Mice Heterozygous for Tumor Necrosis Factor-α Converting Enzyme Are Protected From Obesity-Induced Insulin Resistance and Diabetes

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OBJECTIVE—Tumor necrosis factor (TNF)-α is known to affect insulin sensitivity, glucose, and lipid metabolism through alternative and redundant mechanisms at both translational and post-translational levels. TNF-α exerts its paracrine effects once the membrane-anchored form is shed and released from the cell membrane. TNF-α cleavage is regulated by TNF-α converting enzyme (TACE), which regulates the function of several transmembrane proteins, such as interleukin-6 receptor and epidermal growth factor receptor ligands. The role of TACE in high-fat diet (HFD)-induced obesity and its metabolic complications is unknown.

RESEARCH DESIGN AND METHODS—To gain insights into the role of TACE in metabolic disorders, we used *Tace*$^{+/-}$ mice fed a standard or high-fat diet for 16 weeks.

RESULTS—We observed that *Tace*$^{+/-}$ mice are relatively protected from obesity and insulin resistance compared with wild-type littermates. When fed an HFD, wild-type mice exhibited visceral obesity, increased free fatty acid and monocyte chemoattractant protein (MCP)1 levels, hypoadiponectinemia, glucose intolerance, and insulin resistance compared with *Tace*$^{+/-}$ mice. Interestingly, *Tace*$^{+/-}$ mice exhibited increased uncoupling protein-1 and GLUT4 expression in white adipose tissue.

CONCLUSIONS—Our results suggest that modulation of TACE activity is a new pathway to be investigated for development of agents acting against obesity and its metabolic complications. ***Diabetes*** **56:2541–2546, 2007**

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AMPK, AMP-activated protein kinase; HFD, high-fat diet; IL, interleukin; Insr, insulin receptor; IRS, insulin receptor substrate; MCP, monocyte chemoattractant protein; Pref, preadipocyte factor; TACE, tumor necrosis factor-α converting enzyme; Timp, tissue inhibitor of metalloproteinase; TNF, tumor necrosis factor; UCP, uncoupling protein; WAT, white adipose tissue.

Several studies have suggested that obesity progressively leads to the recruitment of monocyte/macrophage in the visceral adipose tissue, thus initiating the low-grade inflammatory response that, through the expression of inflammatory cytokines such as tumor necrosis factor (TNF)-α (1,2), will consequently determine insulin resistance leading ultimately to diabetes and macro- and microvascular diseases (1). TNF-α is the major negative regulator of the insulin receptor pathway and of adiponectin expression (3–5). TNF-α is regulated at a post-transcriptional level by TNF-α converting enzyme (TACE; also known as ADAM-17), a transmembrane sheddase/metalloprotease, which increases soluble TNF-α versus membrane TNF-α and therefore induces paracrine and systemic inflammation (6–8). Tissue inhibitor of metalloproteinase (Timp)3 is able to block soluble TNF-α generation, therefore restraining the TNF system to the autocrine and juxtacrine activities exerted by membrane TNF-α, which acts mainly through the p75 receptor and is less efficient than soluble TNF-α in inducing insulin resistance and atherosclerosis (6–8). We have recently observed that a genetic transmission of Timp3 deficiency is able to impair glucose tolerance (9). Interestingly, Timp3 downregulation was found in adipose tissue of genetic mouse models of obesity, which may lead to the dysregulation of TACE/ADAM-17 activity (10). Therefore, we investigated the role of TACE in metabolic consequences of long-term, high-fat feeding. In this study, we show that *Tace*$^{+/-}$ mice are in part protected by diet-induced obesity and diabetes, identify the tissues where TACE seems to regulate TNF-α shedding, and provide evidence for other substrates involved in TACE metabolic effects.

RESEARCH DESIGN AND METHODS

Tace$^{+/-}$ mice on B6/SV129 background and metabolic tests were described previously (9). Mice were fed a standard laboratory diet (5% calories derived from fat) or a high-fat diet (HFD) (60% calories derived from fat; Research Diet, New Brunswick, NJ) for 16 weeks starting at 4 weeks of age. Hormone, cytokine, and metabolite levels were measured using commercial kits: insulin (Mercodia), adiponectin (Linco Research), free fatty acids (Wako), resistin, monocyte chemoattractant protein (MCP)1, and interleukin (IL)-6 (R&D Systems). Animal studies were approved by the University of Rome "Tor Vergata" animal care and use committee.

Western blots. Preparation of tissue lysates, immunoprecipitation, and immunoblot analysis were performed as described (9), using the following antibodies: anti–TNF-α (Endogen); anti–phosphoThr172 AMP-activated protein kinase (AMPK) (Cell Signaling Technology); anti-AMPK, anti–insulin receptor (Insr)β, anti–insulin receptor substrate (IRS)-1, and anti–IRS-2 (UBI);

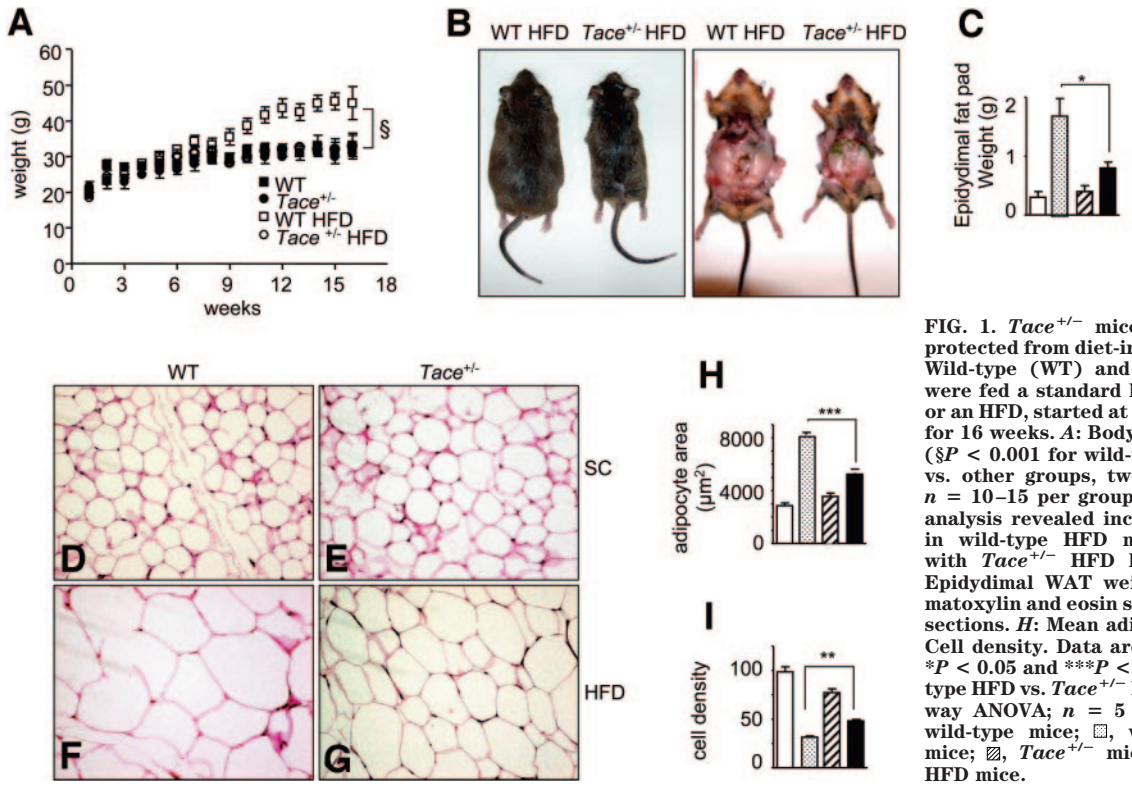

FIG. 1. *Tace*$^{+/-}$ mice are partially protected from diet-induced obesity. Wild-type (WT) and *Tace*$^{+/-}$ mice were fed a standard laboratory diet or an HFD, started at 4 weeks of age, for 16 weeks. *A*: Body weight curves (§$P < 0.001$ for wild-type HFD mice vs. other groups, two-way ANOVA; $n = 10–15$ per group). *B*: Necropsy analysis revealed increased fat pad in wild-type HFD mice compared with *Tace*$^{+/-}$ HFD littermates. *C*: Epididymal WAT weight. *D–G*: Hematoxylin and eosin staining of WAT sections. *H*: Mean adipocyte area. *I*: Cell density. Data are means ± SD. *$P < 0.05$ and ***$P < 0.001$ for wild-type HFD vs. *Tace*$^{+/-}$ HFD mice, one-way ANOVA; $n = 5$ per group. □, wild-type mice; ▨, wild-type HFD mice; ▨, *Tace*$^{+/-}$ mice; ■, *Tace*$^{+/-}$ HFD mice.

and anti–IL-6 receptor, anti–preadipocyte factor (Pref)-1, and anti-phosphotyrosine (Santa Cruz).

RNA extraction and real-time quantitative PCR analysis. Total RNA isolation and reverse transcription was previously described (9). Real-time PCR was carried out on individual samples using an ABI PRISM 7000 Sequence Detection system and TaqMan reagents (Applied Byosystems). Each reaction was carried out in triplicate using standard reaction conditions.

Adipose tissue histological analysis. Epididymal fat was obtained from 5- to 6-month-old mice, and specimens were fixed in 10% paraformaldehyde and embedded in paraffin. Ten-micrometer consecutive sections were then mounted on slides and stained with hematoxylin and eosin. Adipose cell size and density were calculated as described (11).

Statistical analysis. Statistical analyses were performed using ANOVA or unpaired Student's t test as indicated. Values of $P < 0.05$ were considered statistically different.

RESULTS

***Tace*$^{+/-}$ mice are protected from diet-induced obesity.** To assess the role of TACE in the development of obesity and its complications, *Tace*$^{+/-}$ mice and wild-type littermates, fed a standard laboratory diet or an HFD, were used because *Tace*-null mice survive only a few days after delivery due to severe developmental defects (12). Under standard diet conditions, weight gain was comparable in the two mouse models, but *Tace*$^{+/-}$ mice were significantly protected from obesity induced by an HFD (Fig. 1*A*) starting at 9 weeks and more evidently at 16 weeks; two-way ANOVA confirmed that both an HFD ($P < 0.01$) and *Tace* genotype ($P < 0.01$) contributed to the variation in body mass (Fig. 1*A*). Necropsy analysis after 16 weeks of an HFD revealed a higher fat content at 16 weeks in wild-type mice than in *Tace*$^{+/-}$ littermates (Fig. 1*B*). Consistently, epididymal adipose tissue weight was significantly decreased in HFD-fed *Tace*$^{+/-}$ mice compared with that in wild-type littermates (Fig. 1*C*). Histological examination of HFD adipose tissue sections showed a higher density of smaller adipocytes in *Tace*$^{+/-}$ HFD than in wild-type HFD mice (Fig. 1*D–G*); morphometric analysis confirmed that adipocyte size was significantly reduced by TACE haploinsufficiency (Fig. 1*H* and *I*).

Effect of TACE haploinsufficiency on insulin resistance and diabetes in diet-induced obesity. Since *Tace*$^{+/-}$ mice were significantly protected from HFD-induced obesity, we evaluated insulin resistance and glucose tolerance. Fed glucose, fasted, and fed insulin levels were significantly decreased in *Tace*$^{+/-}$ HFD mice compared with those in wild-type HFD mice (Fig. 2*A* and *B*). The homeostasis model assessment of insulin resistance index and free fatty acids were significantly higher in wild-type HFD than in *Tace*$^{+/-}$ HFD mice (Fig. 2*C* and *D*). These results confirmed the protective effect of TACE haploinsufficiency against diet-induced insulin resistance and diabetes. Both intraperitoneal glucose and intraperitoneal insulin tolerance tests (Fig. 2*E* and *F*) suggested that metabolic control was rescued, on an HFD, by TACE haploinsufficiency.

Effect of TACE haploinsufficiency and an HFD on adipose tissue. Next, we explored potential mechanisms explaining the favorable effect on adipose tissue morphology observed in *Tace*$^{+/-}$ mice. Analysis of food intake did not reveal any difference between wild-type and *Tace*$^{+/-}$ mice fed an HFD (data not shown). Analysis of TNF-α shedding revealed a higher content of the 17-kDa soluble TNF-α and of the 50-kDa soluble Pref-1 in wild-type HFD mice than in *Tace*$^{+/-}$ HFD littermates (Fig. 3*A* and *C*). Analysis of proximal insulin signaling revealed no alteration in Insr and IRS protein content. However, wild-type HFD compared with *Tace*$^{+/-}$ HFD littermates showed increased IRS-1 phosphorylation on serine 307, a hallmark of insulin resistance generated by soluble TNF-α (Fig. 3*B*). Observation of increased EGFR (epidermal growth factor receptor) phosphorylation in wild-type HFD mice compared with *Tace*$^{+/-}$ HFD littermates suggests that other TACE substrates belonging to the epidermal growth factor

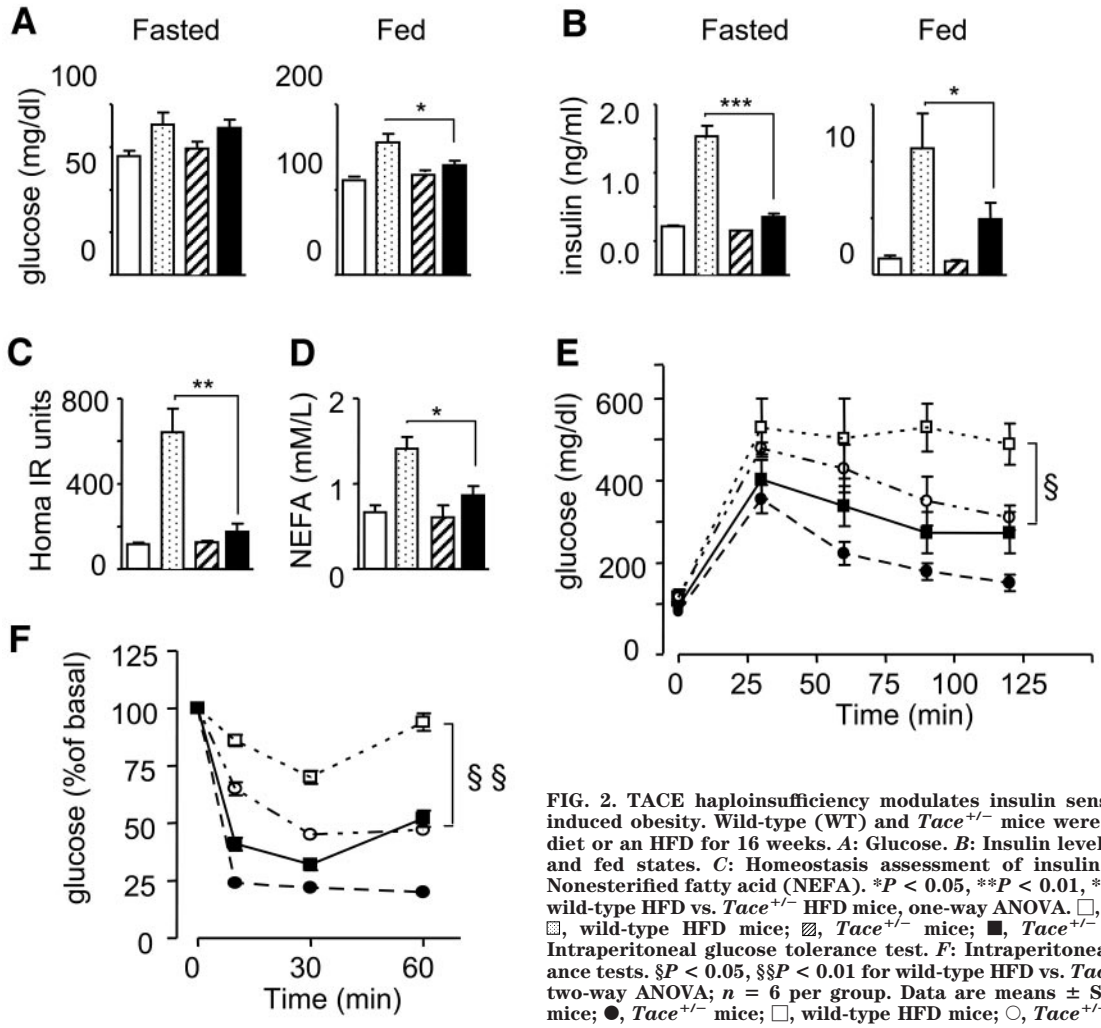

FIG. 2. TACE haploinsufficiency modulates insulin sensitivity in diet-induced obesity. Wild-type (WT) and *Tace*$^{+/-}$ mice were fed a standard diet or an HFD for 16 weeks. *A*: Glucose. *B*: Insulin levels in the fasting and fed states. *C*: Homeostasis assessment of insulin resistance. *D*: Nonesterified fatty acid (NEFA). **P* < 0.05, ***P* < 0.01, ****P* < 0.001 for wild-type HFD vs. *Tace*$^{+/-}$ HFD mice, one-way ANOVA. □, wild-type mice; ▨, wild-type HFD mice; ▨, *Tace*$^{+/-}$ mice; ■, *Tace*$^{+/-}$ HFD mice. *E*: Intraperitoneal glucose tolerance test. *F*: Intraperitoneal insulin tolerance tests. §*P* < 0.05, §§*P* < 0.01 for wild-type HFD vs. *Tace*$^{+/-}$ HFD mice, two-way ANOVA; *n* = 6 per group. Data are means ± SD. ■, wild-type mice; ●, *Tace*$^{+/-}$ mice; □, wild-type HFD mice; ○, *Tace*$^{+/-}$ HFD mice.

receptor ligands family may be involved in this phenotype (Fig. 3*D*). Next, we analyzed genes involved in the regulation of glucose/lipid metabolism, mitochondrial function, and inflammation. In white adipose tissue (WAT), we observed that wild-type mice fed an HFD showed significant lower levels of GLUT4, adiponectin, uncoupling protein-1 (UCP-1), and Pref-1 compared with *Tace*$^{+/-}$ HFD mice (Fig. 3*E*). By contrast, wild-type HFD mice exhibited a significant increase in MCP-1 and CD-68 compared with *Tace*$^{+/-}$ HFD littermates (Fig. 3*E*). Resistin and IL-6 expression was also increased, close to significance, in wild-type HFD mice compared with that in *Tace*$^{+/-}$ HFD littermates (Fig. 3*E*). Serum adiponectin levels in *Tace*$^{+/-}$ HFD mice were higher compared with those in wild-type HFD mice (Fig. 3*F*). Wild-type HFD mice exhibited significantly higher levels of MCP-1 and resistin but not IL-6 ($P < 0.08$, one-way ANOVA) compared with *Tace*$^{+/-}$ HFD littermates.

Effects of HFD on skeletal muscle and liver in *Tace*$^{+/-}$ and wild-type mice. Next, we inquired whether relative protection from obesity may also be dependent on TACE action in muscle and liver. TNF-α shedding and consequently IRS-1 phosphorylation on serine 307 were increased in liver but not in muscle of wild-type HFD mice compared with *Tace*$^{+/-}$ HFD littermates (Fig. 4*A*, *B*, *F*, and *G*). By contrast, we found that levels of membrane IL-6 receptor were downregulated in wild-type HFD mice compared with those in *Tace*$^{+/-}$ HFD littermates in both muscle and liver (Fig. 4*C* and *H*). AMPK phosphorylation on Thr172 in muscle and liver was reduced by an HFD in wild-type but not in *Tace*$^{+/-}$ mice (Fig. 4*D* and *I*). GLUT4 mRNA and fatty acid transport protein-1 levels were lower in muscle of wild-type HFD mice than of *Tace*$^{+/-}$ HFD littermates (Fig. 4*E*) ($P < 0.05$ for wild-type HFD vs. *Tace*$^{+/-}$ HFD mice).

Analysis of phosphotyrosine proteins in WAT, muscle, and liver. Phosphorylation levels of Insr/IRS-1 corresponding bands, assessed by immunoprecipitation with an anti-phosphotyrosine antibody followed by blotting with the same antibody, were significantly lower in WAT and liver and, to a lesser but significant extent, in muscle in wild-type HFD mice than in *Tace*$^{+/-}$ HFD littermates (Supplemental Fig. 1*A*–*D* [available in an online appendix at http://dx.doi.org/10.2337/db07-0360]).

DISCUSSION

Recent studies have suggested that activity of TACE/ADAM-17 or its inhibitor, Timp3, may be altered in humans affected by chronic metabolic and inflammatory diseases because of the observation of increased circulating levels of soluble forms of TACE substrates like TNF-α, soluble TNF receptor-1, and soluble IL-6 receptor (12–14). Inhibition of TACE by KB-R775 led to improved insulin sensitivity in a rat model of type 2 diabetes (15). Furthermore, it

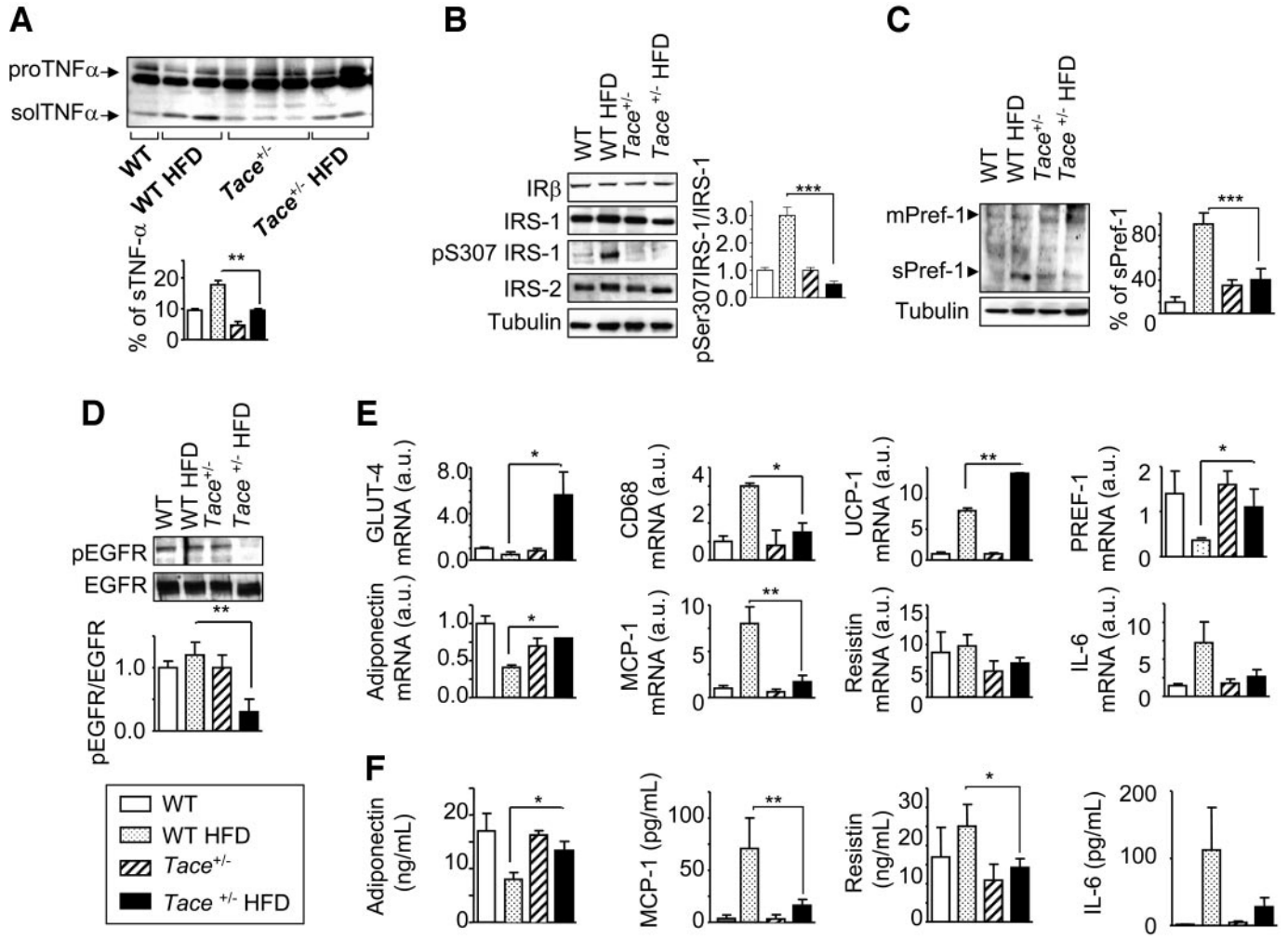

FIG. 3. Differential remodeling of adipose tissue in *Tace*$^{+/-}$ HFD mice compared with that in wild-type (WT) HFD mice. Wild-type and *Tace*$^{+/-}$ mice were fed a standard diet or an HFD for 16 weeks. *A*: Pro–TNF-α to soluble TNF-α conversion in WAT. *B*: Insulin signaling proteins content and IRS-1 phosphorylation on TNF-α–sensitive residue serine 307. *C*: Pref-1 shedding. *D*: Epidermal growth factor receptor phosphorylation. *E*: mRNA expression of metabolic and inflammatory genes. *F*: Serum levels of adipokines. Data are means ± SD. **$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ for wild-type HFD vs. *Tace*$^{+/-}$ HFD mice, one-way ANOVA; $n = 5$ per group.

has been suggested that TACE may regulate adipogenesis via shedding of Pref-1 (16).

Diet-induced obesity usually results first in hyperthophy of preexisting adipocytes and then, after cell sizes have reached a specific threshold, in hyperplasia, during which preadipocytes are recruited to generate small, insulin-sensitive adipocytes (11). On an HFD, *Tace*$^{+/-}$ mice showed reduced fat pad weight and increased small adipocytes number and cell density compared with wild-type mice. These effects may be determined by reduced soluble levels of TNF-α and Pref-1 in adipose tissue of *Tace*$^{+/-}$ mice. Our observation is in keeping with previous results suggesting that increased levels of membrane TNF-α in adipose tissue may be protective against obesity because of modulation of adipose tissue plasticity (4). It is also possible that a restrained release of soluble Pref-1, as observed in *Tace*$^{+/-}$ mice, may help to maintain a higher number of preadipocytes available for continuous differentiation during the chronic HFD. By contrast, the higher availability of soluble Pref-1 protein observed in wild-type mice at the end of the HFD is in keeping with the higher number of large insulin-resistant adipocytes compared with that in *Tace*$^{+/-}$ mice. Altogether, it is conceivable that in wild-type mice the combination of low Pref-1 mRNA expression with high Pref-1 soluble functional form may initially favor preadipocyte differentiation but then limit in the long-term the recruitment of new preadipocytes to form small insulin-sensitive adipocytes (15).

Our data also support a role for TACE in modulating some of the effects ascribed to TNF-α (16,17). The observation that *Tace*$^{+/-}$ mice maintain GLUT4 expression when fed an HFD is relevant because only soluble TNF-α is known to reduce GLUT4 expression in adipose tissue, while membrane TNF-α did not appear to impair insulin sensitivity (18–20). The increase in UCP-1 expression might also contribute to the leaner phenotype of *Tace*$^{+/-}$ mice, since UCP-1 expression was shown to be regulated in vitro by soluble TNF-α (21). UCP-1 expression in WAT was demonstrated to account for some protective effects against obesity and insulin resistance (22).

Adiponectin expression and circulating levels were preserved in *Tace*$^{+/-}$ mice, suggesting that adiponectin effects on fatty acid oxidation are maintained (5), as supported by our finding of unchanged AMPK activity in muscle and liver from *Tace*$^{+/-}$ mice fed an HFD. AMPK is known to be a major regulator of energy expenditure through fat oxidation, and adiponectin is a major stimulus for AMPK activation (23). Moreover, we observed that fatty acid

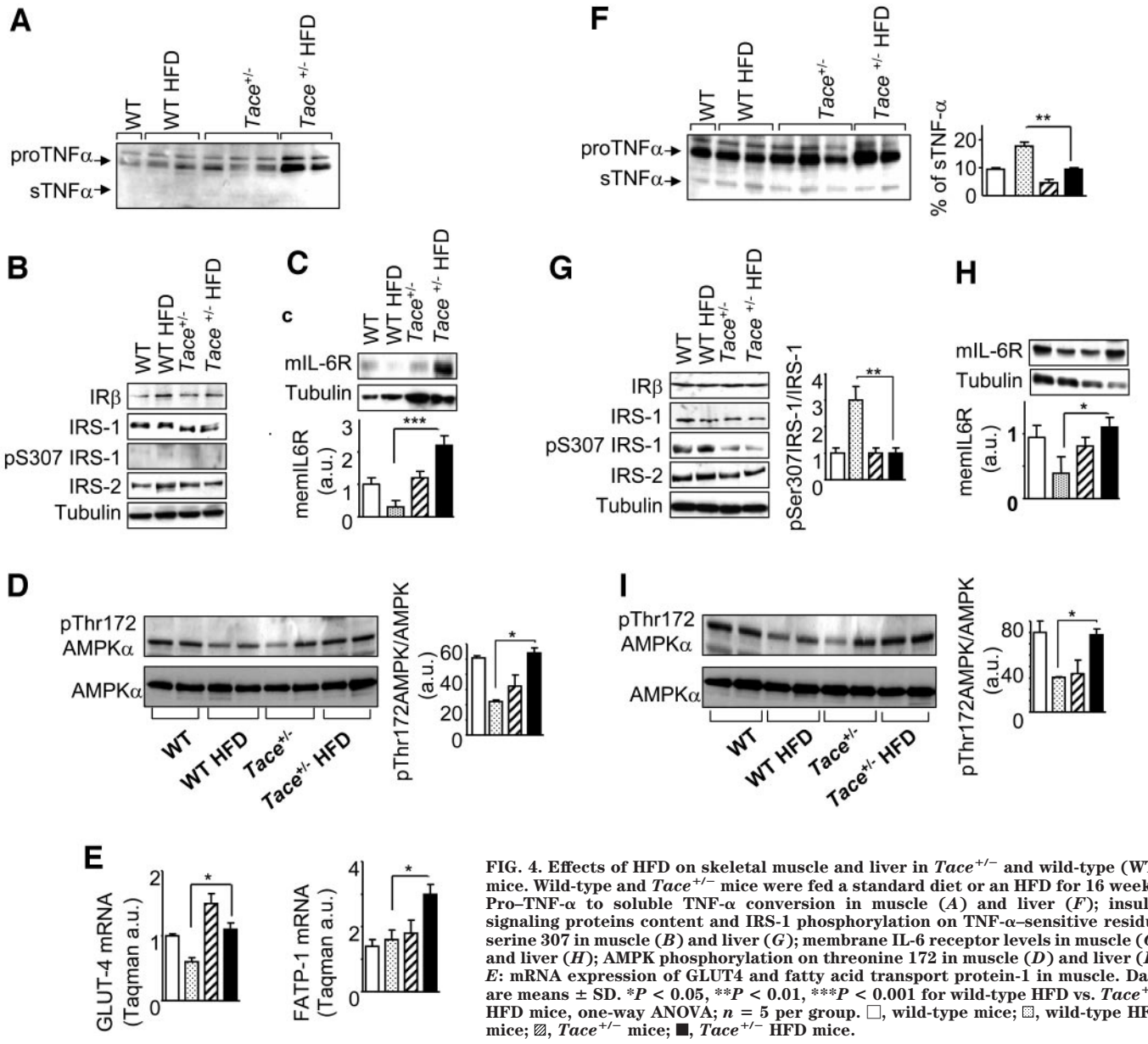

FIG. 4. Effects of HFD on skeletal muscle and liver in *Tace*$^{+/-}$ and wild-type (WT) mice. Wild-type and *Tace*$^{+/-}$ mice were fed a standard diet or an HFD for 16 weeks. Pro–TNF-α to soluble TNF-α conversion in muscle (*A*) and liver (*F*); insulin signaling proteins content and IRS-1 phosphorylation on TNF-α–sensitive residue serine 307 in muscle (*B*) and liver (*G*); membrane IL-6 receptor levels in muscle (*C*) and liver (*H*); AMPK phosphorylation on threonine 172 in muscle (*D*) and liver (*I*). *E*: mRNA expression of GLUT4 and fatty acid transport protein-1 in muscle. Data are means ± SD. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 for wild-type HFD vs. *Tace*$^{+/-}$ HFD mice, one-way ANOVA; *n* = 5 per group. □, wild-type mice; ▨, wild-type HFD mice; ▨, *Tace*$^{+/-}$ mice; ■, *Tace*$^{+/-}$ HFD mice.

transport protein-1 expression in skeletal muscle was preserved in *Tace* heterozygous mice fed an HFD, another finding consistent with previous observations on the links between adiponectin and TNF-α actions (5). Interestingly, we found that both liver and muscle from *Tace*$^{+/-}$ mice exhibit reduced shedding of IL-6 receptor. A higher level of IL-6 receptor may help to maintain insulin-sensitizing effects of gp130 cytokines such as IL-6 itself and CNTF (24), which are known to positively regulate fat oxidation and glucose metabolism in muscle and liver.

Analysis of TNF-α shedding coupled with analysis of mechanisms responsible for TNF-α direct downregulation of insulin signaling, such as IRS-1 phosphorylation on serine 307, also help to identify how modulation of TACE action on TNF-α may downregulate insulin signaling in metabolic tissues. In this model, we propose that a TACE effect is predominant in WAT and liver. Other cytokines such as angiotensin II are known to impair tissue specific insulin action via serine phosphorylation of IRS proteins (1,25).

In conclusion, our data suggest that an interference with the release of TACE substrates such as TNF-α, Pref-1, and IL-6 receptor partially protect from obesity and insulin resistance caused by an HFD, in part as a consequence of a positive effect on adipose tissue plasticity and in part via the modulation of different factors including adiponectin, UCP-1, GLUT4, and AMPK.

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