Published in final edited form as:

Obesity (Silver Spring). 2015 October ; 23(10): 2037–2045. doi:10.1002/oby.21184.

Hematopoietic Cyclooxygenase-2 Deficiency Increases Adipose Tissue Inflammation and Adiposity in Obesity

Nikhil Adi^{1,2}, Curtis Perriotte-Olson^{1,2}, Cyrus Desouza^{2,1}, Ramesh Ramalingam^{1,2}, and Viswanathan Saraswathi^{1,2,3,*}

¹Department of Internal Medicine/Division of Diabetes, Endocrinology, and Metabolism, University of Nebraska Medical Center

²VA Nebraska-Western Iowa Health Care System, Omaha, Nebraska

³Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, Tennessee

Abstract

Objective—Adipose tissue (AT) macrophages mediate AT inflammation in obesity and cyclooxygenase-2 (COX-2) is a major inflammatory gene. We hypothesized that deletion of hematopoietic COX-2 will inhibit AT inflammation in obesity.

Design and Methods—Lethally irradiated wild type (WT) mice were injected with bone marrow (BM) cells collected from WT or COX-2 knock-out (COX-2^{-/-}) donor mice and fed a high fat diet for 16 weeks.

Results—The mice that received BM cells from COX-2^{-/-} mice (BM-COX-2^{-/-}) gained increased body weight, fat mass and visceral AT (VAT) mass. These mice exhibited reduced inflammatory markers in the VAT stromal vascular cells (SVC). However, the inflammatory markers were increased in adipocyte fraction and/or whole VAT. The activation of ERK1/2 MAPK, a pro-inflammatory signaling pathway, was increased in BM-COX-2^{-/-} mice. The molecular markers of adipogenesis were increased in the VAT or adipocyte fraction. Wnt signaling markers which inhibit adipogenesis, including Wnt3A and DVL3, were reduced and Wnt5a/b which promotes inflammation was increased in the VAT and/or adipocytes. Finally, we noted an increase in hepatic TG levels in BM-COX-2^{-/-} mice.

Conclusions—Our data suggest that COX-2 deletion in hematopoietic cells reduces SVC inflammation but increases VAT inflammation and promotes adiposity likely via altered Wnt signaling.

*Address correspondence to: Viswanathan Saraswathi, Research Services, VA Nebraska Western Iowa Health Care System, Omaha, NE. Ph: 402-995-3033; Fax: 402-449-0604; s.viswanathan@unmc.edu.

Conflicts of interest: None

Author Contributions. VS conceived and designed the experiments; VS, NA, and CPO conducted the experiments and analyzed the data; VS and NA wrote the manuscript and RR conducted the experiments. All authors were involved in the writing and/or review of the paper and had final approval of the submitted version.

Introduction

Obesity is a global problem and its pathophysiology is associated with dyslipidemia, insulin resistance, hypertension and a systemic pro-inflammatory state. The mechanisms by which obesity leads to the development of metabolic disorders still remain unclear. Studies in the past decade unraveled the role of adipose tissue (AT) inflammation in mediating the pathogenesis of obesity-linked diseases. It is well-established that immune cells and in particular, macrophages accumulate in AT in obesity and these AT macrophages secrete several pro-inflammatory cytokines such as tumor necrosis factor α (TNF α), interleukin 6 (IL-6), and monocyte chemotactic protein-1 (MCP-1) which play a role in the pathophysiological influence of AT (1). While adipocytes themselves can secrete these inflammatory mediators, the immune cells are considered to be their major source in obese AT (2), [reviewed in (3)]. Although the role of various inflammatory genes in immune cells including TLR4, JNK-1, and CCR5, in reducing AT inflammation in obesity has been reported (4, 5, 6) the effect of COX-2, which plays a crucial role in modulating inflammatory processes, in altering obesity-linked AT inflammation is unclear.

COX exists in two forms, COX-1 and -2; the former is constitutively expressed whereas the latter is induced during inflammation. Both COX-1 and -2 produce prostaglandin and thromboxane metabolites from arachidonic acid, which are known to play a critical role in regulating inflammatory processes [reviewed in (7)]. We previously reported that hematopoietic COX-1 deletion resulted in increased AT inflammation and impaired metabolic homeostasis (8). Regarding COX-2, COX-2^{-/-} mice on a hybrid C57BL/6J $\times$ 129/Ola background and a regular chow diet showed reduced AT inflammation (9). Although pharmacological inhibition of COX-2 using rofecoxib, a COX-2 inhibitor, did not reduce AT inflammation (10), celecoxib or nimesulide, other COX-2 inhibitors, reduced AT inflammation in rats fed a high fat diet (11, 12). It should be pointed out that COX-2 is predominantly expressed by macrophages. Of note, inhibition of COX-2 by nutritional or pharmacological agents has been shown to reduce macrophage inflammatory response (13, 14). Because COX-2 is readily expressed by activated macrophages and because AT macrophages are the significant source of AT inflammation, we hypothesized that deletion of COX-2 in hematopoietic cells (containing precursors of macrophages) will result in reduced AT inflammation in obesity. To test this hypothesis, we conducted a bone marrow transplantation using WT mice as recipients and WT (COX-2^{+/+}) or COX-2^{-/-} mice as donors.

Methods

Mice and diet

COX-2^{+/-} heterozygous mice on pure C57BL/6 genetic background were kindly provided by Dr. Raymond Harris, Vanderbilt University, and were originally purchased from Jackson Laboratories. One month post-bone marrow transplantation (BMT), the mice were fed a high fat diet rich in olive oil containing 50% fat by energy (Dyets Inc.) for a period of 16 weeks. The source of fat in the diet was a fat mixture which was added at 209 g/kg. The fat mixture was derived from 45% coconut oil, 36% olive oil, 15% corn oil, and 10% soy bean oil. In addition, the diet was supplemented with 6% olive oil as we described previously (15)

except that the diet used in the current study did not have added cholesterol. All animal care procedures were carried out with approval from the Institutional Animal Care and Use Committee of Vanderbilt University.

Bone marrow transplantation

We performed BMT to study the specific role of hematopoietic COX-2 in modulating AT inflammation in obesity as we described previously (8). BM was collected from the femurs and tibias of WT (COX-2^{+/+}) and COX-2^{-/-} donor mice. The WT mice (Jackson Laboratories) were used as the recipients for both types of donor marrow (4, 8, 16). About $2-5 \times 10^6$ BM cells were injected into 13 wk old, lethally irradiated, WT recipient mice via the retro-orbital venous plexus. One month post-BMT, the mice were placed on a high fat diet. The control WT recipients that received WT COX-2^{+/+} BM cells will be referred to as BM-COX-2^{+/+} and the WT recipients that received the COX-2^{-/-} marrow will be denoted as BM-COX-2^{-/-} mice. We extracted DNA from the spleen of the recipient mice and PCR for the COX-2^{-/-} genotype was performed to confirm reconstitution of the mutant gene with hematopoietic cells as described previously (8, 17).

Body composition

Total fat mass was measured at baseline and at the end of the feeding period by nuclear magnetic resonance (NMR) spectroscopy using a Bruker Minispec (Woodlands, TX, USA) at the Vanderbilt Mouse Metabolic Phenotyping Center (MMPC).

Plasma variables

Plasma total cholesterol and triglycerides were measured using kits from Raichem. Blood glucose was measured using the Accu-chek Aviva glucometer. Plasma insulin was measured using an insulin assay kit (Mercodia). The homeostasis model assessment of insulin resistance (HOMA-IR) was used as a measure of insulin resistance.

Histology

VAT sections (10 μ m) were stained with toluidine blue O. The liver sections (5 μ m) were stained with hematoxylin and eosin. For immunofluorescence, the VAT sections were incubated with anti-MCP-1 primary antibody (Santa Cruz Biotechnology) followed by incubation with Alexa Fluor 568 conjugated secondary antibody. The sections were mounted with antifade gold containing DAPI (4',6-diamidino-2-phenylindole, Invitrogen). The pictures were taken at 10X or 20X magnification using Nikon eclipse 80i inverted fluorescence microscope. Hepatic lipids and VAT MCP-1 were quantified using the ImageJ program. Briefly, 20X images were taken from 3 areas in each slide. To quantify hepatic lipids, a total of 15-27 areas were analyzed with 5-9 samples in each group. For the quantification of MCP-1, a total of 9 areas were analyzed with 3 samples per group.

Isolation of stromal vascular cells (SVCs)

SVC and adipocyte fractions were isolated as we described earlier (8). Briefly, we incubated minced VAT with collagenase (1 mg/mL) for 1 h. The cell suspension was filtered through a

100 μ m nylon filter. After centrifugation, the SVC pellet and the floating adipocytes were separated.

Real-time PCR

Real-time PCR analysis was carried out using primers from Applied Biosystems. We have analyzed macrophage and inflammatory markers as well as markers of adipogenesis. The

CT method was used to quantify the differences between groups for target genes. We used 18S RNA as an internal control.

Western Blotting

VAT and/or liver lysates were probed for MCP-1, matrix metalloproteinase-12 (MMP12), phospho-P42/44 mitogen-activated protein kinase (MAPK), total- P42/44 MAPK, CCAAT/enhancer binding protein α (C/EBP α), peroxisome proliferator-activated receptor gamma (PPAR γ), dishevelled segment polarity protein 3 (DVL3), Axin1, wingless-type MMTV integration site family, member 3A (Wnt3A) and Wnt5A/B. The antibodies for these proteins were obtained from Cell Signaling Tech. The lysates were also probed for anti-cluster of differentiation 68 (CD68, Abcam), glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Millipore), and fatty acid binding protein 4 (FABP4) and plasminogen activator inhibitor-1 (PAI1) from Santa Cruz Biotech.

Measurement of Eicosanoids

The eicosanoid metabolites such as thromboxane B2 (TXB2), 6-keto prostaglandin F1 α (PGF1 α , a stable metabolite of PGI2), and prostaglandin E2 (PGE2) in VAT and liver were determined using gas chromatography/mass spectrometry (GC/MS) at the Eicosanoid Core Laboratory of Vanderbilt University.

Liver lipid profile

Liver triglyceride, cholesterol ester, unesterified cholesterol, and phospholipids were determined by gas chromatography at the Lipid Core Laboratory of Vanderbilt University as we described earlier (18).

Statistical analysis

Results are presented as mean $\pm$ SEM. Statistical significance was determined by one-tailed Student's 't' test using Graph-Pad Prism software to determine statistical significance ($P < 0.05$ was considered significant).

Results

BMT renders mice less susceptible to weight gain (19). Thus, the overall body weight gain was less in these mice compared to what would be expected in high fat diet fed mice (Figure 1A & B). However, we noted that BM-COX-2 $^{-/-}$ mice gained more body weight than controls (1.5-fold, 4.85 g vs 7.36 g in BM-COX-2 $^{+/+}$ versus BM-COX-2 $^{-/-}$ mice) at the end of 16 wk (Figure 1B). Moreover, the fat mass change (Wk16 - BL) assessed by NMR spectroscopy and, VAT mass were significantly higher in these mice (Figure 1C & D). Weekly food intake was not altered between groups (Table 1). The overall food intake in

both groups was lower than normal likely due to total body irradiation as reported earlier (19). There was no significant difference in liver weight, plasma triglycerides and total cholesterol, blood glucose and HOMA-IR between groups (Table 1). A marginal increase in plasma insulin was seen in BM-COX-2^{-/-} mice compared to controls ($P<0.0501$). Together, these results show that hematopoietic deletion of COX-2 led to a marked increase in adiposity without overt changes in systemic metabolic markers.

Since AT macrophages constitute a major fraction of SVCs we examined the expression of macrophage and inflammatory markers in SVCs isolated from VAT. The mRNA expression of CD68, a macrophage marker, is not altered between the two groups. However, there was a significant decrease in the expression of inflammatory markers such as MCP-1 (0.93 ± 0.24 vs 0.48 ± 0.07 relative units, $P<0.05$) and PAI1 (1.43 ± 0.40 vs 0.73 ± 0.12 relative units, $P<0.05$), and a marginal decrease in macrophage inflammatory protein 1 α (MIP1 α , $P<0.052$) in SVCs from BM-COX-2^{-/-} mice compared to controls (Figure 2A). Conversely, analysis of adipocyte fractions revealed that the mRNA expression of PAI1 was significantly higher in adipocytes collected from BM-COX-2^{-/-} mice. The other inflammatory markers were not altered significantly in isolated adipocytes.

Because PAI1 is an important inflammatory mediator secreted by adipocytes and because we noted an increase in PAI1 expression in adipocytes, we next wanted to determine the inflammatory status of whole VAT. Analysis of macrophage and inflammatory markers in the whole VAT revealed that the mRNA expression of CD68, a macrophage marker, was not altered in VAT of BM-COX-2^{-/-} mice compared to controls. Consistent with the adipocyte fraction, the mRNA expression of inflammatory markers, in particular, PAI1 was significantly increased in VAT of BM-COX-2^{-/-} mice (Figure 3A). Of note, the mRNA level of CD68 in adipocyte fraction was not altered between groups suggesting that the increase in PAI1, an inflammatory marker, in VAT is mainly caused by adipocytes and not due to macrophage contamination (Figure 2B). Interestingly, the expression of metallothionein 1 (MT1), an anti-inflammatory gene (20), was significantly reduced ($P<0.05$) in VAT of BM-COX-2^{-/-} mice compared to controls. Toluidine blue O staining of VAT sections did not show a difference in VAT morphology (Figure 3 B&C). Immunofluorescence analysis showed that BM-COX-2^{-/-} mice had a higher level of MCP1 staining (Figure 3 D&E) in VAT (100 ± 6 vs 129 ± 14 arbitrary units in BM-COX-2^{+/+} vs BM-COX-2^{-/-} mice, $P<0.05$). In addition, western blot analysis revealed a significant increase ($P<0.05$) in inflammatory markers such as glycosylated MCP1 (0.62 ± 0.16 vs 1.01 ± 0.13 arbitrary units), PAI1 (0.04 ± 0.01 vs 0.06 ± 0.01 arbitrary units), and MMP12 (0.01 ± 0.0 vs 0.03 ± 0.01 arbitrary units) in BM-COX-2^{-/-} mice compared to controls (Figure 3F). Because activation of MAPKs, in particular, ERK1/2, is tightly linked to activation of inflammatory pathways, we next analyzed ERK1/2 activation in VAT. We noted a significant increase in ERK1/2 phosphorylation in BM-COX-2^{-/-} mice compared to controls (Figure 3G). Analysis of COX-2-derived prostaglandin and thromboxane metabolites showed that the AT levels of eicosanoids such as PGE2, 6-keto-PGF1 α , and TXB2 did not differ significantly between groups (Figure 3H). Overall, these results show that hematopoietic deletion of COX-2 reduces inflammatory markers in SVCs. However, this was not associated with reduced AT inflammation. Instead there was an increase in AT inflammation in BM-COX-2^{-/-} mice.

As BM-COX-2^{-/-} mice showed increased adiposity we assessed the molecular markers of adipogenesis. The protein levels of PPAR γ and FABP4 did not change between groups whereas C/EBP α was significantly higher in the VAT of BM-COX-2^{-/-} compared to BM-COX-2^{+/+} mice (Figure 4 A&B). In addition, the mRNA expression of adipsin which is downregulated in obesity (21), is significantly lower in the VAT (1.24 ± 0.24 vs 0.80 ± 0.08 relative units, $n=6-9$, $P<0.05$) of BM-COX-2^{-/-} mice compared to BM-COX-2^{+/+} controls. Moreover, the mRNA expression of FABP4, another adipogenic marker, is significantly higher in the adipocyte fraction collected from BM-COX-2^{-/-} mice compared to controls (Figure 4E). Together, these data show that deleting COX-2 in hematopoietic cells can promote adipogenesis.

Because Wnt signaling pathways can modulate the adipogenic process (22), we assessed the expression of key intermediates of the Wnt pathway. We noted a significant decrease in the protein levels of DVL3 (0.053 ± 0.023 vs 0.001 ± 0.00 arbitrary units, $P<0.05$) and Wnt3a (0.010 ± 0.002 vs 0.004 ± 0.00 arbitrary units $P<0.01$) in BM-COX-2^{-/-} mice compared to controls. Of note, DVL3 and Wnt3a activate canonical Wnt signaling and inhibit adipogenesis. In addition, there was a 2.3-fold increase in the level of Axin1 (0.039 ± 0.005 vs 0.086 ± 0.016 arbitrary units, $P<0.05$), a negative regulator of canonical Wnt signaling (23) in BM-COX-2^{-/-} compared to control mice. Interestingly, Wnt5a/b which can activate non-canonical Wnt pathway and is associated with inflammatory response (23, 24) was increased by 3.1-fold ($P<0.05$) in BM-COX-2^{-/-} mice (Figure 4 C&D). Analysis of adipocyte samples for the mRNA expression of Wnt signaling markers showed that the expression of Axin1, Wnt 5a, and Frizzled-4 (Fzd4), was significantly increased in BM-COX-2^{-/-} mice with a concomitant increase in FABP4 indicating that altered Wnt signaling may have a role in increasing adipogenesis in these mice (Figure 4E).

Because obesity is often associated with hepatic steatosis and because we noted an increase in AT mass in BM-COX-2^{-/-} mice, we studied the impact of hematopoietic COX-2 deletion on hepatic steatosis. The H & E staining of liver sections showed that hepatic lipid accumulation was increased in BM-COX-2^{-/-} mice compared to controls (Figure 5 A&B). Digital analysis of hepatic lipids showed that lipid accumulation was significantly higher in BM-COX-2^{-/-} compared to control mice (3.2 ± 0.4 vs 4.3 ± 0.4 arbitrary units in BM-COX-2^{+/+} vs BM-COX-2^{-/-} mice, $P<0.05$). In addition, liver triglyceride content was significantly increased in BM-COX-2^{-/-} mice (Figure 5C). Next, we noted that the mRNA and protein levels of CD68 were significantly reduced in the liver of BM-COX-2^{-/-} mice (Figure 6 A&B). The hepatic inflammatory markers were not altered between groups (Figure 6 A&B). In addition, the levels of eicosanoids such as 6-keto-PGF1 α , PGE2, and TXB2 did not differ significantly between groups (Figure 6C). Overall, these results show that macrophage accumulation but not liver inflammation is significantly reduced in the liver of BM-COX-2^{-/-} mice.

Discussion

In the present study, we provide novel evidence that the deletion of COX-2 only in hematopoietic cells reduces inflammatory markers in AT SVCs. Interestingly, it was not associated with reduced inflammation in whole AT. In fact, AT inflammation was rather

increased in BM-COX-2^{-/-} mice compared to controls. Our data also demonstrate that hematopoietic deletion of COX-2 induces adiposity. The adipogenic and pro-inflammatory effects resulting from deleting COX-2 in hematopoietic cells is likely mediated via altered Wnt signaling in AT. Finally, the increased adiposity is associated with increased triglyceride accumulation in liver. Taken together, our data suggest that hematopoietic COX-2 plays an important role in reducing AT inflammation and promoting lipid metabolism and that deleting COX-2 in these cells can induce inflammation and adipogenesis partly via altered Wnt signaling (Figure 7).

An interesting observation of our study is that the reduction in inflammatory markers in AT stromal cells is not reflected in whole AT of BM-COX-2^{-/-} mice. This is an important observation as immune cells are considered to be predominant mediators of AT inflammation and it is generally accepted that the inflammatory phenotype of AT immune cells will be reflected in AT (2), [reviewed in (3)]. However, emerging evidence suggests that it may not be always the case. For example, Orr *et al* have shown that mice lacking TLR4 in hematopoietic cells had increased accumulation of anti-inflammatory macrophages in AT (4). In this study, they showed that an increase in anti-inflammatory markers in SVCs was neither associated with a reduction in inflammatory markers in adipocyte fraction nor with a reduction in systemic insulin resistance. Therefore, there seems to be discordance between macrophage/adipocyte inflammatory status and the overall metabolic outcome. Our data underscore the role of adipocytes (non-hematopoietic cells) in modulating adipose inflammation even in the absence of stromal cell inflammation in obesity. It should also be pointed out that global COX-2^{-/-} mice have been shown to exhibit reduced AT inflammation (9). The difference between our study and this study can be attributed to the difference in experimental conditions (i.e. whole body versus hematopoietic deletion of COX-2).

While MCP1 mRNA was not altered, a marked increase in MCP1 protein was noted in VAT. The discordance between MCP1 mRNA and protein expression in VAT in our study could be due to difference in gene translation or post-translational modifications as suggested by others (25). In fact, MCP1 is known to be modified by O-glycosylation (26, 27). This notion is further substantiated by our data showing that glycosylated MCP1 is significantly increased in the VAT of BM-COX-2^{-/-} mice. Not only MCP1 but also the protein levels of PAI1 and MMP12, other inflammatory markers, are significantly higher in BM-COX-2^{-/-} mice compared to controls. Together, our data suggest that hematopoietic deletion of COX-2 reduces SVC inflammation yet increases the overall inflammatory status in whole VAT.

Another interesting finding of the present study is that BM-COX-2^{-/-} mice exhibited a clear increase in adiposity. Adiposity is shown to be reduced or unaltered in global COX-2^{-/-} mice depending on the mouse strain (9, 28). Conversely, overexpression of COX-2 reduces adiposity (29) indicating that COX-2 can protect against obesity. Notably, inhibition of COX-2 augmented 3T3-L1 preadipocyte differentiation under *in vitro* conditions indicating increased adipogenesis (30). Similarly, we provide evidence that deleting COX-2 only in the hematopoietic cells can promote adiposity in mice. Not only the adipose tissue mass but also the molecular markers of adiposity were significantly altered in BM-COX-2^{-/-} mice.

The lower body weight gain noticed in our study compared to other obesity studies can be attributed to several reasons. First, the total body irradiation procedure during BMT can render the mice resistant to weight gain (19). Next we used female mice which are less susceptible to obesity. Finally, the high fat diet used in our study is rich in olive oil and the fat source in this diet is a mixture of different plant oils whereas lard is a source of added fat in a regular high fat diet. Regardless, we noted that the overall body weight gain was significantly higher in BM-COX-2^{-/-} mice compared to controls.

Regarding potential mechanisms, studies have shown that the canonical Wnt/ β -catenin pathway negatively regulates adipogenesis and thus inhibition of canonical Wnt signaling would lead to increased adipogenesis (31, 32). Accordingly, we noted a significant decrease in markers of canonical Wnt signaling in BM-COX-2^{-/-} mice compared to controls. Evidence suggests that a cross-talk exists between canonical and non-canonical Wnt signaling and activation of one pathway can inhibit the other. For example, inhibition of canonical Wnt signaling through the extracellular inhibitor Dickkopf results in activation of non-canonical Wnt signaling (33). Moreover, non-canonical Wnt signaling mediated by Frizzled 8 antagonizes canonical Wnt signaling in hematopoietic stem cells (34). Along those lines, while canonical Wnt signaling markers were reduced, we noted an increase in Wnt5a/b which mostly activates non-canonical Wnt pathway, in BM-COX-2^{-/-} mice compared to controls. Wnt5a/b can inhibit canonical Wnt/ β -catenin pathway and promote adipogenesis (35, 36). Wnt5a has also been shown to induce inflammation in adipocytes, human VAT, and endothelial cells (24, 37, 38). Together, these data suggest that the increased adiposity and AT inflammation in BM-COX-2^{-/-} mice could be due to reciprocal regulation of canonical and non-canonical Wnt pathways.

Future studies are needed to address some important questions. First, an intriguing question remains as to what causes AT inflammation in the absence of COX-2 in hematopoietic cells. Evidence suggests that although COX-2 is pro-inflammatory in the early phase of inflammation, COX-2-derived eicosanoids produced at the later stage of inflammation are pro-resolving (39). However, analysis of VAT eicosanoid levels did not show a significant difference in prostaglandin and thromboxane metabolites in this study. It should also be pointed out that COX-2 inhibition can inhibit the biosynthesis of resolvins, the anti-inflammatory lipid mediators (40). Therefore, the role of altered resolvins levels in mediating AT inflammation in the absence of COX-2 in hematopoietic cells cannot be ruled out. Next, determining the response of these mice to acute challenge with insulin and glucose are important to understand the role of hematopoietic COX-2 in modulating systemic metabolic homeostasis. Also, it is important to determine whether increased adiposity in these mice is due to impaired energy expenditure. Despite these limitations, results from the present study clearly demonstrate that deletion of COX-2 in hematopoietic cells leads to increased adiposity, AT inflammation, and hepatic steatosis. These findings underscore the role of hematopoietic COX-2 in modulating both inflammation and metabolism in a state of nutrient excess.

Acknowledgments

The eicosanoid metabolites were analyzed with the help of Dr. Ginger Milne at the Eicosanoid Core Laboratory of Vanderbilt University. The authors gratefully acknowledge the technical support provided by Dr. Alyssa Hasty to perform the bone marrow transplantation procedure in this study. This paper is the result of work conducted with the resources and the facilities at the VA-Nebraska Western Iowa Health Care System, Omaha.

Funding. The project was supported by the American Heart Association Scientist Development Grant (0930335N) to VS. VS is also supported by the NIH-Nebraska Center for Nanomedicine/COBRE Grant (2P20GM103480). Lipid profiles were analyzed at the Lipid Core Laboratory of the Mouse Metabolic Phenotyping Center at Vanderbilt University (DK59637).

References

1. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *The Journal of clinical investigation*. 2003; 112:1821–1830. [PubMed: 14679177]
2. Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. *Endocrinology*. 2004; 145:2273–2282. [PubMed: 14726444]
3. Surmi BK, Hasty AH. Macrophage infiltration into adipose tissue: initiation, propagation and remodeling. *Future lipidology*. 2008; 3:545–556. [PubMed: 18978945]
4. Orr JS, Puglisi MJ, Ellacott KL, Lumeng CN, Wasserman DH, Hasty AH. Toll-like receptor 4 deficiency promotes the alternative activation of adipose tissue macrophages. *Diabetes*. 2012; 61:2718–2727. [PubMed: 22751700]
5. Solinas G, Vilcu C, Neels JG, Bandyopadhyay GK, Luo JL, Naugler W, et al. JNK1 in hematopoietically derived cells contributes to diet-induced inflammation and insulin resistance without affecting obesity. *Cell metabolism*. 2007; 6:386–397. [PubMed: 17983584]
6. Kitade H, Sawamoto K, Nagashimada M, Inoue H, Yamamoto Y, Sai Y, et al. CCR5 plays a critical role in obesity-induced adipose tissue inflammation and insulin resistance by regulating both macrophage recruitment and M1/M2 status. *Diabetes*. 2012; 61:1680–1690. [PubMed: 22474027]
7. Garavito RM, DeWitt DL. The cyclooxygenase isoforms: structural insights into the conversion of arachidonic acid to prostaglandins. *Biochimica et biophysica acta*. 1999; 1441:278–287. [PubMed: 10570255]
8. Saraswathi V, Ramnanan CJ, Wilks AW, Desouza CV, Eller AA, Murali G, et al. Impact of hematopoietic cyclooxygenase-1 deficiency on obesity-linked adipose tissue inflammation and metabolic disorders in mice. *Metabolism: clinical and experimental*. 2013; 62:1673–1685. [PubMed: 23987235]
9. Ghoshal S, Trivedi DB, Graf GA, Loftin CD. Cyclooxygenase-2 deficiency attenuates adipose tissue differentiation and inflammation in mice. *The Journal of biological chemistry*. 2011; 286:889–898. [PubMed: 20961858]
10. Lijnen HR, Van Hoef B, Lu HR, Gallacher DJ. Rofecoxib impairs adipose tissue development in a murine model of nutritionally induced obesity. *Thrombosis and haemostasis*. 2008; 100:338–342. [PubMed: 18690356]
11. Hsieh PS, Jin JS, Chiang CF, Chan PC, Chen CH, Shih KC. COX-2-mediated inflammation in fat is crucial for obesity-linked insulin resistance and fatty liver. *Obesity*. 2009; 17:1150–1157. [PubMed: 19247274]
12. Hsieh PS, Lu KC, Chiang CF, Chen CH. Suppressive effect of COX2 inhibitor on the progression of adipose inflammation in high-fat-induced obese rats. *European journal of clinical investigation*. 2010; 40:164–171. [PubMed: 20039930]
13. Chao CL, Weng CS, Chang NC, Lin JS, Kao ST, Ho FM. Naringenin more effectively inhibits inducible nitric oxide synthase and cyclooxygenase-2 expression in macrophages than in microglia. *Nutrition research*. 2010; 30:858–864. [PubMed: 21147369]

14. Steenport M, Khan KM, Du B, Barnhard SE, Dannenberg AJ, Falcone DJ. Matrix metalloproteinase (MMP)-1 and MMP-3 induce macrophage MMP-9: evidence for the role of TNF-alpha and cyclooxygenase-2. *Journal of immunology*. 2009; 183:8119–8127.
15. Saraswathi V, Gao L, Morrow JD, Chait A, Niswender KD, Hasty AH. Fish oil increases cholesterol storage in white adipose tissue with concomitant decreases in inflammation, hepatic steatosis, and atherosclerosis in mice. *The Journal of nutrition*. 2007; 137:1776–1782. [PubMed: 17585030]
16. Saberi M, Woods NB, de Luca C, Schenk S, Lu JC, Bandyopadhyay G, et al. Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. *Cell metabolism*. 2009; 10:419–429. [PubMed: 19883619]
17. Zhang S, Yajima H, Huynh H, Zheng J, Callen E, Chen HT, et al. Congenital bone marrow failure in DNA-PKcs mutant mice associated with deficiencies in DNA repair. *The Journal of cell biology*. 2011; 193:295–305. [PubMed: 21482716]
18. Saraswathi V, Hasty AH. The role of lipolysis in mediating the proinflammatory effects of very low density lipoproteins in mouse peritoneal macrophages. *Journal of lipid research*. 2006; 47:1406–1415. [PubMed: 16639077]
19. Turner RT, Kalra SP, Wong CP, Philbrick KA, Lindenmaier LB, Boghossian S, et al. Peripheral leptin regulates bone formation. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2013; 28:22–34.
20. Inoue K, Takano H, Shimada A, Satoh M. Metallothionein as an anti-inflammatory mediator. *Mediators of inflammation*. 2009; 2009:101659. [PubMed: 19436762]
21. Flier JS, Cook KS, Usher P, Spiegelman BM. Severely impaired adipon expression in genetic and acquired obesity. *Science*. 1987; 237:405–408. [PubMed: 3299706]
22. Laudes M. Role of WNT signalling in the determination of human mesenchymal stem cells into preadipocytes. *Journal of molecular endocrinology*. 2011; 46:R65–72. [PubMed: 21247979]
23. Rao TP, Kuhl M. An updated overview on Wnt signaling pathways: a prelude for more. *Circulation research*. 2010; 106:1798–1806. [PubMed: 20576942]
24. Kim J, Kim J, Kim DW, Ha Y, Ihm MH, Kim H, et al. Wnt5a induces endothelial inflammation via beta-catenin-independent signaling. *Journal of immunology*. 2010; 185:1274–1282.
25. Ekekezie II, Thibeault DW, Garola RE, Truong WE. Monocyte chemoattractant protein-1 and its receptor CCR-2 in piglet lungs exposed to inhaled nitric oxide and hyperoxia. *Pediatric research*. 2001; 50:633–640. [PubMed: 11641460]
26. Jiang Y, Valente AJ, Williamson MJ, Zhang L, Graves DT. Post-translational modification of a monocyte-specific chemoattractant synthesized by glioma, osteosarcoma, and vascular smooth muscle cells. *The Journal of biological chemistry*. 1990; 265:18318–18321. [PubMed: 2211704]
27. Jiang Y, Tabak LA, Valente AJ, Graves DT. Initial characterization of the carbohydrate structure of MCP-1. *Biochemical and biophysical research communications*. 1991; 178:1400–1404. [PubMed: 1872855]
28. Fain JN, Ballou LR, Bahouth SW. Obesity is induced in mice heterozygous for cyclooxygenase-2. *Prostaglandins & other lipid mediators*. 2001; 65:199–209. [PubMed: 11444591]
29. Vegiopoulos A, Muller-Decker K, Strzoda D, Schmitt I, Chichelnitskiy E, Ostertag A, et al. Cyclooxygenase-2 controls energy homeostasis in mice by de novo recruitment of brown adipocytes. *Science*. 2010; 328:1158–1161. [PubMed: 20448152]
30. Yan H, Kermouni A, Abdel-Hafez M, Lau DC. Role of cyclooxygenases COX-1 and COX-2 in modulating adipogenesis in 3T3-L1 cells. *Journal of lipid research*. 2003; 44:424–429. [PubMed: 12576525]
31. Longo KA, Wright WS, Kang S, Gerin I, Chiang SH, Lucas PC, et al. Wnt10b inhibits development of white and brown adipose tissues. *The Journal of biological chemistry*. 2004; 279:35503–35509. [PubMed: 15190075]
32. Ross SE, Hemati N, Longo KA, Bennett CN, Lucas PC, Erickson RL, et al. Inhibition of adipogenesis by Wnt signaling. *Science*. 2000; 289:950–953. [PubMed: 10937998]
33. Caneparo L, Huang YL, Staudt N, Tada M, Ahrendt R, Kazanskaya O, et al. Dickkopf-1 regulates gastrulation movements by coordinated modulation of Wnt/beta catenin and Wnt/PCP activities,

- through interaction with the Dally-like homolog Knypek. *Genes & development*. 2007; 21:465–480. [PubMed: 17322405]
34. Sugimura R, He XC, Venkatraman A, Arai F, Box A, Semerad C, et al. Noncanonical Wnt signaling maintains hematopoietic stem cells in the niche. *Cell*. 2012; 150:351–365. [PubMed: 22817897]
35. Kanazawa A, Tsukada S, Kamiyama M, Yanagimoto T, Nakajima M, Maeda S. Wnt5b partially inhibits canonical Wnt/beta-catenin signaling pathway and promotes adipogenesis in 3T3-L1 preadipocytes. *Biochemical and biophysical research communications*. 2005; 330:505–510. [PubMed: 15796911]
36. van Tienen FH, Laeremans H, van der Kallen CJ, Smeets HJ. Wnt5b stimulates adipogenesis by activating PPARgamma, and inhibiting the beta-catenin dependent Wnt signaling pathway together with Wnt5a. *Biochemical and biophysical research communications*. 2009; 387:207–211. [PubMed: 19577541]
37. Fuster JJ, Zuriaga MA, Thi-Minh Ngo D, Farb MG, Aprahamian T, Yamaguchi TP, et al. Non-canonical Wnt signaling promotes obesity-induced adipose tissue inflammation and metabolic dysfunction independent of adipose tissue expansion. *Diabetes*. 2014
38. Catalan V, Gomez-Ambrosi J, Rodriguez A, Perez-Hernandez AI, Gurbindo J, Ramirez B, et al. Activation of noncanonical Wnt signaling through WNT5A in visceral adipose tissue of obese subjects is related to inflammation. *The Journal of clinical endocrinology and metabolism*. 2014; 99:E1407–1417. [PubMed: 24840810]
39. Serhan CN, Yacoubian S, Yang R. Anti-inflammatory and proresolving lipid mediators. *Annual review of pathology*. 2008; 3:279–312.
40. Schwab JM, Chiang N, Arita M, Serhan CN. Resolvin E1 and protectin D1 activate inflammation-resolution programmes. *Nature*. 2007; 447:869–874. [PubMed: 17568749]

What is already known about this subject?

- Immunometabolism has evolved as a novel field linking the role of immune cells in modulating metabolism.
- Adipose tissue macrophages can modulate adipose tissue inflammatory phenotype in obesity.
- Global COX-2 deletion has been shown to reduce metabolic inflammation in obesity.

What does this study add?

- The role of COX-2 in hematopoietic cells containing precursors of immune cells, in particular, macrophages, in modulating adipose inflammation is unknown. Our study provides evidence that deletion of COX-2 in hematopoietic cells leads to increased adipose inflammation.
- Our study also demonstrates that hematopoietic deletion of COX-2 results in increased adiposity.
- Our study indicates that altered Wnt signaling in visceral adipose tissue may play a role in mediating adiposity and adipose inflammation seen in mice lacking COX-2 in hematopoietic cells. Together, our data suggest the role of hematopoietic COX-2 in regulating metabolic inflammation in obesity.

Figure 1.

Body weight and adiposity markers in mice. Weekly body weight (A) and the body weight gain (B) were measured. Fat mass was measured by NMR. The change in fat mass (Wk 16-baseline) is shown (C). VAT mass (D) was determined at the time of sacrifice (n=6-9 each group). * $P < 0.05$ and # $P < 0.01$ vs the COX-2^{+/+} controls.

Figure 2. Macrophage and/or inflammatory markers in SVCs and adipocytes collected from VAT. Gene expression was analyzed by real-time PCR in the SVCs (A) and adipocytes (B). Values are mean $\pm$ SEM of 6-9 mice in each group. * $P < 0.05$ vs the COX-2+/+ controls.

Figure 3.

Macrophage and inflammatory markers in VAT. VAT macrophage and inflammatory markers were analyzed by real-time PCR (A). VAT sections were stained with Toluidine blue (B & C) and immunofluorescence analysis was performed for MCP1 (D & E). AT lysates were subjected to immunoblotting (F & G) for MCP1, PAI1 and MMP12 (normalized to GAPDH) and phosphorylated ERK1/2 (normalized to total ERK1/2). Values are mean $\pm$ SEM of 6-9 mice in each group. AT samples were analyzed for eicosanoid levels (H). Values are mean $\pm$

SEM of 3-7 samples in each group. * $P < 0.05$, # $P < 0.01$, and * $P < 0.0001$ vs the COX-2^{+/+} controls.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 4.

VAT markers of adipogenesis and Wnt Signaling. Protein lysates collected from VAT were analyzed for markers of adipogenesis (A & B) and Wnt signaling (C & D). All proteins were normalized to GAPDH. Expression of Wnt signaling and adipogenic markers was analyzed in adipocytes by real time PCR (E). Values are mean $\pm$ SEM of 6-9 mice in each group.

* $P < 0.05$; # $P < 0.01$; Δ $P < 0.001$ vs the COX-2^{+/+} controls.

Figure 5. Analysis of liver lipid accumulation. Liver sections were stained with H & E (A & B). The levels of cholesterol esters (CE), free fatty acids (FFA), phospholipids (PL), triglycerides (TG) and unesterified cholesterol (UC) were analyzed (C). Values are mean $\pm$ SEM of 6-9 mice in each group. * $P < 0.05$ vs the COX-2^{+/+} controls.

Figure 6.

Liver macrophage and inflammatory markers. Gene expression for macrophage and inflammatory markers were analyzed by real-time PCR (A). Liver lysates were probed for CD68 and MCP1 (B) and liver eicosanoid levels were measured (C). Values are mean $\pm$ SEM of 6-9 mice in each group. * P <0.05 vs the COX-2+/+ controls.

Figure 7.

Hematopoietic deletion of COX-2 increases adiposity and AT inflammation in obesity. BM-COX-2 deletion leads to inhibition of canonical Wnt signaling pathway which, in turn, can activate the adipogenic process. BMT COX-2 deletion also activates the non-canonical Wnt pathway which may lead to increased adiposity as well as AT inflammation in obesity. Both increased adiposity and AT inflammation may contribute to the development of hepatic steatosis in BM-COX-2^{-/-} mice.

Table 1
Effect of hematopoietic COX-2 on metabolic parameters in mice on a high fat diet

Measurements	COX-2 +/+	COX-2 -/-
n	6	9
Body weight (g)	25.5 ± 1.28	26.9 ± 0.83
Body weight gain (g)	4.85 ± 0.69	7.15 ± 0.60 *
Cumulative food intake (g/mouse/wk)	12.2	12.1
Liver weight (g)	1.09 ± 0.11	1.29 ± 0.07
Total Cholesterol (mg/dL)	130.5 ± 3.76	138.4 ± 3.04
Triglycerides (mg/dL)	46.08 ± 3.76	44 ± 1.57
Glucose (mg/dL)	188 ± 8	179.3 ± 8
Insulin (µg/L)	0.44 ± 0.10	0.64 ± 0.06
HOMA-IR	5.64 ± 0.95	7.03 ± 0.61

* $P < 0.05$ vs. the COX-2+/+ controls. Values are mean ± SEM of 6-9 samples per group for all except HOMA-IR (n=4-8) and food intake. Food intake was measured in a cage of 5 mice in each group.