Cell Metabolism
Article

Alternative M2 Activation of Kupffer Cells by PPARδ Ameliorates Obesity-Induced Insulin Resistance

Justin I. Odegaard,[1,2,5] Roberto R. Ricardo-Gonzalez,[1,2,5] Alex Red Eagle,[1,3] Divya Vats,[1] Christine R. Morel,[1] Matthew H. Goforth,[1] Vidya Subramanian,[4] Lata Mukundan,[1] Anthony W. Ferrante,[4] and Ajay Chawla[1,2,*]
[1]Division of Endocrinology, Metabolism and Gerontology, Department of Medicine
[2]Graduate Program in Immunology
[3]Department of Genetics
Stanford University School of Medicine, Stanford, CA 94305-5103, USA
[4]Department of Medicine, Naomi Berrie Diabetes Center, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA
[5]These authors contributed equally to this work
*Correspondence: achawla@stanford.edu
DOI 10.1016/j.cmet.2008.04.003

SUMMARY

Macrophage infiltration and activation in metabolic tissues underlie obesity-induced insulin resistance and type 2 diabetes. While inflammatory activation of resident hepatic macrophages potentiates insulin resistance, the functions of alternatively activated Kupffer cells in metabolic disease remain unknown. Here we show that in response to the Th2 cytokine interleukin-4 (IL-4), peroxisome proliferator-activated receptor δ (PPARδ) directs expression of the alternative phenotype in Kupffer cells and adipose tissue macrophages of lean mice. However, adoptive transfer of PPARδ$^{-/-}$ (*Ppard*$^{-/-}$) bone marrow into wild-type mice diminishes alternative activation of hepatic macrophages, causing hepatic dysfunction and systemic insulin resistance. Suppression of hepatic oxidative metabolism is recapitulated by treatment of primary hepatocytes with conditioned medium from PPARδ$^{-/-}$ macrophages, indicating direct involvement of Kupffer cells in liver lipid metabolism. Taken together, these data suggest an unexpected beneficial role for alternatively activated Kupffer cells in metabolic syndrome and type 2 diabetes.

INTRODUCTION

Chronic inflammation is causally linked to obesity, insulin resistance, and type 2 diabetes (Hotamisligil, 2006; Shoelson et al., 2006). Increased adiposity promotes macrophage infiltration into adipose tissue (Weisberg et al., 2003; Xu et al., 2003), perpetuating local inflammation and causing insulin resistance (Kamei et al., 2006; Kanda et al., 2006; Weisberg et al., 2006). However, not all adipose tissue macrophages (ATMs) exhibit the inflammatory phenotype. For instance, macrophages resident in the adipose tissue of lean animals are alternatively activated and have decreased inflammatory potential (Lumeng et al., 2007; Odegaard et al., 2007a). Consistent with this observation, we have recently shown that alternatively activated ATMs exert beneficial effects during times of caloric excess. Specifically, the absence of alternatively activated ATMs, as in macrophage-specific peroxisome proliferator-activated receptor γ null (Mac-PPARγ$^{-/-}$) mice, promotes mitochondrial dysfunction, leading to obesity and insulin resistance (Odegaard et al., 2007a). While these studies have elucidated antidiabetic activities of alternatively activated ATMs in metabolic syndrome, the functions of these cells in other metabolic tissues remain poorly understood.

Inflammatory activation of Kupffer cells, the resident macrophages in liver, has been implicated in both obesity-induced insulin resistance and fatty liver disease. Genetic ablation of IκB kinase β (IKKβ), an upstream kinase required for activation of nuclear factor κB (NF-κB), in myeloid cells reduces macrophage-mediated inflammation and improves systemic and hepatic insulin sensitivity (Arkan et al., 2005). Conversely, inflammatory activation of Kupffer cells by lipopolysaccharide (LPS) promotes hepatotoxicity in obese mice (Li and Diehl, 2003). While these and other studies have delineated the pathogenic activities of inflammatory Kupffer cells, resident hepatic macrophages display tremendous plasticity in their activation programs, ranging from the proinflammatory classical state to the anti-inflammatory alternative state (Gordon, 2003; Herbert et al., 2004). Despite the great therapeutic potential of alternatively activated Kupffer cells, their functions in metabolic disease remain enigmatic.

PPARs, members of the nuclear receptor superfamily (Chawla et al., 2001), control systemic fatty acid metabolism by transcriptional activation of target genes (Evans et al., 2004; Kliewer et al., 2001). Since a variety of fatty acids and fatty acid metabolites can bind and activate these receptors (Xu et al., 1999), PPARs act as fatty acid sensors to alter metabolic pathways in response to changes in fuel availability (Kliewer et al., 2001). Surprisingly, in alternatively activated murine macrophages, PPARγ is required for mitochondrial biogenesis and β-oxidation of fatty acids (Odegaard et al., 2007a). Despite an absolute requirement for oxidative metabolism in alternative macrophage activation (Vats et al., 2006), macrophage-specific disruption of PPARγ primarily affects alternative activation of ATMs, while sparing

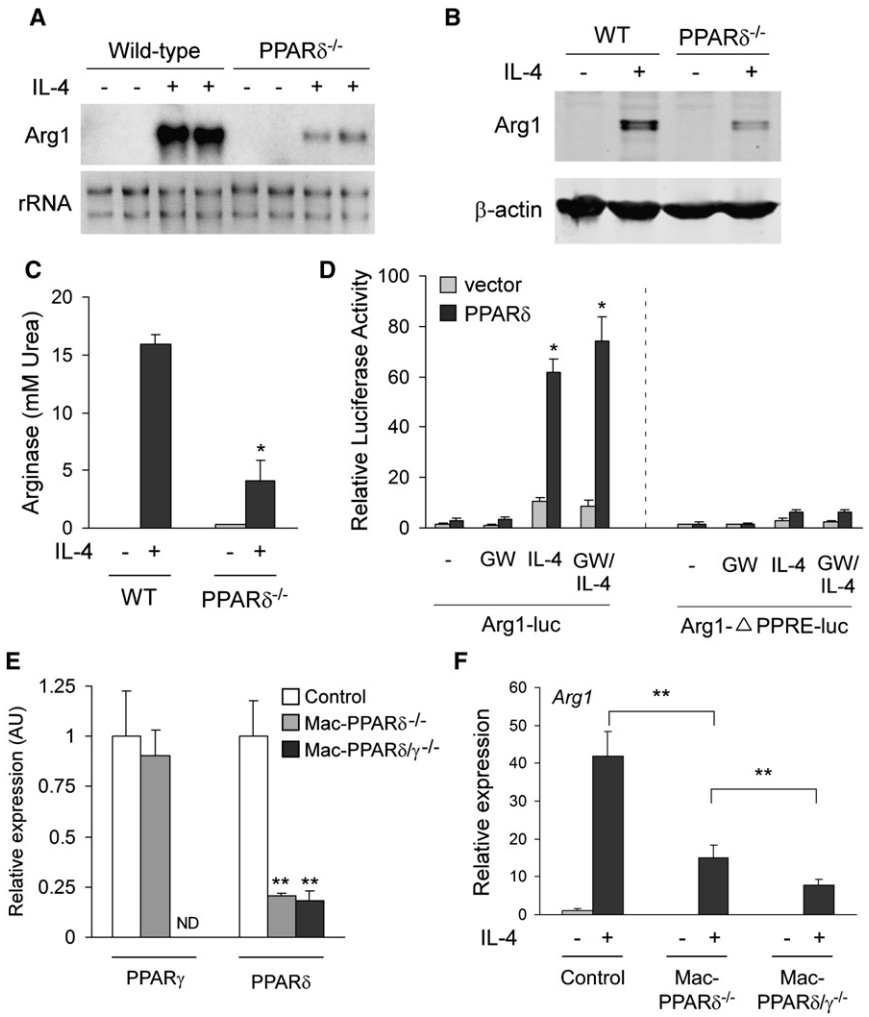

Figure 1. PPARδ Regulates Expression of Arginase 1 in Alternatively Activated Macrophages

(A–C) Decreased levels of arginase 1 (*Arg1*) mRNA (A), protein (B), and enzymatic activity (C) in IL-4-stimulated PPARδ$^{-/-}$ (*Ppard*$^{-/-}$) bone marrow-derived macrophages (BMDMs).

(D) Activation of the *Arg1* promoter by PPARδ/RXR heterodimers and IL-4. Deletion of the PPAR response element in the arginase 1 enhancer abolishes transcriptional activation by PPARδ and IL-4.

(E and F) Attenuated induction of arginase 1 in PPARδ/γ double-knockout macrophages.

(E) Real-time analysis of PPARδ and PPARγ expression in BMDMs generated from control, macrophage-specific PPARδ$^{-/-}$ (Mac-PPARδ$^{-/-}$), and Mac-PPARδ/γ$^{-/-}$ mice. Primers specific for exon 4 of PPARδ or exon 1 of PPARγ were used to quantify excision efficiency in BMDMs. ND, not detected.

(F) qRT-PCR analysis of *Arg1* mRNA in various genotypes.

Data are presented as mean ± SEM. *p < 0.05; **p < 0.01.

Kupffer cells (Odegaard et al., 2007a). This observation suggests that distinct transcriptional regulators and signaling pathways control depot-specific maturation of alternatively activated macrophages; however, the identity of these factors is unknown.

In response to interleukin-4 (IL-4), macrophages enact a coordinated program of oxidative metabolism and mitochondrial biogenesis necessary for alternative macrophage activation (Vats et al., 2006). One critical feature of this metabolic switch is the substantial increase in fatty acid influx. Because fatty acids and fatty acid-enriched lipoproteins can transcriptionally activate the nuclear receptor PPARδ in macrophages (Chawla et al., 2003; Evans et al., 2004; Xu et al., 1999), we investigated its functions in alternative macrophage activation. We report here that PPARδ is absolutely required for full expression of the effector phenotype of alternatively activated macrophages. Genetic deletion of PPARδ (*Ppard*) in bone marrow cells not only impairs alternative activation of tissue macrophages but also predisposes animals to the development of insulin resistance and metabolic syndrome. Surprisingly, PPARδ deficiency has minimal impact on ATM biology. In contrast, Kupffer cells deficient in PPARδ display marked impairment in alternative activation, resulting in hepatic dysfunction and insulin resistance. Together, our findings identify an unexpected role for PPARδ in Kupffer cell biology, thereby delineating a molecular mechanism by which this receptor and alternatively activated macrophages ameliorate obesity-induced insulin resistance.

RESULTS

PPARδ Regulates Arginase 1 Expression in Alternatively Activated Macrophages

To understand the role of PPARδ in alternative macrophage activation, bone marrow-derived macrophages (BMDMs) from wild-type and PPARδ-deficient mice were stimulated with IL-4 for 48–72 hr, a time period that promotes maximal expression of the alternatively activated phenotype (Munder et al., 1999). Notably, induction of mRNA and protein for arginase 1 (*Arg1*), a signature gene induced during alternative macrophage activation (Gordon, 2003), was dramatically reduced in PPARδ$^{-/-}$ macrophages (Figures 1A and 1B). Consistent with impaired induction of arginase 1, cytosolic arginase activity was reduced by ~75% in PPARδ$^{-/-}$ macrophages (Figure 1C). To assess whether PPARδ can directly regulate arginase 1, transient transfections were performed with the arginase 1 reporter construct containing the identified PPAR response element (Odegaard et al., 2007a; Pauleau et al., 2004). Strikingly, cotransfection of PPARδ greatly augmented IL-4's transcriptional activity on this promoter (~4-fold), which was not further potentiated by GW501516, a synthetic agonist of PPARδ (Oliver et al., 2001) (Figure 1D). However, deletion of the PPAR binding site completely abolished the ability of IL-4 and PPARδ to activate this promoter (Figure 1D), indicating that PPARδ regulates arginase 1 in alternatively activated macrophages via this site.

We have previously shown that macrophage-specific PPARγ plays an essential role in the regulation of arginase 1 expression during alternative macrophage activation (Odegaard et al.,

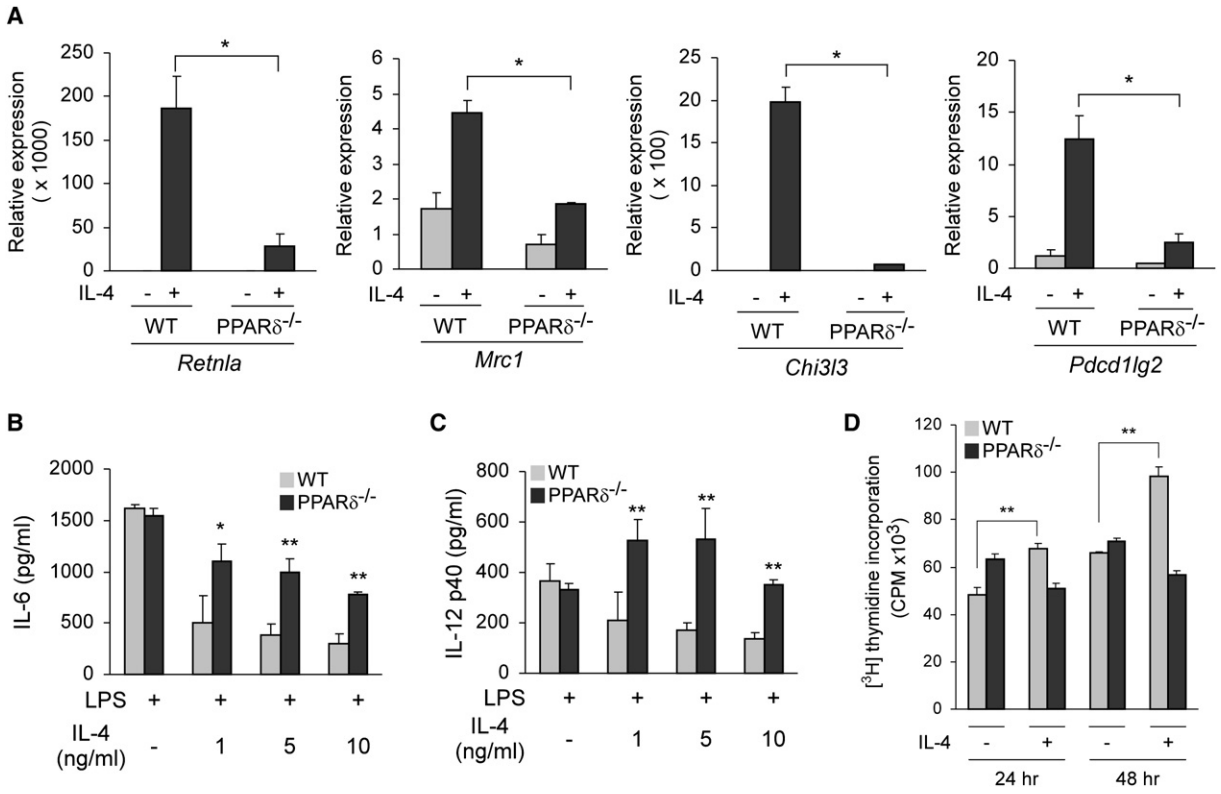

Figure 2. PPARδ Is Required for Expression of the Immune Phenotype of Alternative Macrophage Activation

(A) Decreased expression of markers of alternative activation in IL-4-stimulated PPARδ$^{-/-}$ BMDMs. Relative expression of alternative activation mRNAs was quantified by qRT-PCR. *Retnla*, resistin-like α; *Mrc1*, mannose receptor; *Chi3l3*, chitinase 3-like 3; *Pdcd1lg2*, programmed cell death 1 ligand 2.

(B and C) PPARδ is required for suppression of IL-6 (B) and IL-12 (C) production in alternatively activated BMDMs.

(D) PPARδ is required for the mitogenic response to IL-4 in BMDMs.

Data are presented as mean ± SEM. *p < 0.05; **p < 0.01.

2007a). Since our current data also indicate a requirement for PPARδ in the regulation of this facet of alternative activation, we next investigated the hierarchical relationship between these two nuclear receptors. For these experiments, we generated BMDMs from control (PPARδ$^{flox/flox}$), Mac-PPARδ$^{-/-}$ (PPARδ$^{flox/flox}$;LysM^{Cre}), and Mac-PPARδ/γ$^{-/-}$ (PPARδ/γ$^{flox/flox}$;LysM^{Cre}) mice, strains recently generated in the laboratory on the C57BL/6J background (Figure 1E). As shown in Figure 1F, IL-4 failed to stimulate *Arg1* mRNA expression in macrophages deficient in both PPARγ and δ, indicating that both nuclear receptors are required for optimal expression of this gene during alternative macrophage activation.

PPARδ Regulates Immunologic Phenotype of Alternatively Activated Macrophages

To further evaluate the requirement of PPARδ in expression of the alternative phenotype, we examined other characteristics of alternative activation in PPARδ$^{-/-}$ macrophages. As shown in Figure 2A, IL-4 failed to induce mRNAs encoding *Retnla* (resistin-like α), *Mrc1* (mannose receptor), *Chi3l3* (chitinase 3-like 3), and *Pdcd1lg2* (programmed cell death 1 ligand 2) in PPARδ$^{-/-}$ macrophages (Gordon, 2003; Loke and Allison, 2003; Raes et al., 2002). This was further substantiated by markedly lower cell surface expression of dectin-1 (Clec7a) and mannose receptor in PPARδ$^{-/-}$ macrophages (see Figure S1A available online). Supporting a critical role for PPARδ in alternative macrophage activation, macrophages deficient in PPARδ were also refractory to IL-13-mediated alternative activation (Figure S2).

Because treatment of macrophages with IL-4 dampens their inflammatory response (Herbert et al., 2004; Odegaard et al., 2007b), we next investigated whether PPARδ was also required for this facet of alternative activation. In a dose-dependent manner, pretreatment with IL-4 suppressed LPS-induced secretion of the inflammatory cytokines IL-6 and IL-12 in wild-type, but not PPARδ$^{-/-}$, macrophages (Figures 2B and 2C). Consistent with a global decrement in the acquisition of the alternative phenotype, macrophages lacking PPARδ had a markedly lower mitogenic response to IL-4 as assessed by incorporation of [^{3}H]thymidine (Figure 2D). Lastly, in support of the central role of PPARδ and γ in acquisition of the alternative phenotype, comparative analyses of BMDMs from control, Mac-PPARδ$^{-/-}$, and Mac-PPARδ/γ$^{-/-}$ mice revealed that both receptors are required for optimal alternative macrophage activation (Figure S3). Taken together with our previous findings, these results suggest that both PPARδ and PPARγ coordinate the macrophage's transcriptional response to the Th2 cytokines IL-4 and IL-13. PPARγ primarily regulates metabolic programs in alternatively activated macrophages (Odegaard et al., 2007a), whereas PPARδ is

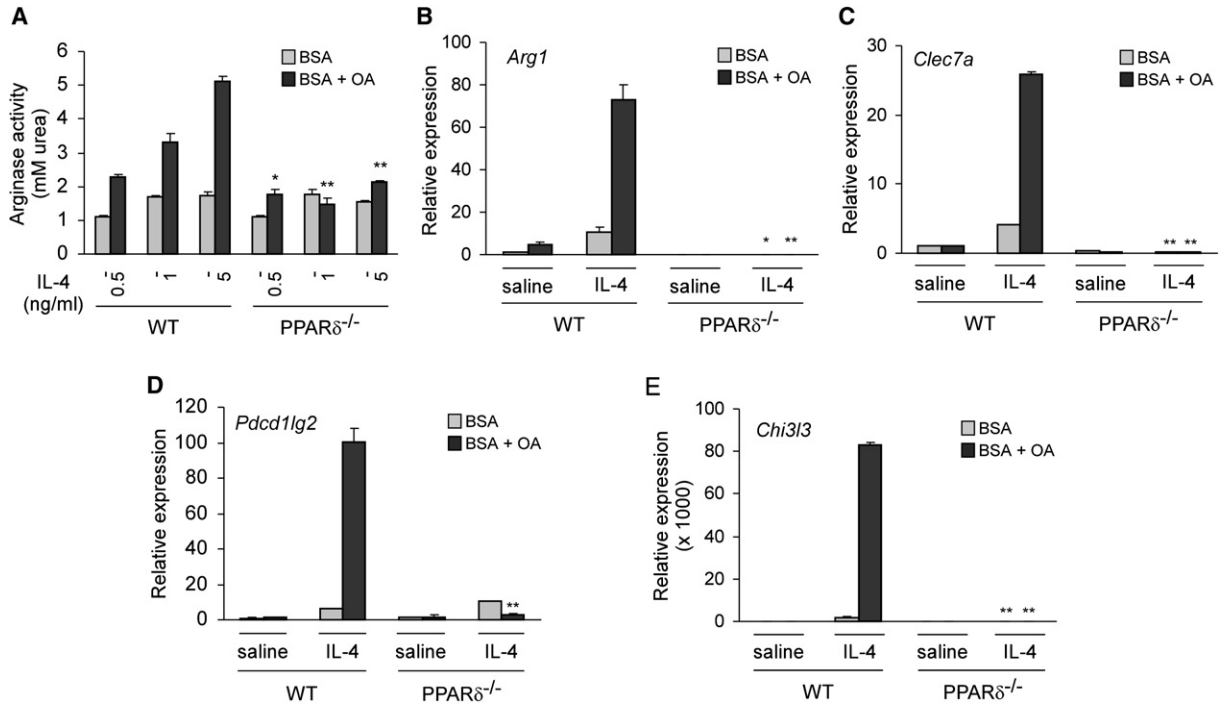

Figure 3. Conjugated Oleic Acid Potentiates Alternative Macrophage Activation

(A) Oleic acid potentiates IL-4 induction of arginase activity in wild-type, but not PPARδ$^{-/-}$, BMDMs.

(B–E) PPARδ is required for potentiation of alternative activation by oleic acid. BMDMs from 129/SvJ wild-type and PPARδ$^{-/-}$ mice were stimulated with IL-4 for 48 hr in the presence of BSA or BSA:oleic acid conjugate. qRT-PCR analyses were performed on mRNAs for *Arg1* (B), *Clec7a* (dectin-1) (C), *Pdcd1lg2* (D), and *Chi3l3* (E).

Data are presented as mean ± SEM. *p < 0.05; **p < 0.01.

required for the full expression of their immune phenotype, including expression of pattern recognition receptors (Mrc1 and Clec7a) and costimulatory molecules (Pdcd1lg2) and suppression of macrophage inflammatory response.

To further explore whether PPARδ ligands can initiate or potentiate alternative macrophage activation, we treated wild-type macrophages with PPARδ activators under three different experimental settings. First, to determine whether PPARδ activation can induce a Th2 bias in an elicited macrophage population, wild-type mice were injected intraperitoneally (i.p.) with vehicle or the synthetic PPARδ agonist GW0742 for 5 days (Graham et al., 2005), and markers of alternative activation were quantified in biogel-elicited macrophages. As shown in Figure S4A, GW0742 treatment led to a dramatic increase in the expression of signature genes for alternative macrophage activation. Second, to determine whether chronic treatment with PPARδ agonist induces an alternative bias in BMDMs, wild-type bone marrow progenitor cells were treated with the PPARδ agonist GW501516 (100 nM) for 7 days. Consistent with the Th2-polarizing effects of PPARδ in macrophages, stimulation with GW501516 induced markers of alternative activation in BMDMs (Figure S4B). Lastly, to explore whether PPARδ agonist can synergize with Th2 cytokines in promoting alternative macrophage activation, wild-type BMDMs were stimulated with vehicle, IL-4 (2 ng/ml), GW501516 (100 nM), or a combination of IL-4 (2 ng/ml) and GW501516 (100 nM). As shown in Figure S4C, BMDMs costimulated with IL-4 and GW501516 had markedly higher levels of transcripts encoding alternative activation signature genes. In aggregate, these data suggest that synthetic ligands for PPARδ can work alone or in combination with endogenous Th2 signals to promote an alternative bias in macrophage activation.

Unsaturated Fatty Acids Potentiate Alternative Macrophage Activation via PPARδ

Tissue macrophages, specifically those present in liver and adipose tissue, are exposed to high levels of fatty acids. Because a large influx of fatty acids accompanies alternative macrophage activation, we reasoned that these incoming fatty acids might activate PPARδ, a genetic sensor of fatty acids (Chawla et al., 2001; Evans et al., 2004). To test this hypothesis, BMDMs were stimulated with varying doses of IL-4 in the presence of bovine serum albumin (BSA) or BSA conjugated to 100 μM oleic acid. As shown in Figure 3A, BSA:oleic acid conjugate synergized with IL-4 to induce arginase activity in macrophages. Notably, this synergistic effect of oleic acid was completely abolished in macrophages lacking PPARδ, indicating that monounsaturated fatty acids might potentiate the broader program of alternative activation. Indeed, oleic acid enhanced alternative macrophage activation in a PPARδ-dependent manner as evidenced by the induction of *Arg1*, *Clec7a*, *Pdcd1lg2*, and *Chi3l3* mRNAs (Figures 3B–3E). Taken together with recent findings of Shi et al. (2006), these data indicate that local availability of fatty acids can alter the activation potential of macrophages. While

saturated fatty acids, such as lauric and palmitic acids, ligate toll-like receptor 4 (TLR4) to promote proinflammatory activation of macrophages (Kim et al., 2007; Shi et al., 2006), monounsaturated fatty acids enhance expression of the anti-inflammatory alternative state by activating the nuclear receptor PPARδ.

PPARδ Is Required for Alternative Activation of Resident Macrophages

To verify whether PPARδ is essential for alternative activation of resident macrophages, we analyzed macrophage signatures in metabolic tissues of lean mice. As expected, the signature genes of alternatively activated macrophages, including *Clec7a*, *Retnla*, *Tgfb1*, *Jag1*, and *Mrc1*, were dramatically reduced in white adipose tissue (WAT) of PPARδ$^{-/-}$ mice (Figure S5A). Surprisingly, genetic deletion of PPARδ also impaired alternative activation of hepatic macrophages, the Kupffer cells, as evidenced by decreased expression of *Arg1*, *Clec7a*, *Jag1*, *Pdcd1lg2*, and *Chia* mRNAs (Figure S5B). Furthermore, treatment of lean wild-type mice with GW0742, a highly selective agonist for PPARδ, increased expression of alternative macrophage activation markers in both WAT and liver (Figures S5C and S5D). Thus, in contrast to PPARγ (Odegaard et al., 2007a), PPARδ transcriptional signaling is required for the maintenance of alternative activation of ATMs and Kupffer cells in lean mice.

Hematopoietic PPARδ Protects against Diet-Induced Insulin Resistance

Having established that PPARδ regulates the immune program of alternative macrophage activation, we investigated whether mice lacking PPARδ in the macrophage compartment were at an increased risk of developing metabolic syndrome. To conduct these experiments, we adoptively transferred wild-type and PPARδ-deficient bone marrow into lethally irradiated wild-type animals (Weisberg et al., 2003). After reconstitution for 4 weeks, age-matched cohorts were placed on a high-fat diet (HFD) for 22 weeks to promote maximal infiltration of macrophages into WAT (Xu et al., 2003). qPCR analysis of genomic DNA confirmed >99% replacement of wild-type marrow by PPARδ$^{-/-}$ cells (Figure S6A). Importantly, in this time frame, even the relatively radioresistant Kupffer cells are replaced with new cells from the bone marrow (Figures S6B and S6D) (Kennedy and Abkowitz, 1997). Moreover, isolation of F4/80 (Emr1)-positive macrophages from WAT and liver confirmed that a majority of tissues macrophages were derived from PPARδ$^{-/-}$ hematopoietic cells (Figures S6C and S6D). Remarkably, reconstitution of wild-type mice with PPARδ$^{-/-}$ bone marrow (PPARδ$^{-/-}$ BMT) led to development of glucose intolerance on HFD (Figure 4A). In addition, compared to mice transplanted with wild-type bone marrow (WT BMT), PPARδ$^{-/-}$ BMT mice were more resistant to glucose lowering by exogenous insulin (Figure 4B). Consistent with this decrease in insulin sensitivity, fasting insulin levels were ~2-fold higher in PPARδ$^{-/-}$ BMT mice (Figure 4C). However, fasting levels of circulating lipids were not significantly different between the two cohorts of transplanted mice (Table S1). To identify the sites of insulin resistance, WT BMT and PPARδ$^{-/-}$ BMT mice were injected with insulin via their inferior vena cava, and tissues were quickly harvested for biochemical analyses of insulin signaling (Arkan et al., 2005; Cai et al., 2005). As shown in Figures 4D–4F, phosphorylation of Akt was markedly reduced in liver, quadriceps, and WAT of PPARδ$^{-/-}$ BMT mice, indicating onset of global insulin resistance. Since reduction in oxidative phosphorylation (OXPHOS) is causally linked to development of insulin resistance in mice and humans (Mootha et al., 2003; Patti et al., 2003; Petersen et al., 2003; Vianna et al., 2006), we looked for evidence of mitochondrial dysfunction in all three tissues. Notably, RT-qPCR analyses revealed a profound decrease (30%–50%) in expression of enzymes for fatty acid oxidation (*Acox1*, *Acadm*, and *Acadl*) and OXPHOS (*Ndufs1*, *Sdhb*, *Uqcrc1*, *Cox4i1*, and *Atp5j*) in livers of PPARδ$^{-/-}$ BMT mice (Figure 4G). Similarly, genes important in oxidative metabolism (*Acox1* and *Cpt1b*) and electron transport (*Ndusf1*, *Uqcrc1*, and *Cox4i1*) were suppressed by 60%–90% in quadriceps of PPARδ$^{-/-}$ BMT animals (Figure 4H). Importantly, in both tissues, expression of transcriptional regulators controlling mitochondrial oxidative metabolism (*Tfam*, *Nrf1*, *Pgc1a*, and *Pgc1b*) was dramatically reduced (30%–80%). However, metabolic programs for β-oxidation and OXPHOS were largely intact in WAT of PPARδ$^{-/-}$ BMT mice (Figure S7C), suggesting that hematopoietic PPARδ deficiency primarily suppresses oxidative metabolism in myocytes and hepatocytes.

Hematopoietic PPARδ Protects against Diet-Induced Obesity

We recently demonstrated that alternatively activated ATMs protect against diet-induced obesity (Odegaard et al., 2007a), prompting us to evaluate changes in adiposity in PPARδ$^{-/-}$ chimeric animals. At 18 weeks on HFD, dual-energy X-ray absorptiometry (DEXA) indicated that weight-matched PPARδ$^{-/-}$ BMT mice had significantly higher fat content (43.4% ± 0.39% versus 37.8% ± 1.81%; $p < 0.01$) and slightly lower lean body mass (18.2 ± 0.47 g versus 19.9 ± 0.52 g; $p < 0.05$) than WT BMT controls (Figure 5A). Furthermore, gross postmortem analyses showed marked enlargement of adipose depots (Figure 5B), paralleling the ~50% increase in epididymal fat pad mass, ~7-fold increase in serum leptin, and 20% reduction in serum adiponectin (Figure 5C; Table S1). However, in contrast to adipocyte cell size in Mac-PPARγ$^{-/-}$ mice (Odegaard et al., 2007a), adipocytes were significantly larger in PPARδ$^{-/-}$ BMT mice (Figure 5D; Figure S7A), suggesting that adipocyte hypertrophy likely accounts for the increase in total adiposity. Because adipocyte morphology is markedly different in these two models, we next quantified ATM content and activation. In striking contrast to Mac-PPARγ$^{-/-}$ mice, ATM content trended toward being higher in PPARδ$^{-/-}$ BMT mice without significant change in the signature of alternatively activated macrophages (Figures 5E and 5F; Figure S7B). Moreover, unlike WAT of Mac-PPARγ$^{-/-}$ mice, expression analysis of PPARδ$^{-/-}$ BMT WAT showed only a modest decrease in adipocyte programs of lipid accretion, storage, and utilization (Figure S7C), findings that are consistent with the minor increase in inflammatory response in this tissue (Figure 5F) (Weisberg et al., 2003). Direct coculture of adipocytes with macrophages further affirmed that PPARδ$^{-/-}$ macrophages do not significantly impact adipocyte biology as assessed by insulin-stimulated glucose uptake (Figure S7D) and expression of cytokines and adipokines in these cells (Figure S7E). Together, these data suggest that nutritional status is a key determinant of whether PPARδ signaling is required for alternative activation of ATMs. While PPARδ signaling is necessary for alternative activation of ATMs in lean mice (Figure S5A), it is largely

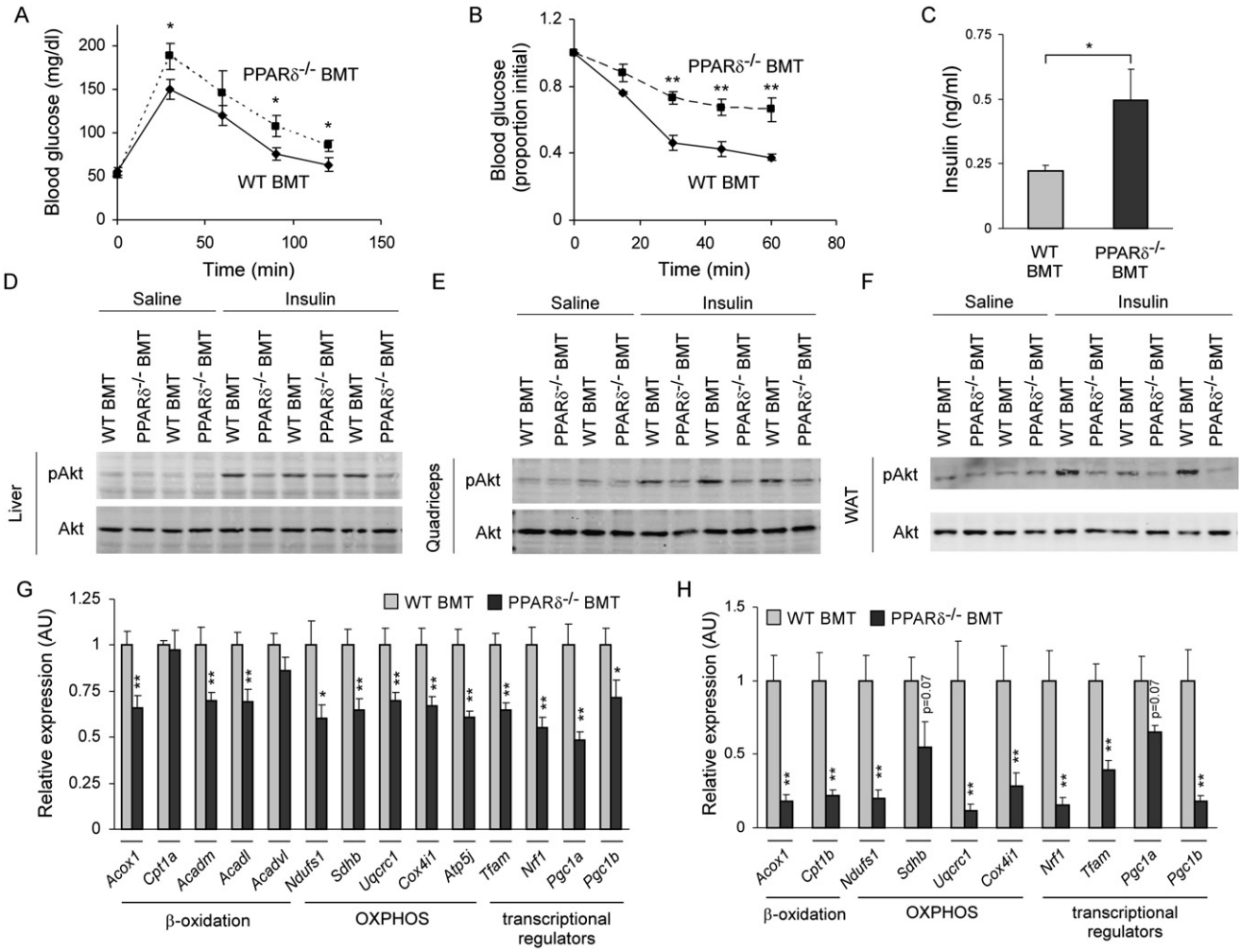

Figure 4. Hematopoietic Deficiency of PPARδ Exacerbates Insulin Resistance and Impairs Glucose Tolerance

(A) Oral glucose tolerance tests (1 g/kg) were carried out after 18 weeks of high-fat diet in male mice reconstituted with wild-type or PPARδ$^{-/-}$ bone marrow (WT BMT and PPARδ$^{-/-}$ BMT, respectively) (n = 5–7 per cohort).

(B) Insulin tolerance tests (0.65 U/kg) were performed in obese mice after a 4 hr fast (n = 5–7 per cohort). Similar results were obtained in two other cohorts of transplanted mice (n = 7 per group).

(C) Fasting serum levels of insulin in WT BMT and PPARδ$^{-/-}$ BMT mice after a 5 hr fast.

(D–F) Impairment in insulin action in PPARδ$^{-/-}$ BMT mice. Total cell lysates were immunoblotted for phospho-Akt (pAkt) or total Akt in liver (D), quadriceps muscle (E), and epididymal white adipose tissue (WAT) (F).

(G and H) Mitochondrial dysfunction in peripheral tissues of PPARδ$^{-/-}$ BMT mice. Relative transcript levels for genes encoding key enzymes in β-oxidation and oxidative phosphorylation (OXPHOS) and transcriptional regulators controlling these pathways in liver (G) and quadriceps (H) are shown.

Data are presented as mean ± SEM. *p < 0.05; **p < 0.01.

dispensable for maintenance of this macrophage population in WAT of obese mice (Figure 5F). Future experiments utilizing recently generated Mac-PPARδ/γ double-knockout mice will allow us to determine whether induction of PPARγ compensates for loss of PPARδ signaling in ATMs of obese animals. Nonetheless, the lack of significant macrophage phenotype in WAT of PPARδ$^{-/-}$ BMT mice suggests potential involvement of alternatively activated macrophages in other metabolic tissues.

Alternative Activation of Kupffer Cells Preserves Hepatic Function

Kupffer cells in lean PPARδ$^{-/-}$ mice exhibited diminished capacity for alternative activation (Figure S5B), prompting us to examine the number and activation state of hepatic macrophages in obese animals. Although HFD feeding led to a modest increase (~20%) in hepatic macrophage content in PPARδ$^{-/-}$ BMT mice (Figures S8A and S8B), the activation state of resident macrophages was dramatically different between WT BMT and PPARδ$^{-/-}$ BMT mice. Notably, the signature of alternatively activated Kupffer cells was greatly reduced in livers of obese PPARδ$^{-/-}$ BMT mice (Figure 6A). Furthermore, liver arginase activity was also reduced by ~41% in PPARδ$^{-/-}$ BMT mice (Figure 6B), confirming that resident macrophages were gradually being replaced by cells derived from the bone marrow (Figures S6C and S6E). To ascertain whether PPARδ is required for IL-4-mediated alternative activation of Kupffer cells,

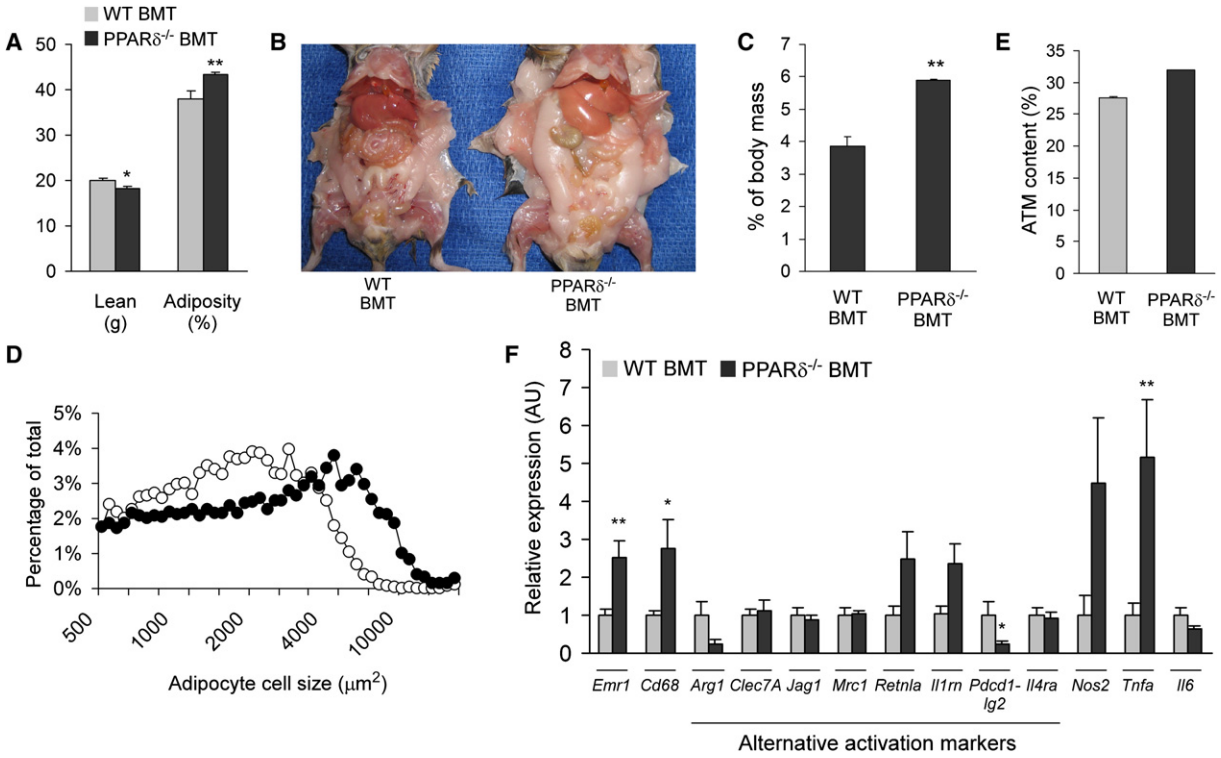

Figure 5. Increased Adiposity in PPARδ$^{-/-}$ BMT Mice

(A) Body composition was quantified by dual-energy X-ray absorptiometry (DEXA) in weight-matched transplanted mice (n = 7 per cohort).

(B) Representative images of necropsied WT BMT and PPARδ$^{-/-}$ BMT mice after 22 weeks of high-fat diet.

(C) Increased epididymal fat pad mass in PPARδ$^{-/-}$ BMT mice (n = 5 per cohort).

(D) Increased adipocyte cell size in PPARδ$^{-/-}$ BMT mice. Adipocyte cell size was measured using dark-field images.

(E and F) Macrophage content and activation in WAT.

(E) Adipose tissue macrophage (ATM) content was determined by immunostaining for the macrophage antigen F4/80. p = 0.16 by paired t test; n = 4 per cohort.

(F) Interrogation of ATM activation state by qRT-PCR. *Emr1*, F4/80; *Cd68*, macrosialin; *Jag1*, jagged 1; *Il1rn*, IL-1 receptor antagonist; *Il4ra*, IL-4 receptor α; *Nos2*, inducible nitric oxide synthase; *Tnfa*, tumor necrosis factor α; *Il6*, interleukin-6.

Data are presented as mean ± SEM. *p < 0.05; **p < 0.01.

wild-type and PPARδ$^{-/-}$ mice were challenged with IL-4 and the activation state of Kupffer cells was interrogated by qRT-PCR. Remarkably, injection of IL-4 induced the expression of the alternative activation signature genes *Arg1*, *Clec7a*, *Chi3l3*, and *Tgfb1* in a PPARδ-dependent manner (Figure 6C), thus proving an absolute requirement for PPARδ in the IL-4-driven program of Kupffer cell alternative activation.

We have previously shown that impairment in alternative macrophage activation is linked to development of mitochondrial dysfunction in metabolic tissues (Odegaard et al., 2007a). That PPARδ$^{-/-}$ chimeric mice have markedly reduced expression of β-oxidation and OXPHOS genes in liver (Figures 4G) suggests that alternatively activated Kupffer cells might directly modulate hepatic metabolism. To test this hypothesis, β-oxidation of fatty acids was analyzed in wild-type primary hepatocytes that had been cocultured with wild-type or PPARδ$^{-/-}$ BMDMs for 72 hr. Strikingly, hepatocytes cocultured with PPARδ$^{-/-}$ macrophages exhibited an ~25% decrease in the rate of fatty acid oxidation compared to those cocultured with wild-type macrophages (Figure S8C). Furthermore, qRT-PCR analyses of cocultured primary hepatocytes demonstrated potent suppression of β-oxidation and OXPHOS pathways by PPARδ$^{-/-}$ macrophages (Figure S8D). Since similar results were obtained when primary hepatocytes were treated with conditioned medium (CM) from wild-type or PPARδ$^{-/-}$ macrophages (Figures 6D and 6E), this suggests that factors secreted by macrophages can directly modulate oxidative metabolism in parenchymal cells. Finally, confirming a causal relationship between PPARδ-deficient Kupffer cells and dysregulation of hepatic metabolism, livers of PPARδ$^{-/-}$ BMT mice exhibited gross and histological evidence of hepatic steatosis (Figure 5B and Figure 6F) and had an ~50% increase in extractable liver triglycerides (Figure 6G).

DISCUSSION

The infiltration and activation of macrophages in metabolic tissues are key events in the pathogenesis of diet-induced obesity and insulin resistance (Qatanani and Lazar, 2007). However, most metabolic tissues, including adipose tissue and liver, contain a resident population of less inflammatory, alternatively activated macrophages (Gordon, 2003) whose functions in obesity-induced metabolic disease remain poorly understood. To address this critical question, we have taken a systematic approach to identify transcriptional regulators that control the

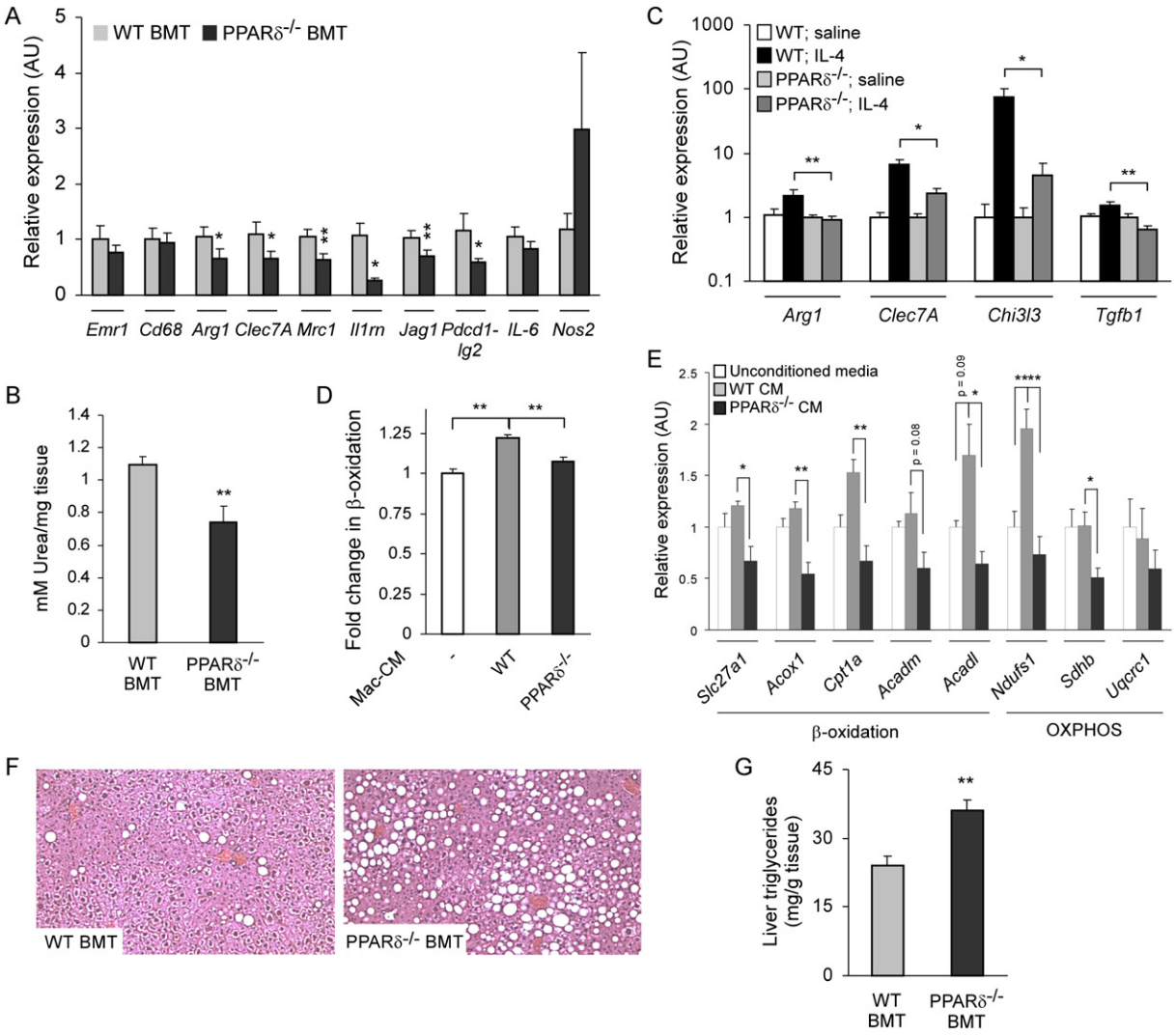

Figure 6. Impaired Alternative Activation of Kupffer Cells and Hepatic Dysfunction in PPARδ$^{-/-}$ BMT Mice

(A) Decreased expression of signature genes for alternative activation of macrophages in PPARδ$^{-/-}$ BMT livers (n = 5–7 per cohort).

(B) Reduction in liver arginase activity in PPARδ$^{-/-}$ BMT mice (n = 5–7 per cohort).

(C) IL-4 is unable to induce alternative activation of Kupffer cells in PPARδ$^{-/-}$ mice (n = 4 per cohort). *Tgfb1*, transforming growth factor β1.

(D and E) Treatment of primary hepatocytes with macrophage conditioned medium (CM) alters their oxidative metabolism. CM from PPARδ$^{-/-}$ macrophages suppresses oxidative metabolism as monitored by β-oxidation of fatty acids (D) and expression of fatty acid oxidation and OXPHOS genes (E).

(F and G) Histologic (F) and biochemical (G) evidence of increased triglyceride accumulation in livers of PPARδ$^{-/-}$ BMT mice.

Data are presented as mean ± SEM. *p < 0.05; **p < 0.01.

maturation of alternatively activated macrophages in tissues. Previously, we demonstrated that PPARγ orchestrates the program of oxidative metabolism in alternatively activated macrophages, a requisite for alternative maturation of ATMs (Odegaard et al., 2007a). In contrast, we now show that PPARδ coordinates the immune phenotype of alternatively activated macrophages, both in vitro and in vivo. Interestingly, hematopoietic deficiency of PPARδ selectively impairs alternative activation of Kupffer cells in obese mice, leading to reduction in oxidative metabolism and insulin sensitivity. Furthermore, direct coculture of primary hepatocytes with PPARδ$^{-/-}$ macrophages recapitulates the liver phenotype of PPARδ$^{-/-}$ BMT mice. Taken together, our findings suggest that PPARδ and PPARγ control distinct aspects of alternative macrophage activation to ameliorate obesity-induced insulin resistance (Figure 7).

While inflammatory activation of Kupffer cells by bacterial byproducts induces hepatic insulin resistance (Arkan et al., 2005; Li and Diehl, 2003), the role of alternatively activated Kupffer cells in liver metabolism has remained enigmatic. Unexpectedly, we found that the PPARδ-regulated program of Kupffer cell alternative activation mediates beneficial effects on metabolic disease, an observation that is supported by three independent lines of evidence. First, in lean animals, genetic deletion of PPARδ reduces the expression of genes that constitute the signature of alternatively activated Kupffer cells. Second, transplantation of PPARδ$^{-/-}$ bone marrow into wild-type mice diminishes

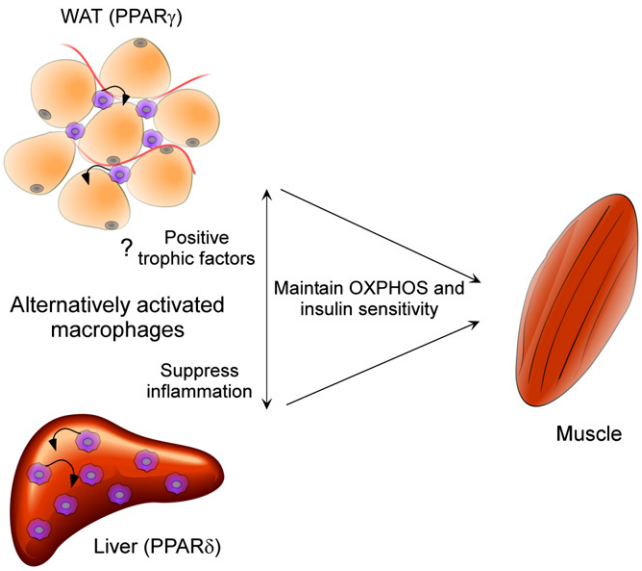

Figure 7. Model Highlighting the Metabolic Functions of Alternatively Activated Macrophages

PPARγ transcriptional signaling is required for maturation of macrophages in adipose tissue, whereas PPARδ controls the expression of the alternative phenotype in Kupffer cells of obese mice. Two potential mechanisms by which alternatively activated macrophages may improve insulin action in obese mice are (1) paracrine inhibition of inflammation and (2) secretion of trophic factors that can directly modulate oxidative metabolism in parenchymal cells (small arrows).

alternative activation of Kupffer cells, leading to mitochondrial dysfunction and insulin resistance in hepatocytes. Third, direct coculture of PPARδ$^{-/-}$ macrophages with primary hepatocytes leads to dramatic suppression of oxidative metabolism in the parenchymal cells. Lastly, since Kupffer cell replacement in PPARδ$^{-/-}$ BMT mice is incomplete (Figure S6D), our studies likely underestimate the metabolic benefits of the alternatively activated Kupffer cells.

Extensive metabolic characterization of PPARδ$^{-/-}$ BMT and Mac-PPARγ$^{-/-}$ mice revealed that the activation state of resident macrophages plays an integral role in regulation of peripheral metabolism (Figure 7). For instance, in Mac-PPARγ$^{-/-}$ mice, loss of alternatively activated ATMs causes impairment in adipocyte function, resulting in reduced oxidative metabolism and insulin sensitivity (Hevener et al., 2007; Odegaard et al., 2007a). Similarly, Kupffer cells in PPARδ$^{-/-}$ BMT mice have diminished capacity to undergo alternative activation, leading to a dramatic reduction in OXPHOS and insulin sensitivity. Hence, these findings suggest that, irrespective of their sites of residence, alternatively activated macrophages have a profound influence on oxidative metabolism and lipid homeostasis in peripheral tissues. Interestingly, the maturation of alternatively activated macrophages is itself regulated by fatty acids and their cognate receptors, PPARγ and PPARδ, thereby positioning these cells to be the cellular integrators of lipid metabolism. In support of this idea, saturated fatty acids (the "bad" fats) bind and activate TLR4 signaling in macrophages, thereby increasing adipose tissue inflammation and insulin resistance (Kim et al., 2007; Shi et al., 2006). In contrast, monounsaturated fatty acids (the "good" fats) activate PPARδ to bias tissue macrophages to the alternative state, thus improving insulin sensitivity. However, the precise molecular mechanisms by which alternatively activated macrophages modulate insulin action and peripheral metabolism remain unclear. On the one hand, attenuation of tissue inflammation by paracrine actions of these cells might lead to improvement in insulin signaling (Bouhlel et al., 2007). On the other hand, alternatively activated macrophages might secrete trophic factors that act in a paracrine or endocrine manner to enhance oxidative metabolism in peripheral tissues (Figure 7). Experimental support for either hypothesis will require further investigation.

While our findings demonstrate a role for alternatively activated ATMs and Kupffer cells in lipid homeostasis, the stimuli driving this program of macrophage activation and the regulation thereof remain unclear. In addition to the Th2 cytokines IL-4 and IL-13, the microenvironment of tissue macrophages might contain other stimuli that support alternative activation (Rauh et al., 2005). In this study, we provide evidence that one such signal is likely to be the composition of fatty acids to which the macrophage is exposed. For instance, monounsaturated fatty acids activate PPARδ to enhance maturation of alternatively activated macrophages in tissues, suggesting that an increase in cytosolic concentration of free fatty acids might promote alternative macrophage activation and improve insulin action. In support of this notion, deletion or inhibition of cytosolic fatty acid-binding proteins improves insulin sensitivity (Furuhashi et al., 2007; Hotamisligil et al., 1996); however, the potential involvement of fatty acid-binding proteins in alternative macrophage activation will require further investigation.

Although our findings suggest subtype specificity for PPARs in acquisition of the metabolic and immune phenotypes of alternatively activated macrophages, it remains unknown whether pharmacologic activation of either receptor can rescue the observed defects in single-receptor knockout macrophages. For instance, it will be important to determine whether treatment of PPARδ$^{-/-}$ macrophages with synthetic activators of PPARγ can compensate for absence of PPARδ signaling. Thus, these types of gain-of-function experiments will be carried out using macrophages deficient in PPARγ, PPARδ, or both PPARγ and PPARδ.

In summary, we have demonstrated a critical role for alternatively activated Kupffer cells in metabolic syndrome. In the absence of PPARδ, Kupffer cells are unable to maintain the alternative phenotype, leading to suppression of oxidative metabolism and worsening of insulin resistance in peripheral tissues. Since our findings indicate that macrophage activation plays a critical role when caloric intake exceeds expenditure, cellular targeting of Kupffer cells to undergo alternative activation might be an effective strategy for treating obesity and insulin resistance.

EXPERIMENTAL PROCEDURES

In Vivo Metabolic Analyses

PPARδ$^{-/-}$ mice used in these studies were generated by the R.M. Evans laboratory and backcrossed onto the 129/SvJ strain for eight generations (Barak et al., 2002). 129/SvJ mice were used as wild-type controls for these experiments. All in vivo studies were initiated at 8–12 weeks of age. For bone marrow transplant studies, whole bone marrow was prepared from wild-type and PPARδ$^{-/-}$ mice and injected intravenously (5 × 10^6 cells/recipient) into lethally irradiated (850 rad) wild-type recipients. After 2–4 weeks of rest for bone

marrow reconstitution, mice were fed a high-fat diet (Bio-Serv F3283) for 18–25 weeks to promote obesity. DEXA was performed using a PIXImus II (GE Healthcare). Oral glucose tolerance tests (1 g/kg) were performed after a 14 hr fast. For insulin tolerance tests, mice were challenged with an i.p. injection of human insulin (0.65 U/kg) after a 4 hr fast. For biochemical analysis of insulin signaling, human insulin (5 U/kg) was injected into the inferior vena cava after a 4 hr fast. Liver and quadriceps were isolated and snap frozen in liquid nitrogen at 2 and 5 min, respectively. Tissue homogenates were immunoblotted for total Akt and phospho-Akt (Ser473) (Cell Signaling). Adipocyte cell size analysis and ATM quantification were performed as described previously (Odegaard et al., 2007a). Serum levels of lipids (total cholesterol and triglycerides), cytokines (TNFα, IL-6, and IL-1β), adipokines (leptin, resistin, and adiponectin), and insulin in fasted mice were quantified using commercially available kits. For liver triglyceride quantification, samples were finely minced and extracted overnight in acetone at 4°C. Extractable lipid content was measured using a commercially available kit (Stanbio) (Qu et al., 2006). To activate Kupffer cells with IL-4, mice were injected with IL-4 complexed with anti-IL-4 antibody, a procedure that has been shown to extend the half-life of this cytokine in vivo (Finkelman et al., 1993). Briefly, soluble complexes of IL-4 (2 μg) with anti-IL-4 antibody (BVD4-11, 10 μg) were injected i.p. into recipient mice on day 1 and day 4, and livers were harvested on day 5 for qRT-PCR analysis.

Quantitative RT-PCR Analyses

Gene expression analyses were performed as described previously (Odegaard et al., 2007a, 2007b). Briefly, total RNA was extracted from homogenized samples using TRIzol reagent (Invitrogen) and used as a template for cDNA synthesis (Marligen). Real-time quantitative PCR assays were carried in triplicate using DNA Engine Opticon 2. Relative expression levels of mRNAs were calculated by the comparative threshold cycle method using L32 (*Rpl32*) as an internal control. All primer sequences were verified by BLASTing against the NCBI mouse genome sequence database to ensure specificity.

In Vitro Macrophage Activation Analyses

BMDMs were cultured from wild-type and PPARδ$^{-/-}$ 129/SvJ as described previously (Odegaard et al., 2007a). Cells were stimulated with recombinant mIL-4 (10 ng/ml) or mIL-13 (10–40 ng/ml) for 48–72 hr in low-glucose medium (1 g/l). Arginase activity was measured by colorimetrically monitoring the evolution of urea from arginine (Rutschman et al., 2001). To assess cytokine production, cells were pretreated with IL-4 for 24–48 hr with subsequent stimulation with LPS (5 ng/ml). Secreted cytokines were quantified by ELISA per the manufacturer's protocols (BD Pharmingen). Cellular proliferation was assessed by measuring [^{3}H]thymidine incorporation over a 16 hr pulse. For the oleic acid experiments, low-glucose medium was supplemented with fatty acid-free BSA (0.35%, Roche) and filter sterilized. Oleic acid (100 μM, Sigma) was added to the medium and allowed to equilibrate for 4–5 hr at 37°C with periodic mixing. Subsequently, differentiated BMDMs were switched to BSA-containing medium and simultaneously stimulated with IL-4 for 24–48 hr. All experiments were repeated independently at least three times.

Immunoblotting and Flow Cytometry

Total cellular proteins were subjected to electrophoresis and immunoblotted for arginase 1 (BD Biosciences) (Rauh et al., 2005). For flow cytometry, BMDMs were treated with vehicle or IL-4 (10 ng/ml) for 48–72 hr, harvested in PBS, and resuspended in PBS containing 2% FCS and 0.2 mM EDTA. Macrophages were blocked with 200 μg/ml normal mIgG, stained with antibodies directed against dectin-1 or mannose receptor, and analyzed on a FACSCalibur system.

Transient Transfections

Transient transfection experiments were carried out as described previously (Vats et al., 2006). Briefly, plasmid DNAs were introduced into RAW264.7 cells by electroporation (300V, 1000 μF), and cells were allowed to recover for 2 hr in macrophage serum-free medium (Invitrogen). Luciferase activity was quantified 16 hr after stimulation with IL-4 (10 ng/ml) or GW501516 (100 nM) using a dual-luciferase reporter assay kit (Biotium). All experiments were performed in triplicate and repeated at least three times.

Macrophage-Hepatocyte Coculture

Primary hepatocytes were isolated and cultured using Invitrogen liver media and reagents. Briefly, the livers of anesthetized 8- to 12-week-old wild-type mice were sequentially perfused with liver perfusion and digest media via catheterization of the inferior vena cava. Digested livers were then resected and dissociated in liver wash buffer. Hepatocytes were purified over 50% Percoll and allowed to recover overnight on collagen-coated plates in Hepatozyme serum-free medium. Wild-type and PPARδ$^{-/-}$ BMDMs were seeded onto hepatocyte cultures at a 1:10 ratio and cocultured for a further 72 hr. Fatty acid oxidation assays were performed using a sodium hydroxide trap in a modified tissue culture flask (Vats et al., 2006). Parallel coculture plates were assayed for gene expression by qRT-PCR. Similarly, fatty acid oxidation and hepatic gene expression were assessed in primary hepatocytes 72 hr after treatment with wild-type or PPARδ$^{-/-}$ macrophage conditioned medium. All experiments were repeated independently at least three times.

Macrophage-Adipocyte Coculture

3T3-L1 cells were differentiated as described previously (Lumeng et al., 2007). On day 10, wild-type and PPARδ$^{-/-}$ BMDMs were seeded onto adipocyte cultures to an approximate density of 1:10 and cultured in macrophage medium for 48 hr. For glucose uptake, cultures were washed and stimulated with insulin (100 nM) or medium alone; glucose uptake was subsequently measured as described previously (Odegaard et al., 2007a).

Isolation of Tissue Macrophages from Transplanted Mice

Kupffer cell-enriched and stromal vascular fractions were isolated from livers and adipose tissue of transplanted mice as described previously (Weisberg et al., 2003). Macrophages present in these fractions were subsequently isolated using F4/80-coupled Dynabeads (Invitrogen) per the manufacturer's protocol. qRT-PCR analyses were performed for F4/80 and the region encompassing exon 4 of PPARδ, which is absent in PPARδ$^{-/-}$ cells.

PPARδ Ligand Studies

For in vivo analyses, wild-type 129/SvJ mice were given i.p. injections of vehicle or GW0742 (20 mg/kg/day) for 5 days (Graham et al., 2005). Twenty-four hours after the first injection, 1 ml of biogel beads suspended in sterile PBS was injected into the peritoneal cavity. Four days later, elicited macrophages were isolated by peritoneal lavage and subjected to qRT-PCR analyses. Similarly, the activation state of Kupffer cells and ATMs was determined in mice after treatment with vehicle or GW0742 for 5 days (n = 3–4 per treatment). For in vitro analyses, bone marrow cells from wild-type 129/SvJ mice were treated with GW501516 (100 nM) for 7 days, with medium changes every 2 days. To assess whether PPARδ ligands can synergize with IL-4 to promote alternative macrophage activation, BMDMs from 129/SvJ wild-type mice were prestimulated with low-dose IL-4 (2 ng/ml) for 24 hr followed by treatment with GW501516 for an additional 24 hr.

Statistical Analyses

Data are presented as mean ± SEM. All p values were calculated using two-tailed distribution, two-sample unequal variance Student's t test. *$p < 0.05$; **$p < 0.01$.

SUPPLEMENTAL DATA

Supplemental Data include one table and eight figures and can be found with this article online at http://www.cellmetabolism.org/cgi/content/full/7/6/496/DC1/.

ACKNOWLEDGMENTS

We thank members of the Chawla laboratory and A. Loh for valuable comments and P. Murray for providing key reagents. This work was supported by grants from the NIH (DK076760 and HL076746), Rockefeller Brothers Fund (Goldman Philanthropic Partnerships), and the American Diabetes Association to A.C. A.C. is a Charles E. Culpeper Medical Scholar. Support was provided by the Stanford Medical Scientist Training Program (J.I.O. and A.R.E.), the American Heart Association (J.I.O.), an HHMI Gilliam Fellowship (A.R.E.),

and National Research Service Award AI066402 (R.R.R.-G.). All animal care was in accordance with Stanford University's A-PLAC committee guidelines.

Received: October 11, 2007
Revised: February 28, 2008
Accepted: April 1, 2008
Published: June 3, 2008

REFERENCES

Arkan, M.C., Hevener, A.L., Greten, F.R., Maeda, S., Li, Z.W., Long, J.M., Wynshaw-Boris, A., Poli, G., Olefsky, J., and Karin, M. (2005). IKK-beta links inflammation to obesity-induced insulin resistance. Nat. Med. *11*, 191–198.

Barak, Y., Liao, D., He, W., Ong, E.S., Nelson, M.C., Olefsky, J.M., Boland, R., and Evans, R.M. (2002). Effects of peroxisome proliferator-activated receptor delta on placentation, adiposity, and colorectal cancer. Proc. Natl. Acad. Sci. USA *99*, 303–308.

Bouhlel, M.A., Derudas, B., Rigamonti, E., Dievart, R., Brozek, J., Haulon, S., Zawadzki, C., Jude, B., Torpier, G., Marx, N., et al. (2007). PPARgamma activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties. Cell Metab. *6*, 137–143.

Cai, D., Yuan, M., Frantz, D.F., Melendez, P.A., Hansen, L., Lee, J., and Shoelson, S.E. (2005). Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat. Med. *11*, 183–190.

Chawla, A., Repa, J.J., Evans, R.M., and Mangelsdorf, D.J. (2001). Nuclear receptors and lipid physiology: opening the X-files. Science *294*, 1866–1870.

Chawla, A., Lee, C., Barak, Y., He, W., Rosenfeld, J., Liao, D., Han, J., Kang, H., and Evans, R. (2003). PPAR delta is a very low-density lipoprotein sensor in macrophages. Proc. Natl. Acad. Sci. USA *100*, 1268–1273.

Evans, R.M., Barish, G.D., and Wang, Y.-X. (2004). PPARs and the complex journey to obesity. Nat. Med. *10*, 355–361.

Finkelman, F.D., Madden, K.B., Morris, S.C., Holmes, J.M., Boiani, N., Katona, I.M., and Maliszewski, C.R. (1993). Anti-cytokine antibodies as carrier proteins. Prolongation of in vivo effects of exogenous cytokines by injection of cytokine-anti-cytokine antibody complexes. J. Immunol. *151*, 1235–1244.

Furuhashi, M., Tuncman, G., Gorgun, C.Z., Makowski, L., Atsumi, G., Vaillancourt, E., Kono, K., Babaev, V.R., Fazio, S., Linton, M.F., et al. (2007). Treatment of diabetes and atherosclerosis by inhibiting fatty-acid-binding protein aP2. Nature *447*, 959–965.

Gordon, S. (2003). Alternative activation of macrophages. Nat. Rev. Immunol. *3*, 23–35.

Graham, T.L., Mookherjee, C., Suckling, K.E., Palmer, C.N., and Patel, L. (2005). The PPARdelta agonist GW0742X reduces atherosclerosis in LDLR−/− mice. Atherosclerosis *181*, 29–37.

Herbert, D.R., Holscher, C., Mohrs, M., Arendse, B., Schwegmann, A., Radwanska, M., Leeto, M., Kirsch, R., Hall, P., Mossmann, H., et al. (2004). Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. Immunity *20*, 623–635.

Hevener, A.L., Olefsky, J.M., Reichart, D., Nguyen, M.T., Bandyopadyhay, G., Leung, H.Y., Watt, M.J., Benner, C., Febbraio, M.A., Nguyen, A.K., et al. (2007). Macrophage PPAR gamma is required for normal skeletal muscle and hepatic insulin sensitivity and full antidiabetic effects of thiazolidinediones. J. Clin. Invest. *117*, 1658–1669.

Hotamisligil, G.S. (2006). Inflammation and metabolic disorders. Nature *444*, 860–867.

Hotamisligil, G.S., Johnson, R.S., Distel, R.J., Ellis, R., Papaioannou, V.E., and Spiegelman, B.M. (1996). Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. Science *274*, 1377–1379.

Kamei, N., Tobe, K., Suzuki, R., Ohsugi, M., Watanabe, T., Kubota, N., Ohtsuka-Kowatari, N., Kumagai, K., Sakamoto, K., Kobayashi, M., et al. (2006). Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. J. Biol. Chem. *281*, 26602–26614.

Kanda, H., Tateya, S., Tamori, Y., Kotani, K., Hiasa, K., Kitazawa, R., Kitazawa, S., Miyachi, H., Maeda, S., Egashira, K., and Kasuga, M. (2006). MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. J. Clin. Invest. *116*, 1494–1505.

Kennedy, D.W., and Abkowitz, J.L. (1997). Kinetics of central nervous system microglial and macrophage engraftment: analysis using a transgenic bone marrow transplantation model. Blood *90*, 986–993.

Kim, F., Pham, M., Luttrell, I., Bannerman, D.D., Tupper, J., Thaler, J., Hawn, T.R., Raines, E.W., and Schwartz, M.W. (2007). Toll-like receptor-4 mediates vascular inflammation and insulin resistance in diet-induced obesity. Circ. Res. *100*, 1589–1596.

Kliewer, S.A., Xu, H.E., Lambert, M.H., and Willson, T.M. (2001). Peroxisome proliferator-activated receptors: from genes to physiology. Recent Prog. Horm. Res. *56*, 239–263.

Li, Z., and Diehl, A.M. (2003). Innate immunity in the liver. Curr. Opin. Gastroenterol. *19*, 565–571.

Loke, P., and Allison, J.P. (2003). PD-L1 and PD-L2 are differentially regulated by Th1 and Th2 cells. Proc. Natl. Acad. Sci. USA *100*, 5336–5341.

Lumeng, C.N., Bodzin, J.L., and Saltiel, A.R. (2007). Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J. Clin. Invest. *117*, 175–184.

Mootha, V.K., Lindgren, C.M., Eriksson, K.F., Subramanian, A., Sihag, S., Lehar, J., Puigserver, P., Carlsson, E., Ridderstrale, M., Laurila, E., et al. (2003). PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat. Genet. *34*, 267–273.

Munder, M., Eichmann, K., Moran, J.M., Centeno, F., Soler, G., and Modolell, M. (1999). Th1/Th2-regulated expression of arginase isoforms in murine macrophages and dendritic cells. J. Immunol. *163*, 3771–3777.

Odegaard, J.I., Ricardo-Gonzalez, R.R., Goforth, M.H., Morel, C.R., Subramanian, V., Mukundan, L., Eagle, A.R., Vats, D., Brombacher, F., Ferrante, A.W., and Chawla, A. (2007a). Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. Nature *447*, 1116–1120.

Odegaard, J.I., Vats, D., Zhang, L., Ricardo-Gonzalez, R., Smith, K.L., Sykes, D.B., Kamps, M.P., and Chawla, A. (2007b). Quantitative expansion of ES cell-derived myeloid progenitors capable of differentiating into macrophages. J. Leukoc. Biol. *81*, 711–719.

Oliver, W.R., Jr., Shenk, J.L., Snaith, M.R., Russell, C.S., Plunket, K.D., Bodkin, N.L., Lewis, M.C., Winegar, D.A., Sznaidman, M.L., Lambert, M.H., et al. (2001). A selective peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport. Proc. Natl. Acad. Sci. USA *98*, 5306–5311.

Patti, M.E., Butte, A.J., Crunkhorn, S., Cusi, K., Berria, R., Kashyap, S., Miyazaki, Y., Kohane, I., Costello, M., Saccone, R., et al. (2003). Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. Proc. Natl. Acad. Sci. USA *100*, 8466–8471.

Pauleau, A.L., Rutschman, R., Lang, R., Pernis, A., Watowich, S.S., and Murray, P.J. (2004). Enhancer-mediated control of macrophage-specific arginase I expression. J. Immunol. *172*, 7565–7573.

Petersen, K.F., Befroy, D., Dufour, S., Dziura, J., Ariyan, C., Rothman, D.L., DiPietro, L., Cline, G.W., and Shulman, G.I. (2003). Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science *300*, 1140–1142.

Qatanani, M., and Lazar, M.A. (2007). Mechanisms of obesity-associated insulin resistance: many choices on the menu. Genes Dev. *21*, 1443–1455.

Qu, S., Altomonte, J., Perdomo, G., He, J., Fan, Y., Kamagate, A., Meseck, M., and Dong, H.H. (2006). Aberrant Forkhead box O1 function is associated with impaired hepatic metabolism. Endocrinology *147*, 5641–5652.

Raes, G., De Baetselier, P., Noel, W., Beschin, A., Brombacher, F., and Hassanzadeh Gh, G. (2002). Differential expression of FIZZ1 and Ym1 in alternatively versus classically activated macrophages. J. Leukoc. Biol. *71*, 597–602.

Rauh, M.J., Ho, V., Pereira, C., Sham, A., Sly, L.M., Lam, V., Huxham, L., Minchinton, A.I., Mui, A., and Krystal, G. (2005). SHIP represses the generation of alternatively activated macrophages. Immunity *23*, 361–374.

Rutschman, R., Lang, R., Hesse, M., Ihle, J.N., Wynn, T.A., and Murray, P.J. (2001). Cutting edge: Stat6-dependent substrate depletion regulates nitric oxide production. J. Immunol. *166*, 2173–2177.

Shi, H., Kokoeva, M.V., Inouye, K., Tzameli, I., Yin, H., and Flier, J.S. (2006). TLR4 links innate immunity and fatty acid-induced insulin resistance. J. Clin. Invest. *116*, 3015–3025.

Shoelson, S.E., Lee, J., and Goldfine, A.B. (2006). Inflammation and insulin resistance. J. Clin. Invest. *116*, 1793–1801.

Vats, D., Mukundan, L., Odegaard, J.I., Zhang, L., Smith, K.L., Morel, C.R., Greaves, D.R., Murray, P.J., and Chawla, A. (2006). Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. Cell Metab. *4*, 13–24.

Vianna, C.R., Huntgeburth, M., Coppari, R., Choi, C.S., Lin, J., Krauss, S., Barbatelli, G., Tzameli, I., Kim, Y.B., Cinti, S., et al. (2006). Hypomorphic mutation of PGC-1beta causes mitochondrial dysfunction and liver insulin resistance. Cell Metab. *4*, 453–464.

Weisberg, S.P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R.L., and Ferrante, A.W., Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. J. Clin. Invest. *112*, 1796–1808.

Weisberg, S.P., Hunter, D., Huber, R., Lemieux, J., Slaymaker, S., Vaddi, K., Charo, I., Leibel, R.L., and Ferrante, A.W., Jr. (2006). CCR2 modulates inflammatory and metabolic effects of high-fat feeding. J. Clin. Invest. *116*, 115–124.

Xu, H.E., Lambert, M.H., Montana, V.G., Parks, D.J., Blanchard, S.G., Brown, P.J., Sternbach, D.D., Lehmann, J.M., Wisely, G.B., Willson, T.M., et al. (1999). Molecular recognition of fatty acids by peroxisome proliferator- activated receptors. Mol. Cell *3*, 397–403.

Xu, H., Barnes, G.T., Yang, Q., Tan, G., Yang, D., Chou, C.J., Sole, J., Nichols, A., Ross, J.S., Tartaglia, L.A., and Chen, H. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J. Clin. Invest. *112*, 1821–1830.