Loss of Kupffer cells in diet-induced obesity is associated with increased hepatic steatosis, STAT3 signaling, and further decreases in insulin signaling

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While adipose tissue-associated macrophages contribute to development of chronic inflammation and insulin resistance of obesity, little is known about the role of hepatic Kupffer cells in this environment. Here we address the impact of Kupffer cell ablation using clodronate-encapsulated liposome depletion in a diet-induced obese (DIO) and insulin resistant mouse model. Hepatic expression of macrophage markers measured by realtime RT-PCR remained unaltered in DIO mice despite characteristic expansion of adipose tissue-associated macrophages. DIO mouse livers displayed increased expression of alternative activation markers but unaltered proinflammatory cytokine expression when compared to lean mice. Kupffer cell ablation reduced hepatic anti-inflammatory cytokine IL-10 mRNA expression in lean and DIO mice by 95% and 84%, respectively. Despite decreased hepatic IL-6 gene expression after ablation in lean and DIO mice, hepatic STAT3 phosphorylation, Socs3 and acute phase protein mRNA expression increased. Kupffer cell ablation in DIO mice resulted in additional hepatic triglyceride accumulation and a 30–40% reduction in hepatic insulin receptor autophosphorylation and Akt activation. Implicating systemic loss of IL-10, high-fat-fed IL-10 knockout mice also displayed increased hepatic STAT3 signaling and hepatic triglyceride accumulation. Insulin signaling was not altered, however. In conclusion, Kupffer cells are a major source of hepatic IL-10 expression, the loss of which is associated with increased STAT3-dependent signaling and steatosis. One or more additional factors appear to be required, however, for the Kupffer cell-dependent protective effect on insulin receptor signaling in DIO mice.

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1. Introduction

Omental adipose tissue produces an array of adipokines including leptin, adiponectin, resistin, and proinflammatory cytokines such as IL-6 and tumor necrosis factor alpha (TNF-α) [1]. Levels of these factors are under metabolic regulation and can individually impact local and systemic insulin responsiveness. For instance, levels of the proinflammatory cytokines IL-6 and TNF-α are elevated in the adipose tissue of obese, insulin resistant patients [2–4] and have been shown to directly inhibit insulin signaling through SOCS-3 induction [5] and c-Jun-N-terminal kinase (JNK)-dependent activation [6–8] respectively.

Adipose tissue-associated macrophages are increased in obesity [9–11] and account for almost all of the TNF-α production and a significant fraction of the IL-6 produced by the adipose tissue [11,12]. This can be attributed to the increased inflammatory properties of the newly recruited macrophages [12] as well as a shift in resident adipose tissue-associated macrophage activation state from alternative to classical activation [13]. Obesity increases expression and secretion of monocyte chemoattractant protein 1 (MCP-1/CCL2) in adipocytes and may explain how the adipose tissue actively recruits new macrophages. In fact, knockout mouse models of MCP-1 or its receptor (CCR2) have reduced macrophage accumulation and decreased inflammation within the adipose tissue under high-fat diet conditions [14,15] and in leptin receptor-deficient (Lepdb) mice [16].

Macrophage stimuli can elicit varied and distinct macrophage activation states [17]. Classical (Th1) activation is elicited in response to inflammatory molecules such as LPS or TNF-α in combination with IFN-γ and results in proinflammatory cytokine production and the respiratory burst (characterized by nitric oxide production) [17]. In contrast, alternative (Th2) activation dampens inflammatory responses and is characterized by increased arginase 1 expression and activity as well as increased expression of molecules such as MHC class II, mannose receptor, chitinase 3-like 3 (Ym1/2), and the resistin-like molecule FIZZ1 [17,18].

Recent investigations have focused on the phenotypic shift in adipose tissue-associated macrophage activation state from alternative to classical that occurs during obesity. Lumeng et al. [13] demonstrated that the predominant adipose tissue-associated macrophage...
activation state shifts from the more protective, anti-inflammatory TH2 state to proinflammatory TH1 in obesity. More recently, Odegaard et al. [19] observed that loss of PPAR-γ (in the TH2-prone Balb/c mice) impairs alternative activation of adipose tissue-associated macrophages, resulting in susceptibility to diet-induced obesity and decreased insulin responsiveness via reduced β-oxidative capacity. Kang et al. [20] demonstrated that parenchymal cell-derived IL-13 cannot stimulate alternative activation of PPAR-γ-deficient myeloid cells and results in inflammation and metabolic dysfunction in adipose tissue and liver. Additionally, Odegaard et al. [21] demonstrated that loss of PPAR-γ in lean mice impairs alternative activation of Kupffer cells, which results in decreased oxidative metabolism, hepatic steatosis and increased systemic insulin resistance. Clarification of the involvement of PPAR-γ and PPAR-δ is needed, however, as Marathe et al. [22] reported that neither PPAR-γ nor PPAR-δ was required for alternative activation.

Given the active involvement of the adipose tissue-associated macrophages and circulating monocyte [23] populations in chronic inflammation of obesity, this current study was designed to investigate the role of the hepatic macrophage, or Kupffer cell, in the inflammatory and insulin resistant states of obesity. Kupffer cells are the largest macrophage population in the body [24] and, in addition to fulfilling a variety of other immunologic functions [25,26], are the primary innate immune defense against exposure to foreign antigens from the diet and intestinal tract [26,27].

In contrast to the active characterization of adipose tissue-associated macrophages, there are few studies addressing the role of Kupffer cells in the inflammatory and metabolically dysfunctional obese state. While the work by Odegaard et al. [21] suggested that an alternatively activated Kupffer cell may support hepatic insulin responsiveness, the effect of obesity on the native Kupffer cell population remains undefined. The results of this investigation indicate that in contrast to adipose tissue-associated macrophages, Kupffer cells do not significantly contribute to the chronic proinflammatory environment of obesity. In support of observations by Odegaard et al. [21], Kupffer cells may partially protect hepatocytes from the inflammatory milieu and the subsequent steatosis and insulin resistance associated with high-fat diet-induced obesity.

2. Materials and methods

2.1. Antibodies

Phospho-specific Akt (serine 473), phospho-specific STAT3 (tyrosine705) and STAT3 antibodies were purchased from Cell Signaling Technology (Beverly, MA). An anti-phosphotyrosine antibody was purchased from Millipore (Billerica, MA). Akt1/2 and insulin receptor beta chain antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

2.2. Animals

Male C57BL/6j mice purchased from Jackson Laboratories were housed 4 per cage in a microisolator room on a 12-h light/dark cycle at the University of Rochester. The University Committee on Animal Resources approved all protocols. 4-week-old C57BL/6j male mice were fed standard chow or a high-fat diet (16.6% kcal/gm protein, 59.3% kcal/gm fat, 24.48% kcal/gm carbohydrate) ( Bioserve #S3282, Frenchtown, NJ). The latter resulted in diet-induced obesity. With the exception of the animals used for metabolic challenge tests, mice were utilized for experimentation after 15–17 weeks on the high-fat diet. Interleukin-10 knockout mice (B6.129P2-IL-10tm1Gsn/J) and wild-type controls were purchased from Jackson Laboratories and housed according to the conditions above. High-fat feeding of this model began at 4 weeks of age and experiments were performed after 7–8 weeks.

2.3. Metabolic studies

Metabolic studies were performed after 12–14 weeks of high-fat diet and 36 h following PBS- or clodronate-containing liposome administration. Briefly, mice were fasted overnight and given an intraperitoneal injection of insulin (1.5U/kg), glucose (1.5 g/kg), or pyruvate (1.0 g/kg) dissolved in sterile saline. Blood was taken from tail vein every 15 min and glucose was measured with an Accu-chek Advantage® glucometer (Accu-check). Results were normalized to percent starting (fasted) glucose and area under the curve (AUC) was calculated.

2.4. Kupffer cell ablation and immunostaining

Phosphate-buffered saline (PBS)-containing and clodronate-containing liposome suspensions were prepared as described earlier [28]. All parameters were assessed at 36 h following a 200-μl intraperitoneal injection. Liver slices were formalin-fixed, paraffin-embedded, and sectioned. Sections were stained using rat anti-mouse F4/80 (Invitrogen, Carlsbad, CA) antibody at 1:50 dilution. Detection was achieved using a biotin-conjugated rabbit anti-rat antibody (Vector Laboratories Inc., Burlingame, CA), incubation with streptavidin-horseradish peroxidase (Jackson Labs), exposure to amniocarbazole (Dako, Carpenteria, CA), and hematoxylin blue counterstaining.

2.5. Assessment of in vivo insulin signaling

Following an overnight fast (~15 h), animals were briefly anesthetized using an isofluorane vaporizer (Summit Medical, Salem, OR), and injected intraperitoneally with vehicle (sterile saline) or 1.5 Units/kg Novolin® human insulin (Novo Nordisk Pharmaceuticals, Princeton, NJ). After 10 min, animals were sacrificed. Harvest and processing of frozen tissue for immunoprecipitation and quantitative immunoblotting were carried out as previously described [29].

2.6. Lipid extraction and analysis

Lipid extraction protocol was adapted from [30]. Briefly, frozen liver fractions were weighed and homogenized in chloroform: methanol (2:1 vol/vol). Extracts were passed through fluted filter paper. Saline/0.05% sulfuric acid was added to partition the chloroform at a ratio of 1:5 (vol/vol) of filtered extract. Partitioning of extracts was completed by centrifugation, and the chloroform layer was removed, dried down and resuspended in fresh chloroform. Samples were diluted in 5% (vol/vol) Triton X-100 (Sigma) in chloroform and evaporated. Lids were measured using L-Type Tg and Cholesterol E kits from Wako Chemicals USA (Richmond, VA) in duplicate. Total lipid was normalized to protein content per wet weight of sample tissue. Oil Red O staining was performed on frozen liver sections and counterstained with hematoxylin.

2.7. Serum collection and analysis

Blood was collected via cardiac puncture, allowed to clot for 30 min, and spun at 7000 rpm for 10 min. Isolated serum was stored at −80 °C. Triglyceride and cholesterol levels were measured by the automated clinical laboratories at the University of Rochester. PAI-1, MCP-1, leptin, resistin, and insulin levels were determined using a LINCplex™ (LINCO Research, Inc, St. Charles, MO) mouse serum adipokine kit on the Bio-Rad Bio-Plex™ 200 Suspension Array System. IL-6 levels were determined using a Luminex® Beadlyte® (Upstate, Lake Placed, NY) assay. Blood glucose was measured from tail vein using an Accu-chek Advantage® glucometer (Accu-check) and HOMA-IR (homeostasis model assessment of insulin resistance).
was calculated: (fasting blood glucose (mmol/l) × fasting blood insulin (μU/ml)/22.5 [31].

2.8. Real-time PCR analysis

RNA was extracted using TRIzol® (Invitrogen) according to the manufacturer’s directions. Reverse transcription was performed using iSCRIPT™ (Bio-Rad). TaqMan probes for Actb, Ccl2, Emr1, Il6, Il10, Socs3, Tnfa, and Hp were purchased from Applied Biosystems and used with TaqMan 2× Master Mix (Applied Biosystems). Primer sequences for assays using Sybr Green (Bio-Rad) can be found in the Supplementary Table. The samples were run on an iCycler IQ real-time PCR detection system (Bio-Rad) and calculations determined as previously described [32].

2.9. Statistical analysis

Statistical analysis was performed using StatView 5 software (SAS Institute, Cary, NC) and Microsoft Excel (2004). Experimental outliers were calculated and removed using interquartile range calculations. Experimental means were compared using ANOVA where sample means from four groups were compared and Student t-test for comparing two groups.

3. Results

3.1. Diet-induced obesity does not increase hepatic Kupffer cell markers or result in classical activation of Kupffer cells

C57BL/6J mice were fed a normal chow (lean) or high-fat diet for 15 weeks to promote diet-induced obesity (DIO). Levels of two macrophage markers, F4/80 and CD68, were used to assess resident hepatic Kupffer cell. Expression of Emr1 (F4/80) and Cd68 remained unaltered in DIO mice (Fig. 1A). Hepatic expression of Ccl2 (MCP-1) (Fig. 1A) and inflammatory cytokines Il6, Tnfa (TNF-α), and Il10 (Fig. 1B) also remained essentially unaltered following high-fat feeding. These data suggest that the Kupffer cell population is not expanded in DIO mice and that the liver may not be directly contributing to the chronic inflammatory state of obesity.

Given the absence of increased inflammatory cytokine production, hepatic markers of alternative activation were examined. Expression of Chi3l3 (chitinase 3-like 3), Mgl1 (macrophage galactose N-acetyl-galactosamine specific lectin 1), and Mr2 (mannose receptor, C type 2) were all modestly elevated in the liver of DIO mice (Fig. 1A). To more directly examine the role of Kupffer cells in obesity and expand upon the observation that alternatively activated Kupffer cells have the potential to protect against obesity-associated inflammation, steatosis, and insulin resistance [21], well-characterized macrophage...
Ablation technique was employed. The majority of intraperitoneally injected clodronate-containing (CLD) liposomes are phagocytized by Kupffer cells (and peritoneal macrophages), resulting in cellular apoptosis [28,34]. Using this approach, nearly complete ablation of Kupffer cells was achieved 36 h post-injection in lean and DIO mice. This was confirmed by a loss of F4/80 staining by immunohistochemistry in clodronate-treated liver sections (Supplementary Fig. 2A) and a 98% (lean) and 96% (DIO) loss of Emr1 expression (Supplementary Fig. 2B). Comparable F4/80 staining in lean and DIO liver from PBS-injected animals supports the interpretation that the Kupffer cell population did not expand with high-fat feeding. CLD liposomes did not disrupt adipose tissue macrophages of lean animals, but a 2-fold increase in DIO adipose tissue-associated Emr1 expression was observed (Supplementary Fig. 2B). This latter effect in DIO mice was not associated with increases in circulating levels of IL-6 (Table 1) or inflammatory cytokine-dependent target gene expression in adipose tissue (Supplementary Fig. 2C).

3.3. Loss of Kupffer cells alters the local hepatic inflammatory environment of lean and DIO mice

Kupffer cell ablation in lean mice reduced hepatic expression of Il6 (73%) and Tnfa (46%) (Fig. 2A). Expression of Il6 was reduced by 52% in DIO mice following Kupffer cell ablation, but Tnfa expression remained unaltered (Fig. 2B). A striking 95% and 84% loss of IL-10 expression in lean and DIO mice, respectively, indicates that Kupffer cells are the primary source of this anti-inflammatory cytokine in the liver (Fig. 2A, B).

Although Kupffer cell ablation did not increase hepatic cytokine production, a selective increase in hepatic STAT3 signaling was

![Fig. 2](image-url)
observed in the liver of lean and DIO mice. Phosphorylation of STAT3 was increased in both lean (Fig. 2C) and DIO (Fig. 2D) mice following Kupffer cell ablation. Acute phase protein family members haptoglobin (Hp), orosomucoid-1 (Orm1), and serum amyloid A (Saa) were approximately 2-, 3-, and 7-fold elevated in lean mice following CLD liposome administration (Fig. 2C). Elevations of these markers were also observed in Kupffer cell ablated DIO mouse livers, with a significant 3-fold increase in suppressor of cytokine signaling (SOCS)-3 (Fig. 2D). In contrast, downstream transcriptional targets of TNF-α or endotoxin via NFκB signaling [35–37] including Traf1, Nfkbia (IκBα), Rela, and Ikbkb (IKKβ) were collectively unchanged in lean liver (Fig. 2C). With the exception of a modest increase in Traf1, they were also unchanged in DIO liver (Fig. 2D). Since IL-6 gene expression within the liver actually trended downward, the increased STAT3-dependent signaling may reflect decreased feedback inhibition of the pathway and/or a response to increased circulating STAT3 agonist.

To explore this possibility, serum cytokine and adipokine markers were assessed in DIO mice following Kupffer cell ablation. Circulating levels of IL-6 and IL-10 in DIO mice remained unaltered following Kupffer cell ablation (Table 1). TNF-α levels were not detectable. No change in the inflammatory marker PAI-1 or adipokine markers (leptin, resistin) was observed. Interestingly, circulating levels of MCP-1 were significantly elevated, possibly reflecting a replenishment mechanism following Kupffer cell ablation. While cholesterol levels were elevated, circulating triglyceride levels remained unchanged.

3.4. Kupffer cell ablation in DIO mice results in increased hepatic lipid accumulation

The reports of Odegaard et al. [21] and Kang et al. [20] suggested that cross-talk between Kupffer cells and hepatocytes could influence hepatic lipid metabolism. It was further proposed that Kupffer cells may protect hepatocytes from steatosis. To test this hypothesis, the impact of Kupffer cell ablation on obesity-associated alterations in lipid metabolism was examined. Lipid accumulation as a result of high-fat feeding was visible by Oil Red O staining in DIO liver sections compared to the lean controls (Fig. 3A). This increase is displayed quantitatively in Supplementary Fig. 1F. No qualitative or quantitative change in lipid accumulation was observed in livers of lean mice following Kupffer cell ablation (Fig. 3A and B). Kupffer cell ablation in DIO mice, however, increased hepatic triglyceride accumulation beyond that caused by high-fat feeding alone (Fig. 3A and B). Although serum cholesterol was increased in DIO mice upon Kupffer cell ablation, total hepatic cholesterol remained unaltered (PBS = 1.00 ± 0.11, CLD = 1.03 ± 0.14, p = 0.86). These results support the proposal that Kupffer cells partially protect hepatocytes from dysregulation of hepatic lipid metabolism and steatosis associated with obesity. Protein and gene expression of several lipid metabolic targets was analyzed in the livers of DIO control and DIO Kupffer cell-ablated mice and displayed in Supplementary Fig. 3. Of the oxidation, synthesis, and secretion-related markers, only PPARγ mRNA was elevated (Supplementary Fig. 3B). Since the modest elevation was not

![Fig. 3](https://example.com/figure3.png)

**Fig. 3.** Kupffer cell ablation in DIO mice is associated with altered hepatic lipid content. Hepatic lipid content in normal chow-fed (lean) and diet-induced obese (DIO) mice was assessed 36 h following Kupffer cell ablation. (A) Representative Oil Red O stained liver sections (20x) are displayed from mice treated with PBS- or CLD-containing liposomes. (B) Lipids were extracted from frozen livers of lean (mean ± S.E. n = 8) and DIO (mean ± S.E. n = 6) mice. Triglyceride content (mg/wet weight) was normalized to protein content (mg/wet weight).
accompanied by changes in any other targets, the significance of this observation is unclear.

3.5. Kupffer cell ablation in DIO mice impairs hepatic insulin signaling

Given that several reports link IL-6-induced STAT3-dependent signaling and hepatic lipid accumulation with insulin resistance [5,29,38], it would be predicted that hepatic insulin signaling would decrease in a Kupffer cell-ablated liver. To test this hypothesis, hepatic response to an insulin bolus was assessed following Kupffer cell ablation. Despite increased STAT3-dependent signaling in Kupffer cell-ablated lean mice, no change in insulin-stimulated insulin receptor autophosphorylation or serine phosphorylation of the downstream signaling mediator Akt was observed (Fig. 4A). In DIO mice, where both STAT3-dependent signaling and increased steatosis were observed, insulin receptor autophosphorylation and Akt serine phosphorylation were reduced 30% and 40%, respectively, upon Kupffer cell ablation (Fig. 4B). These results indicate that increased triglyceride in combination with increased STAT3-dependent signaling is associated with suppressed hepatic insulin signaling following Kupffer cell ablation in DIO mice.

3.6. Kupffer cell ablation in DIO mice and systemic response to insulin

Given that clodronate-treated DIO mice displayed impaired hepatic insulin signaling, systemic response to metabolic stimuli was assessed in these mice. Kupffer cell ablation in DIO mice resulted in an increase in HOMA-IR, an index of insulin resistance (Fig. 5A).

![Fig. 4. Kupffer cell ablation in DIO mice is associated with impaired hepatic insulin signaling. Following Kupffer cell ablation and an overnight fast, lean and DIO mice were given an i.p. insulin bolus (1.5 Units/kg). Tissues were harvested after 10 min. Tyrosine phosphorylation of the insulin receptor (IR) and Akt Ser473 phosphorylation were assessed by Western blot analysis in livers of lean (A) and DIO (B) mice. IR was immunoprecipitated prior to blotting. Each bar represents the mean ± S.E. of n ≥ 7 (lean) and n ≥ 9 (DIO). Mass blots are used to confirm comparable loading. Representative blots are shown. PBS = PBS-containing liposomes. CLD = clodronate-containing liposomes. *p ≤ 0.05; **p ≤ 0.01.](image)
This increase is primarily due to a 3-fold increase in fasting insulin levels (Table 1). In agreement with this result, Kupffer cell ablation of DIO mice modestly impaired insulin-stimulated glucose uptake, but area under the curve (AUC) did not show a significant effect (Fig. 5B). Response to a glucose bolus was similar in PBS and clodronate-treated DIO mice (Fig. 5C). A pyruvate tolerance test revealed prolonged circulating glucose in response to an i.p. injection of pyruvate in the clodronate-treated animals, but this effect did not reach significance (Fig. 5D). These results indicate that Kupffer cell ablation in DIO mice is associated with increased insulin resistance but only minor impairment in metabolic challenge tests.

3.7. Systemic loss of IL-10 during diet-induced obesity

We hypothesized that loss of Kupffer cell-derived IL-10 may locally or systemically contribute to dysregulation of STAT3 signaling and lipid metabolism in diet-induced obesity. To address this, IL-10 knockout mice (IL-10KO) and matched controls (WT) were subjected
DIO IL-10 knockout mice do not display altered hepatic insulin responsiveness. IL-10 knockout (IL-10KO) and wild-type (WT) mice were fed a high-fat diet for 8 weeks. (A) HOMA-IR was calculated from glucose and insulin concentrations of 16-h fasted WT and IL-10KO mice (mean ± S.E. of n ≥ 4). (B) Western blot analysis for phosphorylation of STAT3 (Y705) was performed on whole cell extracts from WT and IL-10KO mouse liver. Hepatic expression of IL-6-responsive genes and TNF-α-responsive genes was assessed by realtime RT-PCR (mean ± S.E. of n = 6). (C) Hepatic cytokine expression was assessed by realtime RT-PCR (mean ± S.E. of n = 6). Circulating IL-6 levels were measured by a Luminex® Beadlyte® assay (mean ± S.E. of n = 6). (D) Hepatic triglyceride content (mg/wet weight) was quantified in WT and IL-10KO mice and normalized to protein (mg/wet weight) (mean ± S.E. of n = 11). High-fat-fed WT and IL-10KO mice were fasted overnight prior to an i.p. injection of insulin (1.5 Units/kg). Tissues were harvested after 10 min. (E) Tyrosine phosphorylation of the insulin receptor (IR) and Akt Ser473 phosphorylation in livers of WT and KO mice were assessed by Western blot analysis (mean ± S.E. of n = 6). IR was immunoprecipitated prior to blotting. IR and Akt mass blots are included for comparison. *p ≤ 0.05; **p ≤ 0.01.
to a high-fat diet for 8 weeks. This shorter feeding protocol, compared to Kupffer cell ablation studies (15–17 weeks), was employed to minimize colitis that develops progressively in the IL-10KO mouse model [39]. Induction of insulin resistance was confirmed by increased HOMA-IR in high-fat diet-fed WT and IL-10KO mice compared to lean controls (Fig. 6A). No significant difference in HOMA-IR or body weight (DIO WT = 30.3 ± 1.1; DIO IL-10KO = 33.4 ± 1.3; p = 0.09) was seen, however, between obese WT and IL-10KO mice. Systemic deletion of IL-10 in an obese mouse resulted in increased hepatic STAT3 phosphorylation and 2.5- and 2-fold increases in hepatic Socs3 and Saa expression, respectively (Fig. 6B). These increases occurred in the absence of increased hepatic Il6 message or an increase in circulating IL-6 levels (Fig. 6C). Circulating IL-6 levels actually decreased in obese IL-10KO mice. Although the magnitude of hepatic acute phase protein gene induction was less than that observed in lean and DIO mice following Kupffer cell ablation (Fig. 2C, D), these data again suggest an association between loss of IL-10 and increased hepatic STAT3-dependent signaling in high-fat diet induced obesity. Despite a modest reduction in Relu, hepatic expression of most Nfkb-responsive genes remained unaltered (Fig. 6B). A statistically significant increase in hepatic triglyceride content was also observed in the livers of the high-fat diet-fed IL-10KO mice compared to their WT counterparts (Fig. 6D). Systemic loss of IL-10 in an obese state, however, did not alter hepatic insulin signaling compared to WT controls (Fig. 6E). Thus, while loss of IL-10 by gene deletion or Kupffer cell ablation is associated with altered hepatic STAT3 signaling, acute phase protein induction, and increased hepatic lipid accumulation, loss of additional Kupffer cell factors are required to further impair obesity-associated hepatic insulin signaling.

4. Discussion

Obesity-mediated insulin resistance has been shown to have a strong inflammatory component. Several groups, including Weisberg et al. [11] and Xu et al. [9], have suggested an important role for the adipose tissue-associated macrophage in mediating this inflammatory state and subsequent insulin resistance. In contrast to the characterization of the adipose tissue-associated macrophages, the effect of obesity on the Kupffer cell population is poorly understood. The current study investigated the role of liver macrophages, or Kupffer cells, in a diet-induced obese mouse model of hepatic inflammation and insulin resistance. Our results support the conclusion that Kupffer cells do not contribute to the proinflammatory environment of obesity, but modulate STAT3-dependent signaling and obesity-associated impairment of hepatic lipid metabolism and insulin resistance.

Here we report similar levels of macrophage markers, Emr1 and Cd68, in livers of lean and obese mice, suggesting no expansion of the Kupffer cell population in obesity. F4/80 staining of liver samples from lean and DIO mice supports this interpretation. This is also supported by the observations of Weisberg et al. [11] and Xu et al. [9] who reported similar Kupffer cell numbers despite an increased adipose tissue-associated macrophage population. Cintra et al. [40] and Cai et al. [41], however, report increased Kupffer cell numbers in mice placed on a high-fat diet for 2 and 3 months, respectively. The use of the Swiss mouse strain by Cintra et al. [40] could account for some of this difference, as mouse strains display varied phenotypes. A variation in diet composition, such as the ratio of saturated to unsaturated fat [42], could play a role in the obesity-associated effects seen by Cai et al. [41], but this dietary information was not provided.

In addition to no change in Kupffer cell markers in the liver following high-fat feeding, the absence of a change in hepatic inflammatory cytokine expression suggests that Kupffer cells are not actively contributing to the proinflammatory environment of diet-induced obesity. A collective increase in expression of alternative activation markers suggests that hepatic macrophages do not respond to the Th1 polarizing environment of obesity. These data are in contrast to adipose tissue-associated macrophages which undergo a Th2 to Th1 switch [13] and actively increase production of inflammatory cytokines. Other hepatic immune populations like CD4+ NK1.1 cells can mediate alternative activation of Kupffer cells in response to inflammation [43]. Perhaps they also mediate a similar Kupffer cell activation in response to the adipose tissue-derived cytokines and hepatic lipid accumulation.

Kupffer cell-ablated and IL-10KO DIO mice displayed increased hepatic STAT3-dependent signaling. In all cases, this occurred in the absence of increased hepatic IL-6 message, but a dramatic (≥90%) reduction of IL-10. Since IL-10 is an antagonist of IL-6 signaling, we hypothesize that Kupffer cell-derived IL-10 could modulate this response. This observation does not rule out a role for other Kupffer cell-derived direct or indirect cellular mediators of hepatocyte STAT3 signaling. Kupffer cell-derived molecules could be inducing inhibitors of IL-6 or other STAT3-dependent effectors within the hepatocyte, such as protein inhibitor of activated STAT3 (PIAS3), SH2-domain-containing tyrosine phosphatase (SHP2), or p38 stress kinase [44–46]. Alternatively, increased shedding or expression of soluble IL-6 receptor alpha (soluble gp80) following Kupffer cell ablation, could sensitize hepatocytes to circulating IL-6 and increase acute phase protein production [47,48]. Endotoxin stimulation of CD14 has been reported to activate the acute phase response, especially in the obese state [49], but this seems unlikely in our models as Nfkb transcriptional markers remained unaltered.

Both loss of Kupffer cells and systemic absence of IL-10 exacerbated hepatic triglyceride accumulation (steatosis) associated with obesity. This suggests that Kupffer cells and/or IL-10 production impart partial protection against pathologic accumulation of hepatic lipid. In support of this premise, inhibition of Kupffer cells has been shown to decrease PCE2 release within the liver leading to increased hepatic lipid synthesis [50]. In agreement with our DIO IL-10KO model, den Boer and associates also observed that systemic loss of IL-10 during obesity results in hepatic triglyceride accumulation [51]. Loss of IL-10 and increased hepatic STAT3-dependent signaling in our model is not sufficient, however, for increased hepatic lipid accumulation, as lipid content remained unaltered following Kupffer cell ablation in lean mice. A high-fat diet appears to also be necessary. These observations do not rule out the possibility that other Kupffer cell-derived factors could be directly involved in hepatic triglyceride synthesis or secretion during obesity.

Experimental evidence demonstrates that chronic inflammation and altered lipid metabolism associated with obesity directly impair insulin signaling. While many cytokines are involved in the chronic inflammation of obesity, IL-6 appears to be a major effector inhibiting insulin signaling in the liver at least in part through SOCS-3 induction [52,53]. Lipid accumulation induces oxidative stress and activates serine kinases that impair the insulin-signaling cascade [54–56]. Our Kupffer cell-ablated DIO mice displayed significant increases STAT3-dependent signaling and hepatic triglyceride accumulation. As a result, we hypothesized that insulin signaling would be impaired in this model. Hepatic response to an insulin bolus following Kupffer cell ablation was impaired 30–40% in association with a 3-fold increase in expression of Socs3 in the DIO mice. Despite an increase in HOMA-IR, systemic response to insulin, glucose, or pyruvate was not significantly altered following ablation in DIO mice. These data would suggest that Kupffer cells promote local hepatic insulin action. Since Kupffer cell ablation in lean mice elevated STAT3-dependent gene expression in the absence of impaired insulin signaling, increased STAT3-dependent signaling alone is not sufficient to impair insulin signaling in this model.

Recently, Cintra et al. [40] observed that treatment of DIO Swiss mice with IL-10 neutralizing antibodies or suppressing its expression using antisense techniques increased inflammation and impaired insulin responsiveness. In support of this premise, increased IL-10 expression levels in humans have been correlated with increased
insulin sensitivity [57]. While infusion of IL-6 into mice impaired insulin responsiveness during a hyperinsulinemic, euglycemic clamp study, co-infusion of IL-10 was shown to overcome the IL-6 effect [58]. In contrast to this, insulin signaling remained unaltered in our DIO IL-10KO mouse model. This agrees with the observation by den Boer et al. [51] that DIO IL-10 knockout mice displayed altered hepatic lipid metabolism, but unaltered insulin action compared to high-fat-fed wild-type controls. Additional work is needed to clarify the relationship between IL-10, inflammation, and insulin signaling.

Our observations indicate that while IL-10 is associated with modulation of hepatic STAT3-dependent signaling and lipid metabolism in DIO mice, one or more additional Kupffer cell mediators is necessary to partially preserve insulin responsiveness. Reactive oxygen species and ER stress have been directly implicated in the induction of insulin resistance in hepatocytes [55,59]. Kupffer cell-produced anti-oxidative molecules are potential direct or indirect protective mediators. Activation of the powerful anti-oxidant heme oxygenase-1 (HO-1) [60] reduces obesity-associated inflammation and improves systemic insulin responsiveness in mouse and rat models [61,62]. Kupffer cells produce HO-1, which has been demonstrated to protect against endotoxemia and oxidative stress in a rat model of ischemia-reperfusion [63]. Kupffer cell ablation may alter synthesis of another powerful anti-oxidant, lipoic acid, which is decreased in high-fat diet observed by Neyrinck et al [68] in mice receiving twice.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbapap.2009.08.007.

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