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Inflammatory Pathways Regulated by Tumor-Necrosis Receptor Associated Factor 1 Protect from Metabolic Consequences in Diet-Induced Obesity

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Abstract

Rationale—The co-incidence of inflammation and metabolic derangements in obese adipose tissue has sparked the concept of met-inflammation. Previous observations, however, suggest that inflammatory pathways may not ultimately cause dysmetabolism.

Objective—We have revisited the relationship between inflammation and metabolism by testing the role of Tumor-Necrosis Receptor associated Factor (TRAF)-1, an inhibitory adapter of inflammatory signaling of TNFα, IL-1β, and TLRs.

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Disclosures

None.

Methods and Results—Mice deficient for TRAF-1, which is expressed in obese adipocytes and adipose tissue lymphocytes, caused an expected hyper-inflammatory phenotype in adipose tissue with enhanced adipokine and chemokine expression, increased leukocyte accumulation, and potentiated pro-inflammatory signaling in macrophages and adipocytes in a mouse model of diet-induced obesity (DIO). Unexpectedly, TRAF-1$^{-/-}$ mice were protected from metabolic derangements and adipocyte growth, failed to gain weight, and showed improved insulin resistance – an effect caused by increased lipid breakdown in adipocytes and UCP-1-enabled thermogenesis. TRAF-1-dependent catabolic and pro-inflammatory cues were synergistically driven by β3-adrenergic and inflammatory signaling, and required the presence of both, TRAF-1-deficient adipocytes and macrophages. In human obesity, TRAF-1-dependent genes were upregulated.

Conclusions—Enhancing TRAF-1 dependent inflammatory pathways in a gain-of-function approach protected from metabolic derangements in DIO. These findings identify TRAF-1 as a regulator of dysmetabolism in mice and humans and question the pathogenic role of chronic inflammation in metabolism.

Keywords

Metabolic syndrome; adipose; tissue inflammation; adipocytes; lipolysis; TRAF-1

Subject Terms

Basic Science Research; Inflammation; Metabolism; Obesity; Vascular Biology

Introduction

Obesity and its metabolic complications, Type 2 Diabetes, hyperlipidemia, and hepatic steatosis, fuel cardiovascular mortality and morbidity[1]. Many clinical and experimental observations have established an association between visceral obesity and a chronic inflammatory response in obese adipose tissue that enhances the risk of atherosclerosis, myocardial infarction, and stroke[2]. Inflammation of visceral adipose tissue (VAT) results in the secretion of pro-inflammatory cytokines and chemokines from tissue-resident cells[2, 3], which promote the recruitment of immune cells or activate inflammatory signaling networks[4]. While the temporal and functional dynamics of VAT-resident leukocytes have been well defined[5], the inflammatory signaling pathways that cause adipose tissue inflammation are less clear. Particularly, it remains controversial how chronic inflammation affects metabolism. For instance, neutralizing pro-inflammatory pathways driven by TNFα, IL-1β, or IL-6 has been reported to aggravate metabolic complications or not to be effective in clinical trials[6–8]. These results suggest that pro-inflammatory signaling may not be exclusively pathogenic in obesity. We therefore re-visited the role of Tumor-Necrosis Factor Receptor-associated Factors (TRAFs) in VAT-inflammation. TRAFs represent intracellular signaling adapters that can bundle signaling events from up-stream pro-inflammatory signals including TNFα, IL-1β, and TLRs[9]. TRAFs showed controversial outcomes in acute and chronic cardio-metabolic inflammation[10, 11], raising the possibility that inflammation may improve metabolism.

Methods

All data and methods used in the analysis, and materials used to conduct the research will be made available to any researcher for the purpose of reproducing the results or replicating the procedures. All data, methods, materials are available upon personal request at the University Heart Centre of Freiburg (contact: dennis.wolf@universitaets-herzzentrum.de).

A Methods section is available in the Online Supplement.

Results

Genetic deficiency of TRAF-1 protects from diet-induced obesity

To interrogate a role of TRAFs in VAT-inflammation, we screened for gene-expression of the known TRAFs 1–7 in epiVAT of male C57BL/6J mice after consumption of a high fat diet (HFD) or a standard diet (chow) for 12 weeks. *Traf1* expression rose ~10-fold, while the expression of other TRAFs was not or only modestly regulated (Figure 1a,b). TRAF-1 protein and gene-expression were detectable in adipose tissue lymphocytes and adipocytes from epiVAT in confocal microscopy and qPCR (Online Figure I,II). We observed a positive correlation of the *Traf1* gene with *Ccl2* (MCP-1/CCL2) and *Adgre1* (macrophage-marker F4/80) (Figure 1c,d), suggesting that TRAF-1 is associated with adipose tissue inflammation.

We next aimed to clarify whether TRAF-1 directly contributes to DIO in rodents. Therefore, 8-week-old male wild type (WT) and TRAF-1$^{-/-}$ mice consumed a HFD for 12 weeks. TRAF-1-deficient mice were protected from absolute and relative weight gain (Figure 1e–g, Online Figure III) – an effect caused by smaller peripheral fat depositions, decreasing fat mass in MRI imaging and MRI body composition analysis, decreased ectopic lipid accumulation, and lowered levels of the adipocyte-specific leptin, but not by a decrease of lean tissue or organ size (Figure 1h–k, Online Figure III, IV, Online Table I). This loss of fat tissue was not caused by a lower food intake (Online Figure V). In accord with improved DIO, TRAF-1$^{-/-}$ mice demonstrated improved insulin resistance in an in vivo insulin-challenge (Figure 1l,m) and diminished glucose and insulin levels in fasting mice (Figure 1n,o, Online Figure VI). Accordingly, gene-expression for glucose transporter (GLUT)-2 (*Slc2a2*) and insulin receptor (IRS)-2 (*Irs2*) increased in TRAF-1$^{-/-}$ epiVAT (Online Figure VI). In addition, TRAF-1-deficiency improved liver steatosis (Figure 1p, Online Figure IV). While TRAF-1$^{-/-}$ mice on a standard chow diet for 12 weeks had a comparable weight to WT mice, we detected a higher food intake, lower physical activity, and aggravated glucose utilization in these mice, rendering the beneficial metabolic effects in the HFD-group as diet-specific (Online Figure VII). Collectively, our findings identify that TRAF-1 contributes to diet-induced obesity and its metabolic consequences.

TRAF-1-deficiency induces a hyper-inflammatory phenotype in VAT

To test whether the metabolically beneficial phenotype of TRAF-1$^{-/-}$ mice translates into an improved inflammatory response, we quantified the number of leukocytes in epiVAT of WT and TRAF-1$^{-/-}$ mice. Surprisingly, TRAF-1-deficient mice presented higher numbers of leukocytes (Figure 2a) accumulating in crown-like structures in epiVAT (Figure 2b,c),

suggesting enhanced inflammation. Flow cytometry of digested epiVAT confirmed higher numbers of obesity-specific CD11c^{+} pro-inflammatory adipose tissue macrophages (ATMs, Figure 2d–f), which was not caused by larger reservoirs of myeloid cells in the circulation, spleen, or bone marrow (Online Figure VIII). We observed an enhanced phosphorylation of the inflammatory MAP-kinase JNK (Figure 2g, Online Figure IX), higher epiVAT gene-expression for the adipokines TNFα, IL-6, and MCP-1 (Figure 2h), and for chemokines, TNF-superfamily members, and TLRs in adipocytes from TRAF-1$^{-/-}$ epiVAT (Figure 2i,j). FACS-sorted macrophages from epiVAT showed increased gene-expression for *Nos1*, the coding gene for the driver for in vitro pro-inflammatory M1-polarization, iNOS, and *Ccl2, Il6, and Il12a* (Figure 2k). Liver inflammation was not enhanced (Online Figure X), indicating a VAT-specific effect.

To address whether this phenotype in TRAF-1$^{-/-}$ macrophages was caused indirectly by adipocytes, we generated bone-marrow chimeras by transplanting WT/CD45.1 and TRAF-1$^{-/-}$/CD45.2 bone marrow into lethally irradiated WT/CD45.1 mice (Figure 2l). This strategy allowed us to selectively study TRAF-1$^{-/-}$ ATMs in a microenvironment with TRAF-1-competent adipocytes. Flow cytometry of digested epiVAT after 12 weeks of HFD revealed a relative increase of TRAF-1$^{-/-}$CD11c^{+} ATMs (Figure 2m–o) with a pro-inflammatory gene-expression (Figure 2p). ATMs in TRAF-1$^{-/-}$ mice showed amplified inflammatory gene-expression after 4 weeks of HFD even in the absence of obesity (Online Figure XI), indicating that the hyper-inflammatory phenotype precedes metabolic changes. In genetically chimeric mice generated by bone marrow transplantations, only these mice with a deficiency of TRAF-1 in hematopoietic cells, but not in stromal/vascular cells (including adipocytes), showed the hyper-inflammatory VAT-phenotype (Online Figure XII, XIII). These findings establish that TRAF-1-deficiency boosts ATM-inflammation.

TRAF-1$^{-/-}$ deficiency induces lipolysis and increases energy expenditure

To assess how TRAF-1-deficiency protects from obesity, we asked how TRAF-1 alters adipocyte remodeling during obesity. We observed that the average adipocyte diameter and size distribution in sections of epiVAT shifted towards smaller adipocytes in TRAF-1$^{-/-}$ mice (Figure 3a–c). *Traf1* mRNA abundance rose in differentiated adipocyte-like 3T3L1 cells (Figure 3d) and in adipocytes from obese epiVAT (Figure 3e). While we observed no relevant modulation of genes involved in lipid synthesis in epiVAT (data not shown), we detected increased plasma levels of the triglyceride (TG) metabolites free fatty acids (FFAs) (Online-Figure XIVa,b) and an increase of the FFAs/triglyceride ratio in epiVAT-lysates from TRAF-1$^{-/-}$ mice (Online Figure XIVc), indicative of a higher lipid break-down in TRAF-1$^{-/-}$ adipocytes.

Lipid breakdown of TGs into FFAs is regulated by adrenergic signaling and an upregulation of the lipolysis key enzymes hormone-sensitive lipase (HSL) and adipocyte triglyceride lipase (ATGL)[12]. Following an intraperitoneal injection of isoproterenol, a β3-adrenoceptor-agonist, FFAs and glycerol were higher in TRAF-1$^{-/-}$ compared with WT mice (Figure 3g,h), indicating that TRAF-1$^{-/-}$ adipocytes are more prone to lipolysis. This was confirmed in an in vitro lipolysis assay of epiVAT (Online Figure XIVd). Obese TRAF-1$^{-/-}$ adipocytes

from epiVAT showed higher gene-expression of HSL (*Lipe*) and ATGL (*Pnpla2*) (Figure 3g), as well as more Perilipin, which is required for FFA-trafficking (Figure 3i).

An excess of FFAs induces expression of uncoupling-protein-1 (UCP-1) in BAT, which boosts energy expenditure[13]. We detected higher UCP-1 mRNA (*Ucp1*) in iBAT (Figure 3j), augmented energy expenditure, and physical activity in obese TRAF-1$^{-/-}$ mice, but not in lean or TRAF-1$^{-/-}$ mice consuming chow (Figure 3k,l, Online Figure VII, XV). Notably, lipolysis in BAT itself was not changed in TRAF-1$^{-/-}$ mice compared with WT mice (Online Figure XIVe).

Adrenergic signaling and an excess of FFAs initiate thermogenesis[14]. Accordingly, injection of the β3-agonist noradrenalin induced a higher body temperature in TRAF-1$^{-/-}$ compared with WT mice (Figure 3n). During cold adaption, TRAF-1$^{-/-}$ mice had a higher body temperature and increased expression of thermogenic genes in iBAT (Figure 3m, Online Figure XVI), while we did not find consistently increased expression of genes indicating browning of adipose tissue in ingSAT of TRAF-1$^{-/-}$ mice (Online Figure XVII). These data establish an enhanced lipid breakdown and increased energy expenditure as cause for improved obesity in TRAF-1$^{-/-}$ mice.

Finally, we asked how increased inflammation in TRAF-1$^{-/-}$ VAT contributes to this effect: First, neither a selective deficiency of TRAF-1 in BM-derived, nor in stromal/vascular cells, sufficed to increase lipolytic gene-expression to the extend observed in a global deficiency (Online Figure XVIII). Second, only inflammatory priming with TNFα increased gene-expression of adrenergic-receptor-β3 (*Adrb3*) selectively in TRAF-1$^{-/-}$ adipocytes (Online Figure XIXa). Third, Both, adrenergic and inflammatory signaling, synergistically induced pro-inflammatory gene-expression in epiVAT (Online Figure XIXb). Fourth, in *in-vitro* cultivation of epiVAT with conditioned media from macrophages, only the combination of TRAF-1$^{-/-}$ macrophages and TRAF-1$^{-/-}$ epiVAT induced lipolysis to a relevant extend (Online Figure XIXc). These findings indicate that TRAF-1-dependent inflammation in macrophages and lipolytic pathways in adipocytes act synergistically in the context of β-adrenergic signaling and lipolysis.

TRAF-1 associated signaling pathways are enriched in human obesity

To clarify whether the TRAF-1 pathway associates with human obesity, we tested the enrichment of genes associated with the TRAF pathway in a gene array of human subcutaneous adipose tissue RNA from 7 healthy and 16 obese donors[15] by GSEA. We found that genes in the TRAF-1 to -7 and in the TRAF-1 pathway (Online Table II) were higher expressed in obese donors (Figure 4a,b, FDR<0.2). Accordingly, TRAF-1 expression in obese SAT tissue lysates (Figure 4c) and isolated subcutaneous adipocytes (Figure 4d) was higher in donors with a higher BMI. We next asked whether TRAF-1 expression could serve as biomarker for human obesity. We screened mRNA from PBMCs of participants of the TRAFICs study, a collective of 304 individuals with a high prevalence of the metabolic syndrome (Online Table III). We found that TRAF-1 expression was highest in patients diagnosed with 4 or more clinical components of the metabolic syndrome (Figure 4e). TRAF-1 correlated with the BMI (Figure 4f), and fasting plasma lipids (Figure 4g,h), suggesting an association with human obesity and dyslipidemia.

Discussion

A hallmark of adipose tissue inflammation is the accumulation of leukocytes and the expression of inflammatory mediators that perpetuate the inflammatory response by direct effects on tissue-resident cells or by enhanced leukocyte recruitment[4]. Adipose tissue inflammation is associated with adipose tissue remodeling and insulin resistance. Disruption of immune cell function can improve obesity[5]. However, it is not clear, whether inflammation is detrimental under every circumstance. This is best illustrated by the classical pro-inflammatory cytokine TNFα that directly counter-acts insulin signaling in adipocytes. Its inhibition reduced DIO in rodents[16], but the genetic knock-out of its receptors aggravated VAT-inflammation[8], raising the possibility that inflammation may in part be beneficial in DIO. Here, we have chosen to target TRAF-1 that inhibits several pro-inflammatory signaling cascades[9]. Expectedly, TRAF-1-deficiency aggravated inflammation of obese VAT[10], but unexpectedly it improved metabolism, protected from obesity, and decreased ectopic lipid depositions by an induction of lipolytic pathways. This co-incidence of increased inflammation and beneficial adipocyte remodeling is not entirely unexpected: Low-grade inflammation is required to protect from adipocyte dysfunction[17]. Biologically, inflammation-induced lipolysis is needed to release energy resources during stress and infection[18]. In line, TNFα directly stimulates lipolysis in adipoctes[19]. Lipolysis itself may also trigger inflammatory pathways by enhancing myeloid cell infiltration[20]. Yet, rampant VAT-inflammation may surpass these protective effects of low-grade inflammation[21].

TRAF-1-deficient macrophages showed increased expression of inflammatory genes that have been attributed to an in vitro pro-inflammatory M1-like polarization, such as *Nos1*, *Il12a*, and *Il6*. Our findings indicate that the enhanced inflammatory cytokine secretion in TRAF-1$^{-/-}$ macrophages was necessary to increase ADRB3 expression on TRAF-1$^{-/-}$ adipocytes, leading to an enhanced responsiveness to adrenergic signaling. As a result, we detected higher lipolytic gene expression, lipolysis, and a higher release of the TG-metabolites glycerol and FFAs. FFAs increase thermogenesis in BAT by an UCP-1 dependent pathway[13], even in the absence of enhanced lipolysis in BAT itself[22]. Accordingly, we detected more *Ucp1* transcripts in BAT and enhanced thermogenesis.

To confer a metabolic benefit, a deficiency of TRAF-1 was necessary in both, macrophages and adipocytes, while a selective deficiency in hematopoietic cells, including macrophages, sufficed to increase epiVAT-inflammation, but did not protect from obesity. To validate these findings and to test whether a cell-specific inhibition of TRAF-1 would remain effective, future studies will have to employ cell specific strategies, e.g. by Cre/lox-mediated conditional knock-outs. This is also important, because TRAF-1 dependent mechanisms seem to be tissue-specific: TRAF-1-deficiency aggravated LPS-induced lung inflammation[10], while TRAF-1$^{-/-}$ mice on an obesity-prone *Ldlr*$^{-/-}$ background mice were protected from atherosclerosis[11]. Because atherosclerosis is driven by obesity and hyperlipidemia, TRAF-1$^{-/-}$ mice may primarily be protected by the improved metabolism independent of enhanced inflammation, or by a protective phenotype of TRAF-1 deficient macrophages specifically in the atherosclerotic plaque[11]. The effects observed in our study seem to depend on an obese phenotype, since we did not observe hyper-inflammation in VAT or an improved metabolism in TRAF-1$^{-/-}$ mice consuming a chow diet. Yet, an

increased food intake, lowered physical activity, and aggravated glucose utilization in these mice point towards a (modest) general role for TRAF-1 in affecting basal energy metabolism. However, these effects do not explain the phenotype of obese TRAF-1$^{-/-}$ mice. A panel of 1440-SNPs, which revealed a C57Bl/6J-identity of >99.5%, furthermore ruled out an incongruent genetic background as potential bias.

Collectively, we present the unexpected finding that a deficiency of the inhibitory adapter TRAF-1 induced hyper-inflammation in obese VAT and attenuated metabolic derangements. The underlying catabolic pathway was synergistically driven by inflammatory and adrenergic signaling. Our findings identify a dissociation of metabolism and rampant inflammation and propose that pro-inflammatory pathways in macrophages and lipolytic pathways in adipocytes are both limited by TRAF-1 under steady-state conditions. TRAF-1 dependent pathways were up-regulated in human obesity, rendering our results relevant for human disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms

ATM	Adipose Tissue Macrophage
BAT	Brown adipose tissue
FFA	Free-fatty acids
HFD	High-fat diet
SAT	Subcutaneous adipose tissue
VAT	Visceral adipose tissue
prVAT	Peri-renal VAT
epiVAT	Epididymidal VAT
iBAT	Induced BAT
ingSAT	Inguinal SAT
TRAF	Tumor-Necrosis Receptor Associated Factor

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Novelty and Significance

What Is Known?

- Obesity is accompanied by a chronic, low-grade inflammation in adipose tissue.
- It is unclear whether increased adipose tissue inflammation directly aggravates obesity and its metabolic complications, such as insulin resistance.

What New Information Does This Article Contribute?

- A genetic absence of the inhibitory signalling adaptor Tumor-Necrosis Receptor Associated Factor 1 (TRAF-1), which limits several inflammatory pathways, is associated with sustained inflammation of mouse adipose tissue.
- TRAF-1-deficient mice on high fat diet showed increased lipolysis in inflamed adipose tissue, enhanced lipid breakdown in adipocytes, and were protected from obesity and insulin resistance.
- Inflammatory cytokines released by TRAF-1-deficient adipose tissue macrophages were required to promote lipolytic pathways in TRAF-1-deficient adipocytes.

Human obesity and insulin resistance are associated with inflammation in adipose tissue. Therefore, it has been proposed that inflammatory pathways promote obesity and dysmetabolism. In this study, in a gain-of-function model of inflammation by knocking-out the inhibitory anti-inflammatory adapter TRAF-1, we found that enhanced inflammation can synergize with lipolytic pathways to increase the break-down of lipids in adipocytes, reduce adipose tissue depots, and improve obesity and its metabolic complications. Thus, inflammation of obese adipose tissue is not necessarily detrimental, but may instead be required to maintain a beneficial remodelling of adipose tissue. These findings question the overall pathogenic role of inflammation in cardio-metabolic disease.

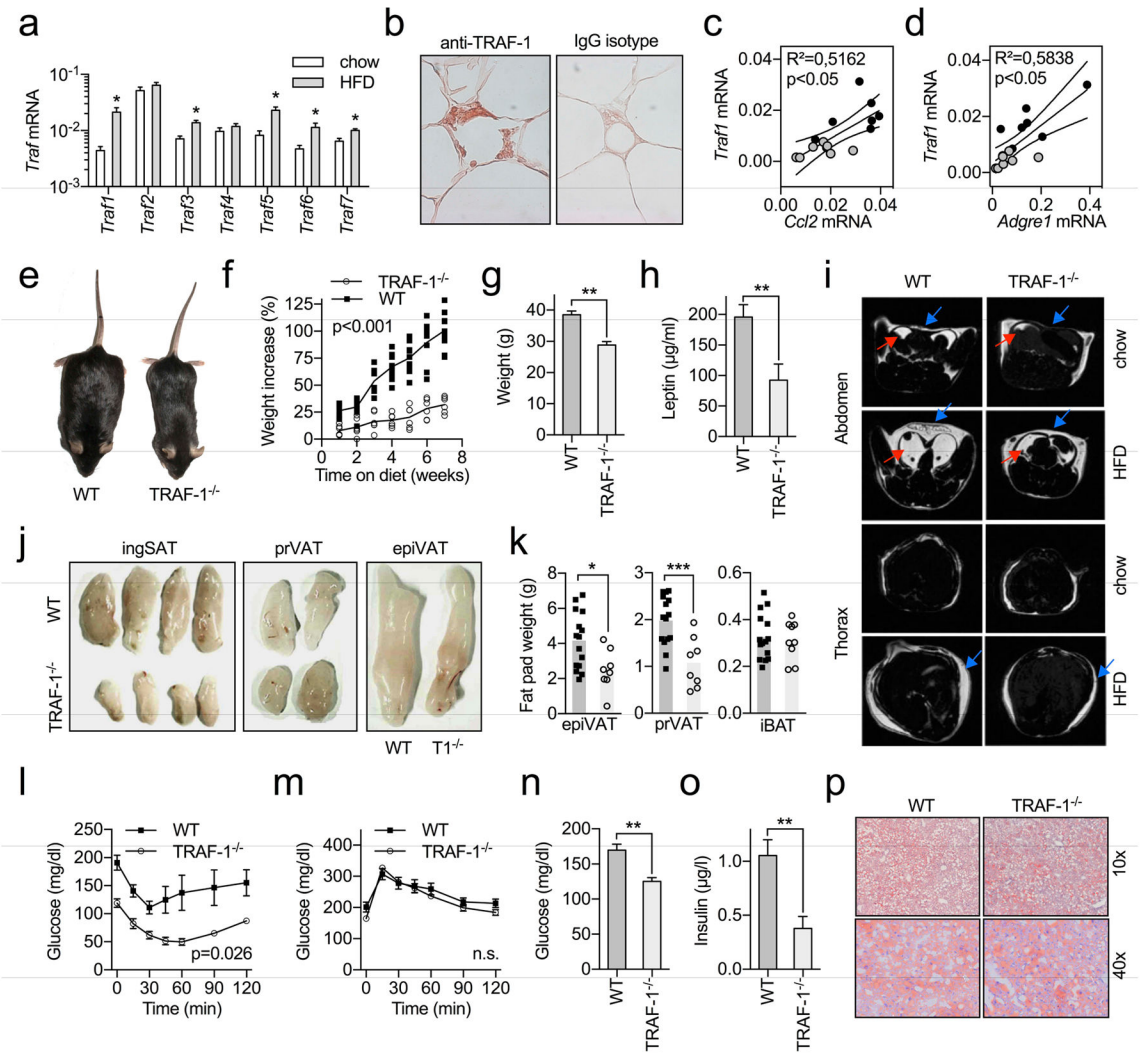

Figure 1. TRAF-1-deficiency protects from DIO

(**a**) mRNA expression of *Traf1* was quantified by qPCR and normalized to *Gapdh* in epiVAT from male WT mice (C57BL/6J) after a chow or a high-fat diet (HFD) for 12 weeks. (**b**) Immunohistochemistry for TRAF-1 or IgG in sections of obese mouse epiVAT. (**c, d**) Correlation of *Traf1* and *Adgre1* or *Ccl2* gene-expression in lean (grey) or obese (black) epiVAT from male WT mice (C57BL/6J) after feeding for 12 weeks. (**e**) 8-weel old male TRAF-1-deficient (TRAF-1$^{-/-}$) or wildtype (WT) mice on a C57BL/6J background consumed a HFD for 12 weeks. (**f**) Increase of body weight (%). (**g**) Total body weight. (**h**) Leptin fasting plasma levels. (**i**) VAT (red arrow) and SAT (blue arrows) was imaged by MRI (white signal). (**j, k**) Fat pad weight. (**l**) Insulin tolerance testing (ITT) and (**m**) glucose tolerance testing (GTT). Injections were based on total body lean tissue weight. (**n, o**) Glucose and Insulin levels in fasting animals. (**p**) Liver sections stained with Oil-Red-O (ORO) and hematoxylin. Significance was assessed by an unpaired T-test (**g,k,h,n,o**), multiple T-Test with Holm-Sidak correction (**a**), repeated 2-way-ANOVA (**f,l,m**). *p<0.05, **P<0.01, ***p<0.001, n=8/8(**a**), n=15/11(**f,g,h,o**), n=15/8(**k**), n=6/6(**l,m**), n=10/10 (**n**), for WT/TRAF-1$^{-/-}$, respectively.

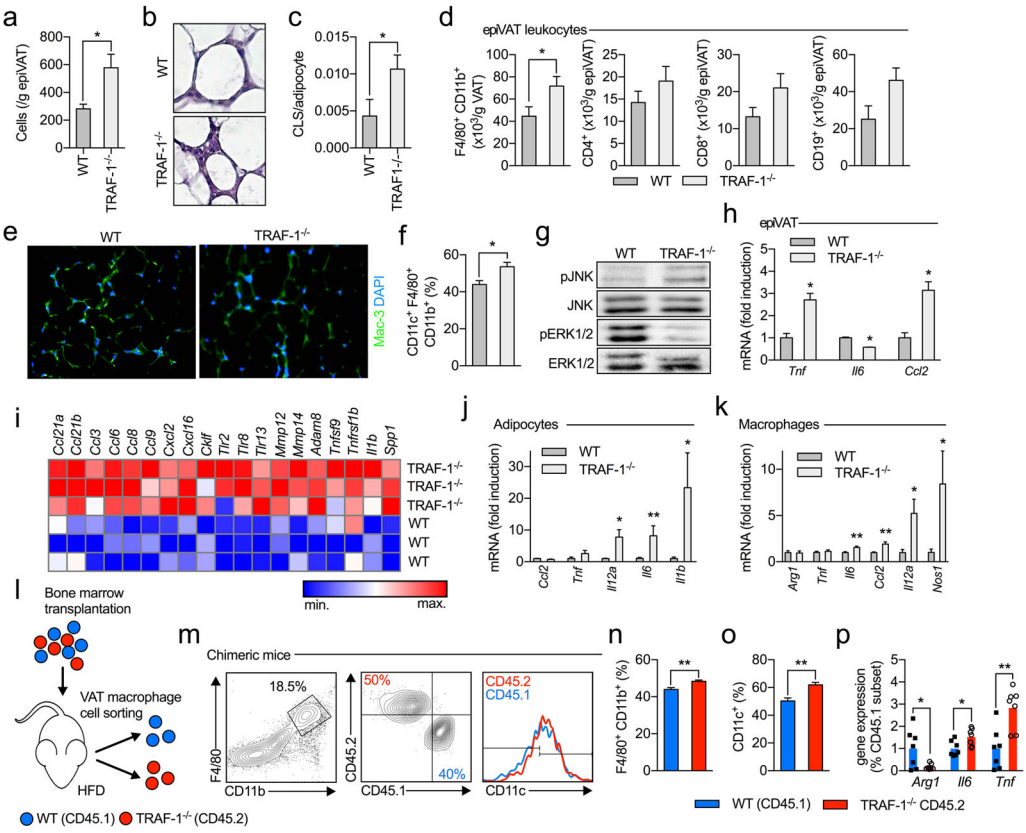

Figure 2. A deficiency of TRAF-1 induces a hyper-inflammatory phenotype in VAT

8-week-old male WT and TRAF-1$^{-/-}$ mice (C57BL/6J) consumed a HFD for 12 weeks. (**a**) Leukocyte numbers (/g tissue) in epiVAT. (**b, c**) H&E-staining of epiVAT sections and quantification of crown-like structures (CLSs). (**d**) Leukocyte subsets were identified by flow cytometry of epiVAT cells after tissue digestion. (**e**) Confocal microscopy of epiVAT sections. Mac-3 was used as macrophage-marker. (**f**) Surface expression of CD11c on ATMs from epiVAT in flow cytometry. (**g**) Western blot on epiVAT-lysates. Gene-expression was quantified by (**h**) qPCR on whole tissue epiVAT mRNA-preparations, (**i**) gene chip array on isolated epiVAT-adipocytes, (**j**) qPCR on selected targets in isolated and TNF-α stimulated epiVAT-adipocytes or (**k**) isolated epiVAT-ATMs. (**l**). To selectively test TRAF-1$^{-/-}$ and WT macrophage gene-expression in the same animal, chimeric animals were generated by bone marrow transplantation from TRAF-1$^{-/-}$(CD45.2) and WT(CD45.1) mice (C57Bl/6J). Mice were fed for 12 weeks with a HFD. (**m**) Gating strategy for cell sorting. (**n**) Infiltration of epiVAT with CD11b^{+}F4/80^{+} macrophages, expressed as % of all epiVAT-leukocytes. (**o**) Expression of CD11c^{+} on epiVAT-ATMs in flow cytometry. (**p**) Gene-expression in isolated epiVAT ATMs. Significance was assessed by a T-test (**a,c,f,h,n–p**), or Mann-Whitney Test (**d,j,k**). *p<0.05, **p<0.01. n=15/11(**a**), n=12/12(**b**), n=12/8(**d**), n=7/7(**f,n,o,p**). n=5/4(**h**), n=8/8(**j,k**), for WT/TRAF-1$^{-/-}$, respectively. Representative pictures from n=7 per group (**g**).

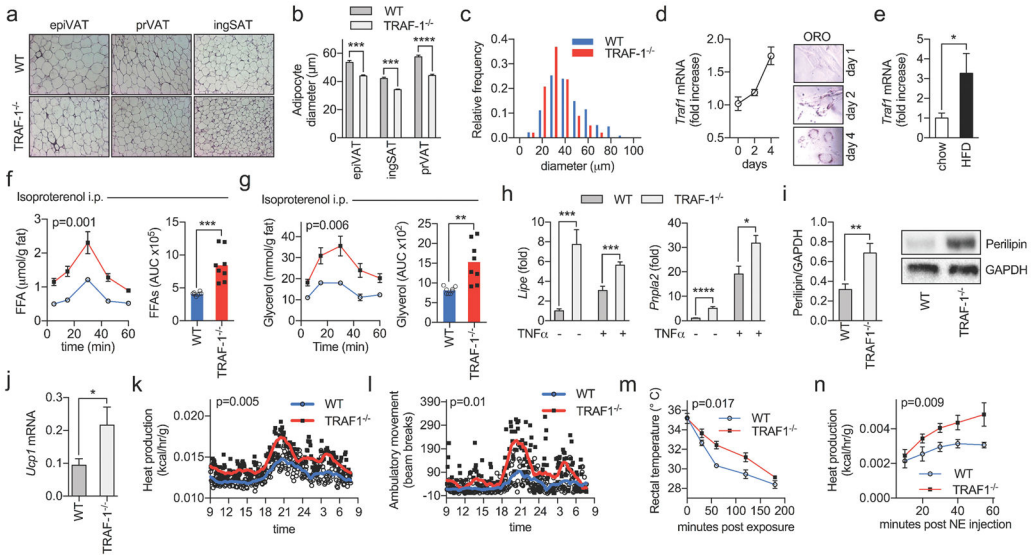

Figure 3. TRAF-1-deficiency induces lipolysis and increases energy expenditure

8-week-old male WT and TRAF-1$^{-/-}$ mice (C57BL/6J) consumed a HFD for 12 weeks. (**a**) H/E-staining in sections of epiVAT, prVAT, and ingSAT. (**b**) Average adipocyte and (**c**) adipocytes cell size distribution in epiVAT tissue sections. (**d**) *Traf1* mRNA-expression in 3T3L-1 differentiation. (**e**) *Traf1* mRNA expression in adipocytes from lean and obese epiVAT (12 weeks HFD). (**f-g**) FFA (**f**) and glycerol (**g**) concentration after i.p. injection of isoproterenol (10mg/kg). The bar graphs show area under the curve (AUC). (**h**) mRNA of genes encoding hormone-sensitive lipase (*Lipe*) and adipocyte triglyceride lipase (*Pnpla2*) in epiVAT-adipocytes at baseline or after stimulation with TNFα (10ng/ml, 4hrs). (**i**) Expression of Perilipin in epiVAT-adipocytes after TNFα stimulation. (**j**) *Ucp1* mRNA-expression in BAT. (**k–l**) Evaluation of the basal energy metabolism in a metabolic chamber (CLAMS). Heat production (**k**) and ambulatory movement (**l**) within 24-hours. (**m**). Rectal temperatures of mice exposed to 4°C for 10 hours. (**n**) Heat production after norepinephrine injection. Significance was assessed by a T-test (**b**,**f**,**g**,**h**,**i**,**j**), or two-way ANOVA (**f**,**g**,**m**,**n**). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. n=8/8(**e,f,g,j**), n=6/6(**h**), n=10/10(**i**), n=5/5, (**k**,**l**), n=6/6(**m**), n=3/3(**n**), for WT/TRAF-1$^{-/-}$, respectively.

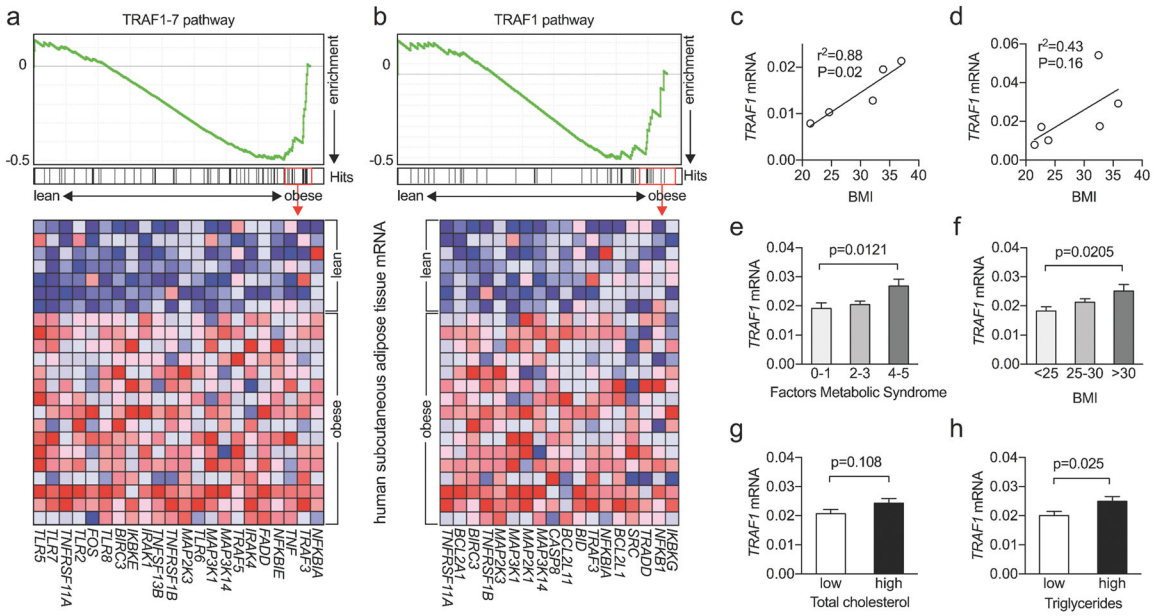

Figure 4. TRAF-1 associated signaling pathways are enriched in human obesity

(**a,b**) Enrichment of genes associated with the TRAF-1–7 (**a**) or the TRAF-1 (**b**) pathway was tested in whole tissue gene-expression of subcutaneous adipose tissue from lean (n=7) and obese (n=16) donors. Expression of the selected signature genes in obese SAT from all individuals is shown in column min.-max. heatmaps. (**c,d**) *TRAF1* mRNA abundance normalized to *GAPDH* in human subcutaneous adipose tissue (**c**) and isolated adipocytes (**d**) was correlated to the body mass index (BMI) of the donor. (**e–h**) *TRAF1* mRNA expression normalized to *GAPDH* in PBMCs of the TRAFICS study participants. Grouping of individuals for clinical factors defining the MS (**e**), BMI (**f**), cholesterol (**g**) and triglyceride (**h**) levels above or below the median. Statistical significance was assessed by a permutation test and false discovery rates (**a,b**), Spearman-correlation (**c,d**), ANOVA with Dunnett's post-test (**e,f**), or an unpaired T-test (**g**,**h**). n=90 (0–1), 133 (2–3), 81 (3).