Obesity and Hypertension

IgG Receptor FcγRIIB Plays a Key Role in Obesity-Induced Hypertension


Abstract—There is a well-recognized association between obesity, inflammation, and hypertension. Why obesity causes hypertension is poorly understood. We previously demonstrated using a C-reactive protein (CRP) transgenic mouse that CRP induces hypertension that is related to NO deficiency. Our prior work in cultured endothelial cells identified the Fcγ receptor IIB (FcγRIIB) as the receptor for CRP whereby it antagonizes endothelial NO synthase. Recognizing known associations between CRP and obesity and hypertension in humans, in the present study we tested the hypothesis that FcγRIIB plays a role in obesity-induced hypertension in mice. Using radiotelemetry, we first demonstrated that the hypertension observed in transgenic mouse-CRP is mediated by the receptor, indicating that FcγRIIB is capable of modifying blood pressure. We then discovered in a model of diet-induced obesity yielding equal adiposity in all study groups that whereas FcγRIIB−/− mice developed obesity-induced hypertension, FcγRIIB+/- mice were fully protected. Levels of CRP, the related pentraxin serum amyloid P component which is the CRP-equivalent in mice, and total IgG were unaltered by diet-induced obesity; FcγRIIB expression in endothelium was also unchanged. However, whereas IgG isolated from chow-fed mice had no effect, IgG from high-fat diet–fed mice inhibited endothelial NO synthase in cultured endothelial cells, and this was an FcγRIIB-dependent process. Thus, we have identified a novel role for FcγRIIB in the pathogenesis of obesity-induced hypertension, independent of processes regulating adiposity, and it may entail an IgG-induced attenuation of endothelial NO synthase function. Approaches targeting FcγRIIB may potentially offer new means to treat hypertension in obese individuals. (Hypertension. 2015;65:456-462. DOI: 10.1161/HYPERTENSIONAHA.114.04670.) • Online Data Supplement

Key Words: C-reactive protein • hypertension • IgG • inflammation • obesity • serum amyloid P component

There is a well-recognized association between obesity, inflammation, and cardiovascular disease. In particular, obesity is a major risk factor for hypertension, and up to four fifths of the 20% increase in the prevalence of hypertension between 1988 to 1994 and 1994 to 2004 in the National Health and Nutrition Examination Surveys have been attributed to increasing body mass index. Regrettably current approaches to combat the underlying obesity, which focus on nutrition and exercise to achieve weight loss and maintenance, have not been effective in the long term. In addition, obesity is a risk factor for the development of hypertension that is resistant to single agent therapy. As such, new therapeutic approaches are needed for the treatment of hypertension in obese individuals.

Circulating levels of the pentraxin C-reactive protein (CRP), which are often interpreted to indicate the presence of low-grade systemic inflammation, are elevated in obese populations including children, adolescents, and young adults. Chronic modest elevations in circulating CRP levels are associated with an increased risk of developing hypertension, though its direct causal role in human hypertension is unclear. We previously demonstrated in studies using a CRP transgenic mouse (TG-CRP), allowing overexpression of the pentraxin, that serum concentrations of CRP comparable with those observed with low-grade systemic inflammation in humans induce hypertension. In that work, we further showed that the CRP-induced hypertension is because of the downregulation of endothelial NO synthase (eNOS) activity in vascular endothelial cells, and impaired endothelial function is an important contributing factor to the development of hypertension in humans. In studies of cultured endothelial cells and endothelial repair and carotid vascular conductance in vivo in mice, we have determined that endothelial actions of CRP are mediated by the IgG receptor Fcγ receptor IIB (FcγRIIB), which we have demonstrated is expressed in endothelial cells. Known ligands for FcγRIIB are CRP, the highly homologous pentraxin serum amyloid P component (SAP), which is the acute phase reactant equivalent of CRP in mice, and IgG, which all bind to identical residues in the extracellular domain of the receptor.

The present studies using mouse models were designed to better understand the pathogenesis of the hypertension that
complicates obesity. Recognizing the associations between CRP and obesity and hypertension, and knowing that FcγRIIB is a biologically relevant CRP receptor, we tested the hypothesis that FcγRIIB mediates obesity-induced hypertension. We first demonstrated that the hypertension caused by elevations in the known FcγRIIB ligand CRP requires the receptor, indicating that FcγRIIB is capable of modifying blood pressure (BP). We then discovered in a model of diet-induced obesity, which yielded equal weight gain and equal increase in body fat with high-fat diet (HFD) feeding in all study groups, that whereas FcγRIIB+/+ mice developed obesity-induced hypertension, FcγRIIB−/− mice were fully protected from hypertension. Thus, independent of any effects on body weight or body composition, FcγRIIB plays a key role in obesity-induced hypertension.

Methods
Detailed methods are available in the online-only Data Supplement.

Animal Models
To study CRP-induced hypertension and FcγRIIB, TG-CRP were crossed with FcγRIIB−/− mice to yield FcγRIIB+/+, FcγRIIB−/−;TG-CRP, FcγRIIB+/−, and FcγRIIB−/−;TG-CRP littermates. All mice were fed standard rodent chow.

To study obesity-induced hypertension, FcγRIIB+/+ and FcγRIIB−/− mice were placed on either control diet (CON) or HFD. Mice were maintained on their respective diet for a minimum of 12 weeks. This resulted in 4 experimental groups: (1) FcγRIIB+/+ CON, (2) FcγRIIB−/− HFD, (3) FcγRIIB−/− CON, and (4) FcγRIIB−/− HFD. Their care and use were approved by the Institutional Animal Care and Use Committees at Baylor College of Medicine and the University of Texas Southwestern Medical Center.

Body Composition
Body composition was measured by dual-energy x-ray absorptiometry.

BP Measurements
Mice underwent tail-cuff BP measurement for 5 consecutive days, using the mean of the last 10 readings on day 5. For radiotelemetry, mice were anesthetized and instrumented with a radiotelemetry device. Mice were allowed to recover until a return of circadian variation in BP and activity were observed. In the CON chow versus HFD experiments, mice were maintained on their respective study diet throughout the radiotelemetry measurements.

Serum CRP, SAP, and IgG
Serum CRP, SAP, and total IgG were quantified by ELISA.

FcγRIIB Expression
Primary aortic endothelial cells were obtained by collagen digestion, and spleens were harvested and splenic cells dispersed by sieving. Cells then underwent fluorescence-activated cell sorting analysis using monoclonal antibody to FcγRIIB (anti-CD16/32b, 2.4G2) and anti-CD31 (PECAM-1 [platelet-endothelial cell adhesion molecule 1]) or anti-CD45R(B220) antibodies for endothelial cell or B-cell selection, respectively.

NO Synthase Activity in Cultured Endothelial Cells
Total IgG was isolated from wild-type mice after 12 weeks of CON versus HFD feeding. Bovine aortic endothelial cells were incubated with either CON IgG or HFD IgG, and NO synthase activation by vascular endothelial growth factor was then evaluated by measuring 14C-L-arginine conversion to 14C-L-citrulline. Additional experiments were performed in bovine aortic endothelial cell transfected with control small interfering RNA or small interfering RNA targeting FcγRIIB.

Statistical Analysis
Statistical analysis for animal experiments was performed using 2-way ANOVA to interrogate the effects of genotype (FcγRIIB−/− versus FcγRIIB+/+) and CRP (wild-type versus TG-CRP) or diet (CON versus HFD) and the interaction effect. For endothelial cell culture experiments, 1-way ANOVA was used with Student–Newman–Keuls post-test analysis. Differences were considered significant at P<0.05.

Results
CRP, FcγRIIB, and BP
We previously demonstrated that modest elevations in CRP causes hypertension in mice. However, the receptor mediating the hypertension invoked by CRP is unknown, and whether FcγRIIB affects BP is unknown. Radiotelemetry measurements of systolic BP (SBP), diastolic BP (DBP), and mean arterial pressure (MAP) were performed in conscious unrestrained mice to determine whether FcγRIIB is necessary for CRP-induced hypertension. In FcγRIIB+/+;TG-CRP mice, SBP and DBP and MAP were all elevated compared with mice not expressing the CRP transgene. CRP caused the mean SBP to increase from 123 to 133 mmHg, mean DBP rose from 92 to 100 mmHg, and mean MAP increased from 108 to 117 mmHg (Figure 1A–1C). In contrast, SBP, DBP, and MAP were not elevated by CRP in FcγRIIB−/− mice. Heart rate was increased in TG-CRP compared with non–TG-CRP mice (Figure 1D); there was no difference in heart rate related to the presence versus absence of FcγRIIB. These findings reveal that FcγRIIB is required for CRP to cause hypertension in mice.

Diet-Induced Obesity Model
Having demonstrated in the TG-CRP mice that FcγRIIB is capable of participation in the pathogenesis of hypertension, we next wished to determine whether the receptor contributes to the disorder in a clinically relevant chronic condition that is commonly complicated by hypertension. Because obesity is a well-recognized risk factor for hypertension in humans and mice placed on HFD develop hypertension, we evaluated the possible role of FcγRIIB in hypertension caused by diet-induced obesity in mice. To accomplish this, starting at 5 to 6 weeks of age FcγRIIB+/+ or FcγRIIB−/− mice were placed on either CON or HFD diet. Beginning 2 weeks after the initiation of the diets, predictably the mice on HFD had greater body weight than the mice on CON diet, and the difference was maintained throughout the 12-week period on the diet (Figure 2A). At 2 and 4 weeks on the diets there was a small difference in weight between HFD-fed FcγRIIB+/+ mice and HFD-fed FcγRIIB−/− mice, with the former weighing slightly more than the latter. However, the difference did not persist >4 weeks on diet.

Body composition was assessed by dual-energy x-ray absorptiometry after 12 weeks of CON or HFD feeding. HFD-fed FcγRIIB+/+ mice and HFD-fed FcγRIIB−/− mice had body weights that were comparable and increased by 35% above the body weights of mice on CON. The vast majority of the increase in body weight was attributed to an increase in adiposity (Figure 2B), with both FcγRIIB+/+ and FcγRIIB−/− mice displaying a 50% increase in percent body fat on HFD (Figure 2C). Importantly, the adiposity that resulted from HFD feeding was equal in FcγRIIB+/+ and FcγRIIB−/− mice.
The known ligands for FcγRIIB are the pentraxins CRP and SAP and the Fc region of IgG, which have shared binding sites. Additional analyses were performed to determine whether the impact of diet-induced obesity on BP, or the impact of FcγRIIB genotype on the BP response to obesity, occurs primarily during daytime or during nighttime, which represent periods of low versus high activity, respectively. Figure 4 shows the measurements during light hours (open bars) and dark hours (filled bars). In all comparisons, BP was increased at night compared with day as expected. SBP was increased during the day in FcγRIIB+/+ HFD-fed mice (Figure 4A), and in contrast there was no impact of HFD on SBP in FcγRIIB−/− mice, either during daytime or during nighttime. Diastolic BP and MAP (Figure 4B and 4C) were raised by HFD in FcγRIIB+/+ mice both during daytime and during nighttime, whereas diet had no effect on either of those parameters during either 12-hour period in FcγRIIB−/− mice. Therefore, increased BP caused by HFD is not related to a change in the diurnal BP pattern, and the presence versus absence of FcγRIIB influences obesity-induced hypertension equally throughout the 24-hour day. Activity monitoring confirmed day–night variation in activity in the 4 experimental groups (Figure 4D). There was a trend for mice on HFD to be less active at night compared with CON-fed mice (P=0.06). Collectively these finding reveal that FcγRIIB participates in the pathogenesis of the hypertension that accompanies diet-induced obesity.

**Diet-Induced Obesity and FcγRIIB Ligands**

The known ligands for FcγRIIB are the pentraxins CRP and SAP and the Fc region of IgG, which have shared binding sites on Fc receptors. Seeking to identify the FcγRIIB ligand that is responsible for HFD-induced hypertension and recognizing that obesity is a chronic inflammatory state, we hypothesized...
that ≥1 of these mediators of inflammatory response is elevated in the setting of HFD feeding and resulting obesity in mice. Analyzing serum from FcγRIIB+/+ mice fed CON versus HFD for 12 weeks, we found that there was no change in serum CRP in response to HFD feeding and the obesity that it causes (Figure 5A). CRP was surprisingly lower in HFD-fed versus CON-fed FcγRIIB−/− mice. Serum SAP also did not change in response to HFD feeding and the obesity that it causes (Figure 5B). However, serum SAP rose modestly with HFD feeding in FcγRIIB−/− mice. Total IgG additionally was similar in CON-fed versus HFD-fed FcγRIIB−/− mice (Figure 5C). Likely related to the lack of the inhibitory Fc receptor in B cells in the FcγRIIB−/− mice, the null mice had marked elevations in total IgG compared with wild-type mice; however, the elevations in total IgG in FcγRIIB−/− were similar in CON-fed versus HFD-fed mice. Thus, there is not a demonstrable change in the circulating level of a known Fcγ receptor ligand in the setting of HFD feeding and resulting obesity in mice.

**FcγRIIB Expression**

Possible effects of HFD on receptor expression were evaluated by fluorescence-activated cell sorting analysis in endothelial cells, in which we previously demonstrated their activation antagonizes eNOS,14 and in B cells in which FcγRIIB attenuates B-cell receptor–mediated processes.23 In both endothelial cells and B cells, FcγRIIB expression was similar in cells obtained from CON versus HFD-fed mice (Figure S1 in the online-only Data Supplement). Thus, diet-induced obesity does not affect FcγRIIB expression, particularly in endothelium in which the receptor is known to alter vascular function.

**Endothelial Actions of IgG**

Observing that circulating levels of CRP, SAP, and IgG, and also FcγRIIB expression were not altered by HFD feeding, possible changes in IgG function were evaluated by studying IgG effects on eNOS in cultured endothelial cells. Treatment with IgG from CON-fed mice did not alter vascular endothelial growth factor activation of eNOS; in contrast, HFD IgG caused marked attenuation of eNOS activation (Figure 6A). To determine whether the actions of HFD IgG are mediated by FcγRIIB, the receptor was silenced by small interfering RNA (Figure 6B). Whereas HFD IgG blunted eNOS activation in control small interfering RNA–transfected cells, it had no effect in FcγRIIB-depleted endothelial cells (Figure 6C).

![Figure 3.](image-url) **Figure 3.** Fcγ receptor IIB (FcγRIIB) is required for obesity-induced systolic and diastolic hypertension. Tail-cuff systolic blood pressure (SBP) was measured in conscious, restrained FcγRIIB+/+ and FcγRIIB−/− mice after control chow (CON) or high-fat diet (HFD) feeding for 16 weeks. The mice were acclimated to the BP monitor for 4 consecutive days before data were collected on the fifth day. Values are mean±SEM (n=12–13). B to F, A separate group of mice underwent carotid artery cannulation with a radiotelemetry device for measurement of SBP (B), diastolic blood pressure (DBP, C), mean arterial pressure (MAP; D), and heart rate (HR; E) after being on their respective diets for 12 weeks. In B to F, values are mean±SEM (n=4–7). In 2-way ANOVA analysis, cuff SBP and heart rate were significant for the effect of diet (P<0.05). More importantly, DBP and MAP had a significant interaction of diet and genotype (P<0.05). The effect of genotype was not significant in any comparison (P>0.05). In post-test analysis, *P<0.05 vs CON diet and †P<0.05 vs FcγRIIB−/− HFD mice.

![Figure 4.](image-url) **Figure 4.** Modulation of blood pressure by high-fat diet (HFD) and Fcγ receptor IIB (FcγRIIB) occur during both light and dark cycles. A to C, Radiotelemetric systolic (A), diastolic (B), and mean arterial pressure (C) measurements in FcγRIIB−/− and FcγRIIB−/− mice after control chow (CON) or HFD feeding were grouped and averaged by 12-hour light (open bars) and 12-hour dark cycles (filled bars). D, Relative activity levels were also quantified. Values are mean±SEM (n=4–7). *P<0.05 vs CON diet and †P<0.05 vs FcγRIIB−/− HFD mice.
Thus, diet-induced obesity yields IgG that antagonizes eNOS via FcγRIIB.

Discussion

Seeking to better understand the basis for obesity-induced hypertension, we designed experiments in mice to determine the role of FcγRIIB in the pathogenesis of the disorder. First testing whether a known ligand for FcγRIIB alters BP via the receptor, we demonstrated that CRP-induced hypertension in the mouse is fully dependent on FcγRIIB. We then discovered in a model of diet-induced obesity with equal increases in body weight and body fat in wild-type and null mice that whereas obese FcγRIIB-null mice developed hypertension, obese FcγRIIB−/− mice were fully protected from hypertension. These observations indicate that independent of any effect on body weight or body composition, FcγRIIB plays a key role in obesity-induced hypertension.

The basis for the CRP-induced hypertension that we now show is mediated by FcγRIIB was elucidated in our previous studies.10 TG-CRP mice displayed exaggerated BP elevation in response to angiotensin II and a reduction in vascular angiotensin II receptor subtype 2 expression. In contrast, the decline in BP with angiotensin receptor subtype 1 antagonism and vascular angiotensin receptor subtype 1 abundance were unaltered in the TG-CRP mice, indicating a selective effect of CRP on vascular angiotensin II receptor subtype 2. Additional experiments showed that the CRP-induced decrease in vascular angiotensin II receptor subtype 2 is caused by NO deficiency, and earlier studies indicated that NO deficiency results from potent antagonism of eNOS by the CRP-FcγRIIB tandem.14 The eNOS antagonism by CRP entails the coupling of FcγRI to FcγRIIB by Src kinase, and the activation of SH2 domain-containing inositol 5′-phosphatase 1, which attenuates signaling downstream of PI3 kinase, thereby preventing Akt activating phosphorylation at Ser473.12,13 Thus, we now know that FcγRIIB activation by CRP initiates a series of alterations in intracellular signaling in endothelial cells, and that the actions of the pentraxin-receptor pair ultimately cause hypertension in mice.

Along with an important role for endothelial dysfunction, altered sympathetic nervous system activity may participate in obesity-related hypertension.24 Interestingly, we observed that heart rate is increased in TG-CRP mice and in HFD-fed obese mice, and that whereas FcγRIIB deletion normalizes BP in the setting of either CRP elevation or diet-induced obesity, it has modest, if any, effect on the heart rate elevation. The finding that FcγRIIB participates in the hypertension but not in the tachycardia observed with raised CRP or obesity, with the heart rate changes likely mediated by sympathetic nervous system activation,25 suggests a peripheral (non-CNS, non–sympathetic nervous system) site of action of FcγRIIB. As such, the distinct impact of the receptor on BP versus heart rate is consistent with a role in hypertension for FcγRIIB in endothelium, which is the cell type implicated in our prior studies of the vascular mechanisms of action of CRP.12–14

Observing the parallel requirement for FcγRIIB in CRP- and obesity-induced hypertension, we were then prompted to identify the FcγRIIB ligand that is operative in the obesity-induced disorder. In wild-type mice we found that there is no change in serum CRP in response to HFD feeding and the obesity that it causes. This is consistent with prior reports that CRP is not elevated in mice in response to a HFD.26,27 Recognizing that the acute phase reactant in mice is the highly homologous pentraxin SAP,16 we also assessed whether SAP is altered by HFD in wild-type mice, and found that it is unaffected. Surprisingly, SAP was modestly increased in FcγRIIB−/− mice.

Figure 5. Impact of high-fat diet (HFD)-induced obesity on serum C-reactive protein (CRP), serum amyloid P component (SAP), and total IgG. Concentrations of CRP (A), SAP (B), and total IgG (C) were measured by ELISA in serum obtained from Fcγ receptor IIB (FcγRIIB)−/− and FcγRIIB−/− mice after control chow (CON) or HFD feeding for 12 weeks. Values are mean±SEM (n=8–12), *P<0.05 vs FcγRIIB+/+, #P<0.05 vs FcγRIIB+/+.
on HFD. However, SAP levels in healthy mice are between 25 and 60 μg/mL and during an acute inflammatory reaction they reach 250 μg/mL.16,28 and the highest SAP concentration observed in HFD-fed FcγRIIB−/− was only 55 μg/mL. Because neither CRP nor SAP levels were elevated by HFD in association with the hypertension that HFD invokes in wild-type mice, we determined serum concentrations of total IgG as it is also a ligand for FcγRIIB. HFD did not affect total IgG in wild-type mice, and predictably total IgG was increased in FcγRIIB−/− mice because the receptor is expressed in B cells in which it negatively regulates IgG production.29 In mice others have observed either no effect on HFD on IgG or an increase in total IgG.30,31 In humans, total IgG may rise with obesity,32 and it falls after weight loss after gastric banding.33 We additionally evaluated possible effects of HFD on FcγRIIB abundance, and found that expression in both endothelial cells, where the receptor alters vascular function, and B cells was not modified by HFD feeding. Alternatively, we discovered that the IgG obtained from HFD-fed mice potently antagonizes eNOS activation, and furthermore that the eNOS antagonism is entirely FcγRIIB dependent. These findings mirror those we previously made on the role of the receptor in eNOS antagonism by CRP,12 and they are consistent with the prior observation that aortic endothelial cells isolated from HFD-fed mice display decreased activating phosphorylation of eNOS at Ser1176 compared with endothelial cells from control diet–fed mice.24 The particular nature of the IgG generated during diet-induced obesity that antagonizes eNOS can now be interrogated in future studies. One possibility is that the culprit is IgG autoantibodies, which increase with obesity and are thought to link obesity with autoimmune disorders.31

Obesity-related hypertension is a perplexing clinical problem, and efforts to combat the underlying obesity regrettably can often be unsuccessful. We have discovered FcγRIIB as a new mediator of obesity-induced hypertension that participates in the disorder in mechanisms that are unrelated to the control of body weight or body composition. Novel therapeutic approaches targeting FcγRIIB-related processes may offer valuable new means to combat obesity-related hypertension independent of strategies to promote weight loss.

Perspectives

The mechanisms that link obesity and hypertension are incompletely understood. The mainstay of clinical management for obesity-related conditions is to encourage weight loss. However, such efforts are often unsuccessful and therapies that complement weight loss regimens would be useful. Having discovered that FcγRIIB is critically involved in obesity-induced hypertension, approaches targeting FcγRIIB may offer potentially new means to treat hypertension in obese individuals.

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Disclosures

None.

References


**Novelty and Significance**

**What Is New?**
- We demonstrate a novel role for the inflammatory receptor, Fcγ receptor IIb (FcγRIIB), in obesity-induced hypertension.

**What Is Relevant?**
- The mainstay of clinical management for obesity-related conditions is to encourage weight loss. However, such efforts are often unsuccessful and therapies that complement weight loss regimens would be useful. Having discovered that FcγRIIB plays an important role in obesity-induced hypertension, approaches targeting FcγRIIB may offer potentially new means to treat hypertension in obese individuals.

**Summary**

We have discovered FcγRIIB participates in the development of obesity-induced hypertension by mechanisms that are unrelated to adiposity. Therefore, novel therapeutic approaches targeting FcγRIIB-related processes may offer valuable new means to combat obesity-related hypertension independent of strategies to promote weight loss.
IgG Receptor FcγRIIB Plays a Key Role in Obesity-Induced Hypertension

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THE IgG RECEPTOR FcγRIIB PLAYS A KEY ROLE IN OBESITY-INDUCED HYPERTENSION

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Supplemental Methods

Animal Models: All experiments were performed in male mice. To study CRP-induced hypertension and FcγRIIB, CRP transgenic mice (TG-CRP) on CF1 background were crossed with FcγRIIB−/− B6:129S mice (Jackson Laboratory)\textsuperscript{1} to yield FcγRIIB\texttextsuperscript{+/+}, FcγRIIB\texttextsuperscript{+/−};TG-CRP, FcγRIIB−/−, and FcγRIIB−/−;TG-CRP littermates. CRP levels were measured by ELISA.\textsuperscript{2} Mice lacking the CRP transgene had serum concentrations <1µg/mL, and serum CRP concentrations in TG-CRP mice were 10-13µg/mL and similar in FcγRIIB\texttextsuperscript{+/+};TG-CRP and FcγRIIB−/−;TG-CRP.\textsuperscript{3} All mice were fed standard rodent chow (Teklad Global 18% Protein Rodent Diet 2018).

To study obesity-induced hypertension, C57BL/6 FcγRIIB\texttextsuperscript{+/+} and FcγRIIB−/− mice were placed on either control diet (CON) (Research Diets, Inc. D12329, with 11 kcal% fat) or high fat diet (HFD) (Research Diets, Inc. D12331, with 58 kcal% fat) beginning at 5-6 weeks of age. Mice were maintained on their respective diet for a minimum of 12 weeks and weights were obtained every 2 weeks. This resulted in 4 experimental groups: 1) FcγRIIB\texttextsuperscript{+/+} CON, 2) FcγRIIB\texttextsuperscript{+/+} HFD, 3) FcγRIIB−/− CON, and 4) FcγRIIB−/− HFD. All mice were maintained in animal facilities with a 12 hour light and 12 hour dark cycle. Their care and use were approved by the Institutional Animal Care and Use Committees at Baylor College of Medicine and the University of Texas Southwestern Medical Center.

Body Composition: After 12 weeks on CON or HFD (at 17-18 weeks of age), body composition was evaluated by dual-energy x-ray absorptiometry (DEXA) in the 4 study groups interrogating HFD-induced hypertension. Mice were anesthetized using
Isoflurane and body composition/densitometry was performed using a Lunar PIXImus x-ray densitometer. Measurements obtained were lean body weight and fat body weight in grams as well as percent body fat.

**Tail Cuff Blood Pressure (BP) Measurement:** In the first series of experiments in FcγRIIB+/+ versus FcγRIIB−/− mice on CON versus HFD, mice underwent BP measurement by tail cuff after 16 weeks on their respective diet using a Visitech System BP-2000 Series II (Apex, NC) BP monitor. BP was measured 20 times over a 30 min period daily for 4 days to train the mice. The mean of the last 10 readings on the following day (day 5) was recorded as the BP value for a given mouse.

**Blood Pressure (BP) Measurement by Radiotelemetry:** In experiments determining the role of FcγRIIB in CRP- or HFD-induced hypertension, radiotelemetry was performed as described previously. In the studies of CRP, instrumentation was done at 12-16 weeks of age, and in the CON chow versus HFD experiments it was done at 17-19 weeks of age after the mice were on their respective diets for 12-13 weeks. Mice were anesthetized under isofluorane and instrumented with a left carotid artery catheter and radiotelemetry device (PA-C10, Data Sciences International, St. Paul, MN). Mice were allowed to recover for a minimum of 6 days (range 6-10 days) and until a return of circadian variation in BP and activity was observed. Then BP, heart rate, and activity were recorded every 15 min for 72 h. Inclusion criteria were pulse pressure ≥20 mmHg, standard deviation for pulse pressure ≤9 mmHg, and the presence of diurnal differences in systolic BP between the light and dark cycles. BP recordings that did not meet these criteria were not used for analysis. In the CON chow versus HFD experiments, mice
were maintained on their respective study diet throughout the radiotelemetry measurements.

**Serum CRP, SAP and Total IgG:** CRP, SAP and total IgG were measured in the serum of FcγRIIB+/+ versus FcγRIIB−/− mice on CON versus HFD after 12 weeks on their respective diets. To avoid the effects of surgical stress, non-instrumented mice were employed, and samples were collected by cardiac puncture. The three parameters were quantified by ELISA (Immunology Consultants Laboratory, Inc., Portland, OR), running all samples in duplicate and employing the following dilutions: 1:200 for CRP, 1:1000 for SAP, and 1:50000 for IgG.

**FcγRIIB Expression:** To evaluate possible effects of HFD on receptor expression, following 12 week feeding of CON versus HFD in wild-type mice, primary aortic endothelial cells were obtained by collagen digestion, and spleens were harvested and splenic cells dispersed by sieving. Cells then underwent fluorescence-activated cell sorting (FACS) analysis using monoclonal antibody to FcγRIIB (anti-CD16/32b, 2.4G2) and anti-CD31 (PECAM-1) or anti-CD45R(B220) antibodies (all from eBiosciences) for endothelial cell or B cell selection, respectively. The anti-CD16/32b antibody recognizes FcγRIIB and FcγRIII; however, we previously demonstrated that mouse endothelial cells express FcγRIIB and not FcγRIII, and in B cells the sole Fcγ receptor expressed is FcγRIIB.

**NO Synthase Activity in Cultured Endothelial Cells:** To evaluate possible effects of HFD on IgG action on endothelial cells, total IgG was isolated from wild-type mice following 12 weeks of CON versus HFD feeding using Melon Gel IgG Purification Resin (Pierce
Biotechnology) according to the manufacturer's instructions. Bovine aortic endothelial cells (BAEC) were incubated with either CON IgG or HFD IgG (10 μg/ml) for 15 min, and NO synthase activation by vascular endothelial growth factor (VEGF, 100 ng/ml) was then evaluated by measuring $^{14}$C-L-arginine conversion to $^{14}$C-L-citrulline using previously described methods. Additional experiments were performed in BAEC transfected with control siRNA or siRNA targeting Fc$\gamma$RIIB to silence the receptor.

**Statistical Analysis:** Statistical analysis for animal experiments was performed using 2-way ANOVA with genotype (Fc$\gamma$RIIB$^{+/+}$ vs. Fc$\gamma$RIIB$^{-/-}$) as the first factor compared and CRP (wild type vs TG-CRP) or diet (CON vs. HFD) as the second factor for comparison. Student-Newman-Keuls was used for post-hoc analysis. For endothelial cell culture experiments, 1-way ANOVA was used with Student-Newman-Keuls post-test analysis. Differences were considered significant at p<0.05.
References


Figure S1: FcγRIIB expression is not altered in high fat diet-induced obesity.

FACS was performed on primary aortic endothelial cells (A) and spleen-derived B cells (B) from control chow (CON) or high fat diet (HFD) fed mice. Antibodies employed were 2.4G2 to detect FcγRIIB, and anti-CD31 (PECAM-1) or anti-CD45R(B220) antibodies to identify endothelial cells or B cells, respectively. Values are mean±SEM, n=4-8.
Figure S1

A

FcγRIIB expression (% of endothelial cells)

Con  HFD

B

FcγRIIB expression (% of B cells)

Con  HFD