binding to soluble TNF-α receptors [35]. Nevertheless, we did not see a clear effect of TNF-α deficiency on adiponectin levels in either CH- or HF-fed mice. This is especially surprising because higher adiponectin mRNA and protein were detected in SC vs EPI fat [36], and this difference in regional fat distribution is very clear in WT HF vs KO HF mice. We conclude that, at least during this early phase of HF feeding, TNF-α deficiency does not increase insulin sensitivity by interacting with adiponectin.

The altered channeling of fat into adipose depots in TNF-α KO mice may have also contributed to their maintenance of insulin sensitivity. After 4 weeks of HF feeding, WT mice had more EPI fat and less SC fat than KO mice. Increases in the size of the EPI fat pad specifically have been linked both to the development of insulin resistance [6,7] and to increased expression of TNF-α mRNA and protein and reduced insulin sensitivity [37,38] in HF-fed mice. Together with the fact that the total WAT was similar in WT-HF and KO-HF mice at this time, these data suggest that TNF-α deficiency may confer protection from the development of HF feeding–induced insulin resistance by channeling lipid flux into the SC fat, which is less metabolically active [39,40]. As above, it is tempting to speculate that part of the mechanism maintaining the insulin sensitivity in the absence of TNF-α is related to an effect on regional fat distribution. This hypothesis indicates the importance of determining whether the redistributed adiposity in TNF-α KO mice is a cause of improved insulin sensitivity or merely a consequence of it.

In addition to the increase in SC fat pad, KO-HF mice also had more MES fat than WT-HF mice, which is at odds with the numerous reports that insulin resistance is more associated with visceral rather than SC fat. This hypothesis, however, is supported mainly by studies performed in obese humans or in rodent models of genetic obesity (see [41,42] for reviews, and thus at a much more advanced stage of adiposity and insulin resistance. The early increase in MES fat in KO-HF mice may in fact reflect the greater insulin sensitivity of these mice compared with WT mice, and the high metabolic activity of this depot could subsequently favor redistribution of fat into SC adipose tissue. Some data appear to be in accord with this idea. First, the basal lipolytic rate in the MES fat is higher than in any other region; and there are rapid changes in the flux of lipid in and out of this pad, reflecting meal-to-meal transients rather than long-term storage of triglycerides [40]. Second, the flux of lipid out of the MES fat seems to be channeled more toward the SC than EPI fat [43,44]. Third, short-term intravenous administration of TNF-α decreased lipolysis almost twice as much in EPI or RP than in SC fat [39]. Fourth, TNF-α has been reported to inhibit de novo lipogenesis and stimulation of free fatty acid release via lipolysis [45], which also could occur differentially across fat depots. Altogether, when challenged with HF feeding, an initial increase in MES fat in KO mice may reflect increased insulin sensitivity and, in the long term, help to retain whole-body insulin sensitivity by facilitating lipid deposition in the SC rather than the EPI fat. The specific mechanisms of this effect require further investigation.

Finally, several data suggest that TNF-α affects insulin sensitivity in humans as well as rodents. In particular, lean, nondiabetic subjects showed improved plasma insulin levels and insulin sensitivity indices after short-term treatment with the anti–TNF-α monoclonal antibody infliximab [46,47], in the absence of changes in BW or adiposity. These findings support the use of TNF-α antibody treatment to reduce the

incipient insulin resistance. Tumor necrosis factor–α antibody infusions, however, have not been proven successful in obese and/or insulin-resistant patients [48,49,50]. Results from these studies, however, should be interpreted with caution, as they involved only a single injection of one dose of antibody and were of short duration (2-4 weeks). Moreover, whether the TNF-α antibody neutralized systemic TNF-α activity or TNF-α locally released has not been investigated. This is important because both humoral and paracrine actions of TNF-α have been implicated in obesity (reviewed by Cawthorn and Sethi [45]). Finally, it may be relevant that insulin-sensitizing drugs such as thiazolidinediones also inhibit TNF-α signaling and expression in the adipose tissue [51]. As the incidence of obesity continues to climb, it is becoming increasingly clear that novel therapeutic approaches need to be developed. Many previous data support the idea that lowering circulating or tissue TNF-α levels may provide such an approach; the data presented here suggest that the efficacy of TNF-α–targeted treatments may be maximal during the early stages of obesity and insulin resistance.

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