Haematopoietic leptin receptor deficiency does not affect macrophage accumulation in adipose tissue or systemic insulin sensitivity

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Abstract

The adipokine leptin is primarily produced by white adipose tissue (AT) and is a potent monocyte/macrophage chemoattractant in vitro. The long form of the leptin receptor (LepR) is required for monocyte/macrophage chemotaxis towards leptin. In this study, we examined the effects of haematopoietic LepR as well as LepR with C–C chemokine receptor 2 (CCR2) deficiency (double knockout (DKO)) on macrophage recruitment to AT after two different periods of high fat diet (HFD) feeding. Briefly, 8-week-old C57BL/6 mice were transplanted with bone marrow (BM) from Lepr+/+, Lepr−/− or DKO donors (groups named BM-Lepr+/+, BM-Lepr−/− and BM-DKO respectively), and were placed on an HFD for 6 or 12 weeks. At the end of the study, macrophage infiltration and the inflammatory state of AT were evaluated by real-time RT-PCR, histology and flow cytometry. In addition, glucose and insulin tolerance were assessed at both time points. Our results showed no differences in macrophage accumulation or AT inflammatory state between the BM–Lepr+/+ and BM–Lepr−/− mice after 6 or 12 weeks of HFD feeding; any effects observed in the BM-DKO were attributed to the haematopoietic deficiency of CCR2. In addition, no changes in glucose or insulin tolerance were observed between groups after either period of HFD feeding. Our findings suggest that although leptin is a potent chemoattractant in vitro, haematopoietic LepR deficiency does not affect macrophage accumulation in AT in early to moderate stages of diet-induced obesity. Journal of Endocrinology (2012) 212, 343–351

Introduction

Accumulation of inflammatory macrophages in adipose tissue (AT) during obesity has been shown to correlate with AT inflammation and subsequent insulin resistance (IR; Weisberg et al. 2003, Xu et al. 2003). In addition, the polarisation of the infiltrating macrophages is known to be an important factor in the inflammatory state of AT (Lumeng et al. 2007, 2008). Thus, understanding the mechanism(s) of macrophage recruitment to AT could lead to the development of therapies against IR and type 2 diabetes. Adipokines, which are hormones secreted from AT, could act as chemoattractants and contribute to macrophage recruitment to AT. The adipokine leptin has emerged as a strong candidate, given its known role in immune regulation (La Cava & Matarese 2004). Leptin is a 16 kDa soluble protein encoded by the Ob gene and is expressed primarily by white AT, such that circulating levels of leptin are positively correlated with levels of adiposity and AT macrophage accumulation (Halaas et al. 1995, Coenen et al. 2007).

Leptin acts primarily through the long form of the leptin receptor (LepR) and plays an important role in weight regulation through its action in the hypothalamus (Leshan et al. 2006). Mice that lack either leptin or the LepR are characterised by severe hyperphagia and morbid obesity, as well as other hormonal abnormalities (Robertson et al. 2008). Previous studies have shown that leptin promotes neutrophil and monocyte chemotaxis as well as the activation of monocytes/macrophages leading to secretion of pro-inflammatory cytokines and the up-regulation of inducible nitric oxide synthase (iNOS; Zarkesh-Esfahani et al. 2001, Dixit et al. 2003, Gruen et al. 2007). We have shown that leptin is a potent monocyte chemoattractant in vitro and that this chemotaxis is LepR-dependent (Gruen et al. 2007). In addition, we have shown that expression of Emr1 (the gene for F4/80) in AT, an indicator of macrophage infiltration, is positively correlated with circulating levels of leptin during obesity (Supplementary Figure 1, see section on supplementary data given at the end of this article; $r^2 = 0.6973; P<0.0001$; Coenen et al. 2007). Given that leptin is primarily secreted from AT and that its circulating levels are positively correlated with increases in macrophage infiltration, we hypothesised that leptin could act as a monocyte chemoattractant that regulates macrophage recruitment to AT during obesity. We designed a bone marrow transplant (BMT) study to determine if haematopoietic deficiency of the functional long form of the LepR, which we have shown to be required for monocyte chemotaxis towards leptin in vitro (Gruen et al. 2007), would lead to decreases in macrophage recruitment to AT during high fat diet (HFD).
feeding. We transplanted bone marrow from Lepr<sup>−/−</sup> and double knockout (DKO) mice into wild type (WT) recipients and placed these mice on an HFD for 6 or 12 weeks. These time points were chosen because of previously shown kinetics of macrophage recruitment to AT (Nishimura et al. 2009). We examined macrophage infiltration into AT by flow cytometry, histology and real-time RT-PCR. Our data provides evidence that haematopoietic deficiency of the LepR does not affect macrophage recruitment to AT or insulin sensitivity during HFD-induced obesity.

Materials and Methods

Mice and diets

All animal care and experimental procedures were performed with approval from the Institutional Animal Care and Use Committee of Vanderbilt University. The Lepr<sup>+/+</sup>, Lepr<sup>−/−</sup> and Ccr2<sup>−/−</sup> mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and were on a C57BL/6 background. DKO donor mice were generated by crossing Ccr2<sup>−/−</sup> with Lepr<sup>−/−</sup> mice. All studies were performed with 8-week-old male donor and recipient mice. Mice were fed ad libitum and given free access to water. Mice were kept on antibiotic water for 1 week prior to BMT until 4 weeks post-BMT. Mice were then placed on a 60% fat diet from Research Diets, Inc. New Brunswick, NJ, USA, (D12492) with a calorie density of 5.24 kcal/g; starting at 4 weeks post-BMT for a total of 6 or 12 weeks (Fig. 1). Because we were only interested in changes between the two genotypes and not the diet effects we did not use chow-fed mice in the original study; however, we performed an additional study in which we showed that HFD-fed Lepr<sup>+</sup> mice have increased macrophage infiltration and become insulin resistant in comparison to Lepr<sup>+</sup> mice fed a chow diet (Supplementary Figure 2, see section on supplementary data given at the end of this article). Thus, we had the ability to identify any changes in these parameters in mice fed HFD for only 6 weeks.

Bone marrow transplantation

Bone marrow cells were collected from Lepr<sup>+/+</sup>, Lepr<sup>−/−</sup> and DKO donor mice and were injected into the retro-orbital venous plexus of lethally irradiated WT recipient mice. Complete reconstitution of the desired bone marrow was confirmed by PCR of splenic cells using primers specific for the db allele and the Neo cassette insertion in the Ccr2<sup>−/−</sup> deficient mice (data not shown). The BM-Lepr<sup>+/+</sup> controls in this study were also used as negative controls for a different study performed simultaneously (Gutierrez et al. 2011). In addition, recipients of Ccr2<sup>−/−</sup> marrow (BM-Ccr2<sup>−/−</sup>) were included in the previous paper. There were no significant differences in any parameter studied between the Ccr2<sup>−/−</sup> and DKO recipients. Thus, only the DKO mice are shown in the current report.

Body weight, body composition and food intake

Body weight and food intake were measured weekly for the duration of the study. Total lean fat and free fluid mass were measured by nuclear magnetic resonance (NMR) using a Bruker Minispec (Woodlands, TX, USA) in the Vanderbilt Mouse Metabolic Phenotyping Centre (MMPC) at baseline, 6 and 12 weeks after placement on an HFD.

Insulin and glucose tolerance tests

Mice were fasted for 5 h and baseline glucose levels were measured using a LifeScan One Touch Ultra Glucometer (Johnson & Johnson, Northridge, CA, USA) via the tail vein. Mice were then injected i.p. with either 0.4 U (6 weeks) and 0.5 U (12 weeks) insulin per kilogram of lean mass; or 2 g (6 weeks) and 1 g (12 weeks) dextrose per kilogram of lean mass. Glucose levels were then assessed at 15, 30, 45, 60, 90 and 150 min post-injection.

Plasma collection and measurements

Mice were fasted for 5 h and bled from the retro-orbital venous plexus using heparinised collection tubes. Plasma was separated by centrifugation and stored at −80 °C. Plasma insulin levels were measured using an ELISA kit from Millipore, Inc. (Billerica, MA, USA). Leptin levels were assessed at the Vanderbilt University Hormone Core.

Real-time RT-PCR

RNA extraction was performed with an RNeasy kit (Qiagen) according to the manufacturer’s instructions. The iScript cDNA synthesis kit (Bio-Rad) was used for reverse transcriptase reactions. Real-time RT-PCR experiments were performed using an q5 thermal cycler. The reactions were carried out using IQ Supermix (Bio-Rad) and FAM-conjugated Assay-on-Demand (Applied Biosystems, Foster City, CA, USA) primer/probe sets normalized to 18S. The following genes were assessed: 18S (4352930E),...
Flow cytometry

Stromal vascular and white blood cells were first incubated with Fc block for 5 min at RT, followed by incubation for 30 min at 4°C with fluorophore-conjugated antibodies: F4/80–FITC (eBioscience, San Diego, CA, USA), Ly6C–FITC (BD Biosciences, Franklin Lakes, NJ, USA), Ly6G–PE (BD Biosciences) and CD11b–APC-Cy7 (BD Bioscience). DAPI (0.2 μg/ml) was added to each sample as a viability stain 10 min before flow cytometry analysis. Stromal vascular cells (SVCs) were processed on an MACS Quant analyzer (Miltenyi Biotech, Bergisch Gladbach, Germany). Blood cells were processed using a 5 Laser LSRII machine in the Vanderbilt Flow Cytometry Core. All data was analysed using FlowJo Software version 7.6.1 (Ashland, OR, USA).

Histology

Epididymal AT was harvested from mice, weighed and a portion was fixed overnight in 10% (v/v) formalin, transferred to 70% (v/v) ethanol and paraffin embedded. Tissue was cut into 7 μm sections and stained with 0.1% (w/v) toluidine blue O (TBO) solution (Newcomer Supply, Middleton, WI, USA) as previously described (Coenen et al. 2007).

Stromal vascular fraction separation

Epididymal AT pads were excised and minced in 3 ml of 0.5% (w/v) BSA in PBS and placed on ice. Subsequently, 3 ml of 2 mg/ml collagenase was added to the minced fat and incubated at 37°C for 20 min with shaking. The cell suspension was filtered through a 100 μm filter and then spun at 300 g for 10 min to separate floating adipocytes from the stromal vascular fraction (SVF) pellet. The SVF pellet was then resuspended in 3 ml ACK lysis buffer and incubated at room temperature (RT) for 5 min for red blood cell lysis, followed by dilution in 10 ml PBS. The cells were then washed twice with PBS.

Figure 2 Weight gain was similar among groups upon HFD feeding. (A) Average weekly body weight of BM-Lepr+/+ , BM-Lepr−/− and BM-DKO mice pre- and post-HFD. (B) Percent adiposity (total fat tissue divided by total body weight) determined by NMR. (C) Epididymal AT weight. (D) Circulating plasma leptin levels assessed by ELISA. Data are presented as mean ± S.E.M. For pre-HFD up to 6 weeks post-HFD, n = 20 mice per group. For 12 weeks post-HFD, n = 9–10 mice per group.

Figure 3 All groups have similar glucose and insulin tolerance. Glucose tolerance tests were performed by injecting mice i.p. with dextrose at a concentration of 2 g (6 weeks) or 1 g (12 weeks) per kilogram of lean mass after a 5 h morning fast. Glucose tolerance curves for all groups after (A) 6 weeks and (B) 12 weeks of HFD feeding. Insulin tolerance tests were performed by injecting mice i.p. with 0.4 U (6 weeks) or 0.5 U (12 weeks) human insulin per kilogram of lean mass after a 5 h morning fast. Insulin tolerance curves for all groups after (C) 6 weeks and (D) 12 weeks of HFD feeding. (E) Fasting insulin levels were measured by ELISA at the pre-HFD, 6 and 12 weeks time points. (F) Fasting glucose levels were assessed at the pre-HFD, 6 and 12 weeks time points. Data are presented as mean ± S.E.M. For GTT and ITT data, n = 5 mice per group for 6 week data and n = 5 mice per group for 12 week data. For fasting insulin and glucose data, n = 8–10 per group. ***p < 0.001 for all groups between Pre-HFD and 6 or 12 weeks.
Statistical analysis

GraphPad Prism 4.0 software, La Jolla, CA, USA was used for all statistical analyses. Data was analysed using one-way ANOVA to test differences between the three experimental groups and two-way ANOVA to compare measurements with two different variables (i.e. glucose and insulin tolerance tests (GTTs and ITTs)). Outliers were excluded from the data for each individual parameter if outside the range of the mean ± 2 s.d. and \( P \leq 0.05 \) was considered significant.

Results

Metabolic profile

Male C57BL/6 mice were transplanted with \( \text{Lepr}^{+/+} \), \( \text{Lepr}^{-/-} \) or DKO marrow followed by HFD feeding for 6 or 12 weeks as described in the Materials and Methods section (Fig. 1). There were no differences in body weight (Fig. 2A) or food intake (data not shown) between the groups at any point during the study. Likewise, there were no differences in adiposity among genotypes at any time although all groups increased in adiposity after 6 and 12 weeks of HFD feeding (Fig. 2B). Epididymal fat pad weight (Fig. 2C) and circulating leptin levels (Fig. 2D) were not different among genotypes at any time point. In accordance with the NMR adiposity data, epididymal AT weight and leptin levels were generally elevated between the 6 and 12 week time points for each group (Fig. 2C and D). Glucose and insulin tolerance tests were performed after 6 weeks (Fig. 3A and C) and 12 weeks (Fig. 3B and D) of HFD feeding. All groups responded similarly to the intraperitoneal glucose and insulin challenges after both periods of HFD feeding. In general, fasting insulin levels were increased for all groups between the baseline measurements and 12 weeks of HFD feeding (Fig. 3E). Fasting glucose levels were significantly increased (\( P < 0.001 \)) in all groups between the pre-HFD and both the 6 and 12 week time points (Fig. 3F).

Blood monocytes

Two phenotypic and functional subsets of mature blood monocytes have been described: Ly6C\text{lo} and Ly6C\text{hi} (Geissmann et al. 2010). The Ly6C\text{hi} circulating monocyte subset is thought to be the source of recruited inflammatory macrophages to inflamed tissues. The circulating levels of blood monocytes and neutrophils were assessed by flow cytometry after 12 weeks of HFD feeding by first gating on

![Graph showing blood monocytes and neutrophils](image-url)
all live CD11b+ cells. The BM-Lepr−/− mice had a ~40% (P<0.05) reduction in circulating Ly6Chigh monocytes, and BM-DKO mice had a ~90% (P<0.001) reduction, when compared with the BM-Lepr+/+ controls (Fig. 4A, B, C and D). No differences were observed in the Ly6Clow monocyte subset or the percentage of circulating neutrophils between groups (Fig. 4E and F).

Macrophage accumulation in AT

The accumulation of macrophages in AT after 6 and 12 weeks of HFD feeding was evaluated by histology, real-time RT-PCR and flow cytometry. TBO staining showed that there were no overt differences in overall immune infiltration between groups after either period of HFD feeding (Fig. 5A, B, C, D, E and F). Real-time RT-PCR gene expression analysis in total AT for the macrophage markers Emr1 and Cd68 showed no differences between groups after 6 weeks (Fig. 5G) or 12 weeks (Fig. 5H) of HFD feeding. Quantification of the percentage of F4/80hi cells in the SVF showed a significant decrease (P<0.05) in the BM-DKO group when compared with the BM-Lepr+/+ and BM-Lepr−/− groups after 6 weeks of HFD feeding (Fig. 6G). There were no differences in the percentage of F4/80hi cells in the AT of any group after 12 weeks of HFD feeding (Fig. 6H). The BM-DKO mice had an increase in a discrete F4/80low myeloid population (Fig. 6C, arrow) which is due to the deficiency in hematopoietic C–C chemokine receptor 2 (CCR2), as we have previously reported (Gutierrez et al. 2011).

AT inflammatory state

The inflammatory state of total AT was determined by assessing the gene expression of several pro- and anti-inflammatory markers by real-time RT-PCR. Analysis of the expression of the inflammatory markers Tnf and Nos2 showed no differences in these genes among groups after 6 weeks.

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**Figure 5** Histology and real-time RT-PCR analysis of macrophages in AT. Representative AT TBO images of (A) BM-Lepr+/+, (B) BM-Lepr−/− and (C) BM-DKO mice after 6 weeks of HFD feeding. Representative AT TBO images of (D) BM-Lepr+/+, (E) BM-Lepr−/− and (F) BM-DKO mice after 12 weeks of HFD feeding. Real-time RT-PCR gene expression analysis of Emr1 (F4/80) and Cd68 in total AT after (G) 6 and (H) 12 weeks of HFD feeding (mean±s.e.m.; n=10 mice per group). Full colour version of this figure available via http://dx.doi.org/10.1530/JOE-11-0338.
weeks on the HFD (Fig. 7A). There was a significant decrease ($P<0.001$) in Nos2 expression in the BM-DKO mice in comparison to the BM-Lepr$^{+/+}$ group when compared with the BM-Lepr$^{+/+}$ and BM-Lepr$^{-/-}$ groups at the 12 week time point (Fig. 7B). Additionally, assessment of the anti-inflammatory ‘M2’ markers Arg1, Mgl1 and Mgl2 showed that there was a significant increase ($P<0.05$) in Arg1 expression in the BM-DKO mice in comparison to the BM-Lepr$^{+/+}$ mice after 6 weeks of HFD feeding (Fig. 7A). Arg1, Mgl1 and Mgl2 were all significantly increased ($P<0.05$) in the BM-DKO group in comparison to the BM-Lepr$^{+/+}$ and/or BM-Lepr$^{-/-}$ groups after 12 weeks of HFD (Fig. 7B). Bone marrow LepR deficiency alone did not impact anti-inflammatory gene expression in AT.

**Discussion**

Inflammatory macrophage infiltration into AT during obesity has been shown to be temporally correlated with local and systemic inflammation and IR. (Weisberg et al. 2003, Xu et al. 2003, Hotamisligil 2006, Lumeng et al. 2007). Several genetic models that either disrupt macrophage recruitment to AT (Kanda et al. 2006, Weisberg et al. 2006, Odegaard et al. 2007, Surmi et al. 2010) or promote an AT macrophage switch towards an ‘M2’ or anti-inflammatory phenotype (Bouhlel et al. 2007, Odegaard et al. 2007, Westcott et al. 2009) have been explored. Despite the great progress made in this field in the past decade, methods to actively decrease macrophage recruitment to AT or diminish their inflammatory impact on this tissue are poorly understood.

We and others have shown that the adipokine leptin plays an important role in macrophage chemotaxis in vitro directly via its ability to act as a macrophage chemoattractant (Grunewald et al. 2007) or indirectly through the up-regulation of adhesion molecules on endothelial cells (Curat et al. 2004). Additionally, leptin has been shown to play a role in chemotaxis of macrophages (Kato et al. 2011) and neutrophil (Caldefie-Chezet et al. 2001, 2003) chemotaxis. These findings have led to the speculation that leptin, which has potent monocyte chemotactic activity, could be an important mediator of monocyte/macrophage chemotaxis to AT, where this adipokine is mainly produced (Surmi & Hasty 2008, Bourliere & Bouloumie 2009, Gutierrez et al. 2009, Maeda et al. 2009, Vona-Davis & Rose 2009, Wood et al. 2009, Conde et al. 2010, Fernandez-Riejos et al. 2010). This is of particular interest during the initial stages of inflammatory macrophage accumulation in AT when recruitment is positively correlated with adiposity and circulating leptin levels (Supplementary Figure 1; Coenen et al. 2007). However, this hypothesis had not been tested in vivo. In this study we assessed whether haematopoietic LepR deficiency led to a disruption in macrophage recruitment to AT during obesity with the hypothesis that BM-Lepr$^{-/-}$ chimeras would have reduced macrophage accumulation and improvements in glucose and insulin tolerance. Given that compensation by other cytokines was possible, we also generated BM-DKO chimeras that have a haematopoietic deficiency in both Lepr and Ccr2.

**Figure 6** Flow cytometry analysis of stromal vascular cells (SVCs). SVCs were isolated and the percentage of live F4/80$^+$ cells was quantified. Representative histograms of F4/80$^+$ cells for (A) BM-Lepr$^{+/+}$, (B) BM-Lepr$^{-/-}$ and (C) BM-DKO mice after 6 weeks of HFD feeding; (D) BM-Lepr$^{+/+}$, (E) BM-Lepr$^{-/-}$ and (F) BM-DKO mice after 12 weeks of HFD feeding. (G and H) Quantification of the percentage of F4/80$^+$ live cells in the SVF of all groups after 6 or 12 weeks of HFD feeding. Data represent mean±s.e.m. of four to five mice per group. Arrow indicates F4/80$^{lo}$ cells found in BM-DKO mice. $^*P<0.05$ between BM-Lepr$^{+/+}$ and BM-DKO mice. $^\wedge P<0.05$ between BM-Lepr$^{-/-}$ and BM-DKO mice.
Our results showed that haematopoietic LepR deficiency led to a significant decrease in the percentage of circulating Ly6C^hi monocytes (Fig. 4B and D). A similar but more dramatic phenotype has been observed in mice with CCR2 deficiency (Tsou et al. 2007, Gutierrez et al. 2011), in which Ly6C^hi monocytes fail to egress from the bone marrow. In fact, the BM-DKO mice showed an identical phenotype to that of the BM-Ccr2^−/− mice (Fig. 4C and D), indicating that having both CCR2 and LepR deficiency does not confer an additive or synergistic effect on the decreases in circulating inflammatory monocyte levels. The mechanism responsible for the reduction of Ly6C^hi monocytes in BM-Lepr^−/− mice is unknown; however, because the levels of circulating monocyte in the BM-DKO mice are identical to those of the BM-Ccr2^−/− mice, it is likely that the BM-Lepr^−/− mutation is not affecting inflammatory monocyte egress but instead their proliferation and/or differentiation in the bone marrow. This hypothesis remains to be tested.

The main end point of this study was to determine if the deficiency of LepR led to a reduction in macrophage recruitment to AT. Previous studies have shown that the initial process of inflammatory macrophage accumulation in AT occurs between 6 and 12 weeks of HFD feeding (Nishimura et al. 2009, Shaul et al. 2010); thus we used these time points to assess macrophage accumulation using multiple approaches. Our histology (Fig. 5A, B, C, D, E and F), gene expression (Fig. 5G and H) and flow cytometry (Fig. 6) data showed no protective effect of LepR deficiency on macrophage accumulation in AT after 6 or 12 weeks of HFD feeding. There was a significant decrease (P<0.05) in the percentage of F4/80hi macrophages in the BM-DKO group after 6 weeks of HFD feeding (Fig. 6G); however, this can be accounted for by the haematopoietic CCR2 deficiency, which leads to a decrease in F4/80hi cells and the accumulation of F4/80lo cells (Gutierrez et al. 2011). In addition, no changes in glucose or insulin tolerance were observed between the three groups (Fig. 3A, B, C and D) despite the fact that all mice became hyperglycaemic and hyperinsulminemic in response to the HFD feeding (Fig. 3E and F). Thus, we can conclude that haematopoietic LepR deficiency alone or in combination with haematopoietic CCR2 deficiency does not affect inflammatory macrophage recruitment to AT or insulin sensitivity after 6 or 12 weeks of HFD feeding.

The negative results are not a consequence of the inability of these mice to have increased macrophage infiltration, become insulin resistant or glucose intolerant during HFD feeding. We performed an additional study in which we show that Lepr^+/+ mice fed an HFD have increased macrophage infiltration, glucose intolerance and IR when compared with Lepr^+/− mice fed a chow diet (Supplementary Figure 2). Thus, within the limits of the sensitivity of our assays, haematopoietic LepR deficiency does not affect macrophage recruitment or insulin sensitivity during obesity.

Similar negative results have been obtained by our group and others when assessing inflammatory macrophage recruitment to AT after deletion of a single chemokine or chemokine receptor (Chen et al. 2005, Inouye et al. 2007, Kirk et al. 2008, Surmi et al. 2010). Accordingly, the current study provides evidence for the redundancy of chemokines and chemokine receptors in the innate immune system. Despite the elimination of two receptors that mediate the function of four different chemokines (leptin, CCL2, CCL7 and CCL8) that are up-regulated in the AT during obesity, macrophage infiltration into AT is not disrupted. This redundancy in the chemokine system contributes to the requirement for leukocyte chemotaxis in normal immune system function, making it ‘robust’ to naturally occurring mutations (Mantovani 1999).

Previously published studies suggest that in vitro leptin treatment leads to the activation of monocyte/macrophages and have shown a dose-dependent induction of inflammatory mediators such as interleukin 6 and tumor necrosis factor α (Zarkesh-Esfahani et al. 2001). Macrophages are primarily responsible for AT inflammation during obesity (Hotamisligil et al. 1993, 1995, Xu et al. 2003); thus, we tested whether haematopoietic LepR deficiency led to changes in the inflammatory state of total AT by real-time RT-PCR. Our results showed no differences in pro- or anti-inflammatory marker gene expression between BM-Lepr^+/+ and BM-Lepr^−/− mice after any period of HFD feeding (Fig. 7A and B). Significant differences were observed in the inflammatory gene expression of BM-DKO mice; however, these are similar to the changes in gene expression found in the BM-Ccr2^−/− mice (Gutierrez et al. 2011). As with macrophage chemotaxis, the effects of leptin on inflammatory gene expression that have been found in vitro are likely compensated in vivo by other cytokines found in the AT environment during obesity.

In conclusion, our study provides evidence that despite the potency of leptin as a monocyte/macrophage chemotactic agent (Gruen et al. 2007) in vitro, haematopoietic deficiency of
its functional receptor does not affect macrophage infiltration in AT. Our current finding that haematopoietic LepR deficiency decreases the number of circulating Ly6C\(\text{hi}\) monocyte levels should be further studied as it could provide a novel role for leptin in haematopoiesis and/or myeloid cell migration. Our results do not rule out the possibility that leptin can affect macrophage infiltration into AT through the up-regulation of adhesion molecules in the endothelial cells of the SVF, as has been previously suggested by Curat et al. (2004).

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/JOE-11-0338.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

D A G designed, performed and analysed all experiments and wrote the manuscript. A H H aided in designing the experiments and edited the manuscript.

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