CYP2J2 Targeting to Endothelial Cells Attenuates Adiposity and Vascular Dysfunction in Mice Fed a High-Fat Diet by Reprogramming Adipocyte Phenotype

Nader G. Abraham, Komal Sodhi, Anne M. Silvis, Luca Vanella, Gaia Favero, Rita Rezzani, Craig Lee, Darryl C. Zeldin, Michal L. Schwartzman

Abstract—Obesity is a global epidemic and a common risk factor for endothelial dysfunction and the subsequent development of diabetes mellitus and vascular diseases such as hypertension. Epoxyeicosatrienoic acids (EETs) are cytochrome P450 (CYP)-derived metabolites of arachidonic acid that contribute to vascular protection by stimulating vasodilation and inhibiting inflammation. Heme oxygenase-1 is a stress response protein that plays an important cytoprotective role against oxidative insult in diabetes mellitus and cardiovascular disease. We recently demonstrated interplay between EETs and heme oxygenase-1 in the attenuation of adipogenesis. We examined whether adipocyte dysfunction in mice fed a high-fat diet could be prevented by endothelial-specific targeting of the human CYP epoxygenase, CYP2J2. Tie2-CYP2J2 transgenic mice, fed a high-fat diet, had a reduction in body weight gain, blood glucose, insulin levels, and inflammatory markers. Tie2-CYP2J2 gene targeting restored HF-mediated decreases in vascular heme oxygenase-1, Cyp2C44, soluble epoxide hydrolase, phosphorylated endothelial nitric oxide synthase, phosphorylated protein kinase B, and phosphorylated adenosine monophosphate protein kinase expression, thus improving vascular function. These changes translated into decreased inflammation and oxidative stress within adipose tissue and decreased peroxisome proliferator–activated receptor-γ, CCAAT/enhancer binding protein alpha, mesoderm-specific transcript, and adipocyte 2 expression and increased uncoupling protein 1 and uncoupling protein 2 expression, reflecting the effect of vascular EET overproduction on adipogenesis. The current study documents a direct link between endothelial-specific EET production and adipogenesis, further implicating the EET-heme oxygenase-1 crosstalk as an important cytoprotective mechanism in the amelioration of vascular and adipocyte dysfunction resulting from diet-induced obesity. (Hypertension. 2014;64:1352-1361.) • Online Data Supplement

Key Words: 11,12-EET ■ AMP-activated protein kinases ■ aP2 protein, human ■ eNOS protein, rat ■ heme oxygenase-1 ■ mesoderm-specific transcript protein

Obesity has become a major and ever increasing epidemic worldwide and is a major risk factor in the development of vascular diseases such as diabetes mellitus and hypertension, and other associated complications, including vascular dysfunction and insulin resistance. Fat acts not only as a reservoir for triglyceride storage but also as a dynamic organ that secretes bioactive factors to influence adjacent vasculature. Adipose tissue is highly vascularized and, vice versa, most vasculature is surrounded by adipose tissue. Thus, it is essential to investigate the role of each in the regulation of adipogenesis and vascular homeostasis. In addition, emerging studies implicate a mechanistic link between vascular and adipocyte dysfunction as it relates to obesity-derived cardiovascular complications. This may be because of an increase in reactive oxygen species. Epoxycosatrienoic acids (EETs) are metabolites of arachidonic acid that are produced by a family of cytochrome P450 (CYP) monooxygenases/epoxygenases. On formation, EETs are subjected to hydrolysis by soluble epoxide hydrolase to their respective dihydroxyepoxytrienoic acids as well as to esterification primarily to glycerophospholipids. Vasodilatory, anti-inflammatory, and antiapoptotic actions of EETs are well established and it is well documented that soluble epoxide hydrolase inhibition significantly increases cellular and circulating EET levels. EET agonists prevent both vascular dysfunction and adiposity in vitro and in mice fed high-fat (HF) diets. EET-mediated increases in heme oxygenase (HO)-1 provide vascular protection and regulate adipogenesis. HO, an essential stress response gene, has 2 isoforms: HO-1 (inducible) and HO-2 (constitutive).

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HO-2 (constitutive). Each catalyzes the degradation of heme to equimolar quantities of biliverdin, carbon monoxide, and iron. As part of a cell’s antioxidant defense system, increased expression of HO-1/HO-2 promotes resistance to injury from heme, a pro-oxidant, and damaging reactive oxygen species. Induction of HO-1 decreases adipogenesis via suppression of transcription factors, including peroxisome proliferator-activated receptor-γ (PPARγ), adipocyte 2 (aP2), and mesoderm-specific transcript (Mest) proteins, while concomitantly increasing adiponectin and improving insulin sensitivity. PPARγ is a master regulator of adipogenesis and activates the expression of genes, such as aP2, to trigger the synthesis of fatty acids and triglycerides. Paternally expressed 1/Mest, when upregulated, results in adipocyte enlargement during adipose tissue expansion that is associated with increased release of inflammatory adipokines and enhanced insulin resistance. Although obesity is associated with oxidative stress and increased levels of reactive oxygen species, we have demonstrated that the expression and activity of both CYP epoxygenases and HO-1 are downregulated in obesity.

EETs and HO-1 are interdependent; 11,12-EET stimulates HO-1 expression and its vasodilatory action is dependent on HO activity. However, the role of EETs on adipocyte HO-1 levels and insulin resistance is largely unknown. HO-1 levels are decreased in models of diabetes mellitus, atherosclerosis, and metabolic syndrome. Because excess fat is a major component of all of these diseases, it is plausible that diminished HO activity contributes to increased oxidative stress, inflammation, and subsequent adipocyte dysfunction. In rodents fed HF diets, the decrease in CYP epoxygenase expression and EET biosynthesis was associated with increased reactive oxygen species production and reduced levels of HO-1 and adiponectin. Specifically secreted by adipocytes, adiponectin has antiatherogenic, antihypertensive, and insulin-sensitizing properties. Furthermore, adipocytes derived from obese mice have decreased EET levels, and the administration of an EET agonist decreased adiposity and increased HO-1 and adiponectin levels.

The protective effects of both EETs and HO-1 are supported by improved vascular function and reduced adipogenesis via targeted expression of key enzymes in specific cell populations. Lentiviral-targeted HO-1 expression in rat endothelium improved vascular function in accordance with decreased oxidative stress and inflammation. Furthermore, selective HO-1 expression in adipocytes attenuated adiposity and vascular dysfunction in mice fed an HF diet. Therefore, studying the effects of HO-1 and EET on adipocyte function is critical to better understanding how adipocyte dysfunction leads to increased inflammation, a known risk factor for vascular dysfunction and, subsequently, the development of hypertension in mammals that consume an HF diet.

Materials and Methods
A detailed description of experimental protocols and materials and methods is included in the online-only Data Supplement.

Results
Effect of Endothelial CYP2J2 Gene Targeting on Body Weight, Visceral Fat, and Subcutaneous Fat Accumulation
We used a B6 transgenic mouse strain (Tie2-CYP2J2-Tr) that, in endothelial cells, selectively expresses a primary CYP
epoxygenase (CYP2J2) responsible for EET biosynthesis, which were fed either a normal or an HF diet during a period of 18 weeks. There was no significant difference in food intake between the wild-type (WT) and CYP2J2 mice. Body weight of WT mice fed an HF diet (WT-HF) was increased by 53% over that of WT mice fed a normal diet (Figure 1A and 1B). This phenomenon was paralleled in HF versus normal diet Tie2-CYP2J2 mice; however, body weight was significantly lower in Tie2-CYP2J2-Tr-HF mice in comparison with WT-HF mice. Measurements of visceral fat paralleled that of body weight with more pronounced differences between WT and transgenic mice; Tie2-CYP2J2-Tr were leaner than WT mice and an HF diet increased visceral fat in WT by ∼4-fold. Although the fold increase in visceral fat in HF-fed Tie2-CYP2J2-Tr was greater, it remained significantly lower than in HF-fed WT mice (Figure 1C). A similar pattern was seen with regard to subcutaneous fat content, which was >2-fold higher in WT-HF mice than in Tie2-CYP2J2-Tr-HF mice (Figure 1D).

Effect of Endothelial CYP2J2 Gene Targeting on Blood Glucose, Insulin, Adiponectin and Interleukin-6 levels

Fasting blood glucose and insulin levels were measured as indicators of systemic complications stemming from excessive body weight gain (Figure 2A and 2B). Blood glucose and insulin levels increased in WT-HF mice in comparison with WT mice. Tie2-CYP2J2-Tr-HF mice displayed lower blood glucose and insulin levels compared with WT-HF mice (Figure 2A and 2B).

Circulating adiponectin was lower in WT-HF mice versus WT controls (Figure 2C). Tie2-CYP2J2-Tr mice exhibited increased adiponectin levels compared with corresponding WT and these levels decreased on an HF diet. Nonetheless, adiponectin levels in Tie2-CYP2J2-Tr-HF mice were significantly higher relative to WT-HF mice. In contrast to the attenuation of serum adiponectin levels, an HF diet increased circulating levels of interleukin-6 (Figure 2D). Interleukin-6 levels increased by 3-fold in WT-HF mice as compared with those in WT controls. Interleukin-6 levels in Tie2-CYP2J2-Tr mice fed a normal diet were comparable with the corresponding WT mice, yet were strikingly reduced in Tie2-CYP2J2-Tr-HF mice when compared with those in WT-HF mice.

Effect of Endothelial CYP2J2 Gene Targeting on Vascular Parameters

Systolic blood pressure, measured by the tail-cuff plethysmography method (see online-only Data Supplement), significantly increased during the 18-week period of HF diet in WT and Tie-CYP2J2-Tr mice (Figure 3A). Systolic blood pressure of Tie2-CYP2J2-Tr mice fed a normal diet was comparable with WT control mice. However, the HF-mediated blood pressure increase in Tie-CYP2J2-Tr mice was significantly attenuated as compared with that in WT mice (109±2 versus 129±5 mmHg; n=10). Vascular function, measured as relaxations to acetylcholine in renal interlobar arteries, was impaired in WT mice fed an HF diet. As seen in Figure 3B, at 10−3 mol/L acetylcholine, the percent of relaxation in WT mice fed a normal diet was 92±8 versus 59±6 in WT-HF mice (P<0.05). Tie2-driven endothelial expression of CYP2J2 restored maximal

![Figure 2. A](http://hyper.ahajournals.org/). Fasting blood glucose, (B) insulin, (C) adiponectin, and (D) interleukin (IL)-6 levels in mice fed a normal diet (wild-type [WT] and CYP2J2) and mice fed a high-fat (HF) diet (WT-HF and CYP2J2-HF; n=10; *P<0.05 vs WT; †P<0.05 vs CYP2J2; and #P<0.05 vs WT-HF).
The effect of Tie2-CYP2J2 transduction and HF diet on phospho-

sion significantly higher than in WT-HF mice (Figure 4C), and although HF diet decreased HO-1 expression by only 40% remain-

ig significantly higher than in WT-HF mice (Figure 4A and 4B). HO-1 expression was 4-fold higher in Tie2-CYP2J2-Tr mice when compared with that in WT mice (Figure 4B). However, Tie2-CYP2J2-Tr-HF mice exhibited increased soluble epoxide hydrolase expression although the increase was tempered compared with that observed in HF-WT mice.

We further evaluated the effect of endothelial-specific CYP2J2 expression on the levels of key cytoprotective molecules in the vasculature. HO-1 expression was 4-fold higher in Tie2-CYP2J2-Tr mice when compared with that in WT mice (Figure 4C), and although HF diet decreased HO-1 levels in both strains, it remained significantly higher in the Tie2-CYP2J2-Tr-HF mice. Phosphorylation of AMPK showed a similar pattern; it was 2.5-fold higher in Tie2-CYP2J2-Tr than in WT and was decreased by HF diet by only 40% remaining significantly higher than in WT-HF mice (Figure 4D). The effect of Tie2-CYP2J2 transduction and HF diet on phosphorylated protein kinase B (pAKT) signaling and eNOS phosphorylation at ser1177 mirrored that of phosphorylated adenosine monophosphate protein kinase (Figure 4E and 4F). Tie2-CYP2J2-Tr mice displayed higher levels of pAKT signaling (2-fold) and phosphorylated endothelial nitric oxide synthase (25%) relative to WT mice. In WT mice, an HF diet decreased pAKT and phosphorylated endothelial nitric oxide synthase levels by 35% and 50%, respectively. Although an HF diet in Tie2-CYP2J2-Tr mice also resulted in a decrease in pAKT and phosphorylated endothelial nitric oxide synthase, these levels remained significantly higher than those in HF-WT mice (Figure 4E and 4F).

Effect of Endothelial CYP2J2 Gene Targeting on Adipocyte HO-1 Expression and Macrophage Infiltration

Immunohistochemical staining of adipose tissue (Figure 5A) and quantification (Figure 5B) of HO-1 expression indicated a reduction in HO-1 protein in WT mice fed an HF diet as compared with WT fed a normal diet (P<0.05). A parallel relationship was observed in Tie2-CYP2J2-Tr-HF mice, which exhibited lower HO-1 levels than Tie2-CYP2J2 mice fed a normal diet (P<0.05). Nonetheless, WT-HF mice expressed less HO-1 than Tie2-CYP2J2-Tr-HF mice (P<0.05).

Because obesity and excessive adipogenesis are positively correlated with increased inflammation,39,40 we assessed infiltration of macrophages (CD68+ cells) to adipose tissue. Immunohistochemical staining (Figure 5C) illustrates an 8-fold increase in the CD68+ stained area in adipose tissue of WT-HF mice compared with WT mice fed a normal diet. Tie2-CYP2J2-Tr mice had decreased CD68+ regions as compared with WT mice (32±3 versus 69±8; P<0.05). In addition, in comparison with WT-HF mice, CD68+ regions in Tie2-CYP2J2-Tr-HF mice were attenuated (199±14; Figure 5D).

Effect of Endothelial CYP2J2 Gene Targeting on Key Signaling Pathways for Adipogenesis and Energy Metabolism

We examined the levels of aP2, Mest, and PPARγ to see whether they could account for the decrease in fat content in adipose tissues from mice with endothelial expression of CYP2J2 fed an HF diet. Western blot analysis demonstrated in WT-HF mice an upregulation of Mest (2-fold), aP2 (1.5-fold), and PPARγ (1.3-fold) versus WT mice fed a normal diet (Figure 6). In contrast, Tie2-CYP2J2-Tr mice displayed reduced expression of each of these adipogenic markers in comparison with WT mice. HF diet increased expression of Mest, aP2, and PPARγ in Tie2-CYP2J2-Tr mice (Figure 6B–6D). However, the levels of these markers remained significant low (except for PPARγ) in comparison with WT-HF mice (Figure 6D).

Uncoupling protein 2 (UCP2) is a marker of energy metabolism and its uncoupled energy is used for heat production in fat tissue. WT mice fed an HF diet exhibited a decreased expression of UCP2 in adipose tissue in comparison with WT mice fed a normal diet (Figure 6A and 6E). Adipocyte UCP2 levels in Tie2-CYP2J2-Tr mice were 2-fold higher than in WT mice and although an HF diet decreased its level, it remained higher than in WT-HF adipose tissue. Measurements of UCP1 protein levels in adipose tissues (Figure S1 in the online-only Data Supplement)
followed the same pattern as that of UCP2. In addition, measurements of gp91phox, a subunit of NADPH oxidase and a marker of oxidative stress, in aortic and adipose tissues indicated that increased endothelial expression of CYP2J2 resulted in reduced oxidative stress following an HF diet (Figure S2).

Figure 4. Western blots and densitometry analyses of vascular tissue (A) Cyp2C44, (B) soluble epoxide hydrolase (sEH), (C) heme oxygenase (HO)-1, (D) phosphorylated adenosine monophosphate protein kinase (pAMPK), (E) phosphorylated protein kinase B (pAKT), and (F) phosphorylated endothelial nitric oxide synthase (peNOS) proteins in mice fed a normal diet (wild-type [WT] and CYP2J2) and mice fed a high-fat (HF) diet (WT-HF and CYP2J2-HF; n=4; *P<0.05 vs WT; #P<0.05 vs WT-HF; and +P<0.05 vs CYP2J2).

Figure 5. Heme oxygenase (HO)-1 and CD68+ expression in visceral fat from mice fed a normal diet (wild-type [WT] and CYP2J2) and mice fed a high-fat (HF) diet (WT-HF and CYP2J2-HF). A and B, HO-1 immunohistochemistry and quantitative analysis. C and D, CD68+ immunohistochemistry and quantitative analysis (bar, 20 μm; n=5; *P<0.05 vs WT; #P<0.05 vs WT-HF; and +P<0.05 vs CYP2J2).
Discussion

This is the first study to demonstrate that targeting endothelial cells in mice fed an HF diet with human CYP2J2 decreased adiposity and vascular dysfunction, improved metabolic parameters, and attenuated serum levels of inflammatory cytokines. These improvements were associated with increased HO-1 expression in both the vasculature and in the adipose tissue. Moreover, the beneficial effects of endothelial targeting of CYP2J2, a major EET-producing enzyme in humans,\(^4^1\) are reflected by the attenuation of adipocyte expression of the adipogenic markers Mest, aP2, and PPAR\(^\gamma\). In addition, increased HO-1 expression correlated with an increase in circulating adiponectin levels, which play a protective role in the development of insulin resistance and inflammation.\(^4^2\) Altogether, these data indicate the existence of a crosstalk between vascular EETs and HO-1 to modulate metabolic function and, thus, may provide a therapeutic target for patients with metabolic syndrome.

The current results support and substantiate our previous findings that indicated the protective effect of EETs on adipose and vascular tissues. Tie2-CYP2J2-Tr mice display increased levels of plasma and tissue EETs.\(^3^8,4^3\) We recently reported a significant reduction of EETs in mice fed an HF diet, which was accompanied by elevated levels of markers of inflammation and oxidative stress; administration of an EET agonist inhibited the expression of these markers of vascular and adipocyte dysfunction.\(^7\) In agreement with that report, our present results show a significant decrease in gp91phox levels in CYP2J2 Tr mice in vascular and adipose tissues as compared with WT mice and, additionally, demonstrated the specific ability of endothelial cell–generated EETs to exhibit cytoprotective effects by attenuating oxidative stress in adipose tissue. EET regioisomers are potent arachidonic acid–derived vasodilators and anti-inflammatory molecules\(^1^1,4^4\) produced by enzymes of the CYP2C and CYP2J gene families.\(^9\) Studies have shown vascular protection against angiotensin II–mediated renal injury by CYP2C23 induction.\(^4^5\) Our previous findings in human mesenchymal stem cells implicated CYP2J2 as the primary source of EET generation\(^1^8\) and provided evidence that EETs are antiadipogenic.\(^1^4\) In vivo, systemic administration of an EET agonist improves insulin sensitivity and lowers adiposity.\(^7\) In addition to this study, we have provided several lines of evidence that the EET-mediated improvement of adipocyte function is a result of increased HO-1 levels.\(^1^4,1^5,1^8,4^6\) We have also shown that EETs stimulate HO-1 expression via the bach1–glycogen synthase kinase-3\(\beta\) pathway.\(^7\) The targeting of CYP2J2 to the vascular endothelium results in decreased body weight gain and improved insulin sensitivity and vascular function. It is likely that the beneficial effect of EET is primarily because of local EET production in the vasculature of the fat tissue. The mechanisms by which vascular endothelial EETs affect adipocyte function are yet to be fully explored. We have shown previously that EETs induce HO-1 in vascular endothelium as well as in adipocytes.\(^1^4,1^8,2^7\) Hence, based on our studies and a recent report suggesting the existence of an EET receptor,\(^6\) we postulate that vascular endothelial EETs affect adipocyte function via a receptor-mediated mechanism in an autocrine and paracrine fashion to increase vascular and adipose HO-1 and consequently reduce vascular dysfunction, inflammation, hypertrophic adipogenesis, and insulin resistance.

Our previous studies demonstrated that HO-1 induction attenuates adipogenesis and improves insulin sensitivity in obesity-induced diabetic rats\(^1^9\) and in obese mice\(^2^5\)
while promoting healthy adipocyte function via adiponectin synthesis and release. A similar improvement in vascular function via targeting HO-1 expression specifically to adipocytes (aP2-HO-1) has been described recently. In addition, aP2-HO-1–transduced mice, fed an HF diet, had a decreased inflammation, lower blood glucose, and increased adiponectin versus WT-HF mice. Increased vascular and adipocyte HO-1 expression in Tie2-CYP2J2-Tr mice in the current study supports these findings and is manifest by an improved vascular response and increased adiponectin levels. Furthermore, recent analysis in diabetic patients shows an impaired HO-1 system, chronic inflammation, and oxidative stress, which was accompanied by diminished adiponectin synthesis and release.

Obesity downregulates both CYP epoxygenases and HO-1; yet with the Tie2-CYP2J2-Tr mouse model, we demonstrate restoration of these 2 key cytoprotective enzyme systems through an increase in EET production specifically in the vascular endothelial cell population. Our data suggest a paracrine effect of endothelial EET on adipocyte function that is reflected by improved vascular function and decreased adipogenesis. Moreover, whereas an HF diet in WT mice led to decreased activation of MAP kinase signaling (ie, phosphorylated adenosine monophosphate protein kinase and pAKT), Tie2-CYP2J2-Tr mice exhibited restored activation to levels well beyond that of WT controls. AMPK activation is controlled by the energy state of the cell (ie, glucose availability) via a decrease in ATP and a rise in cellular AMP. Therefore, high circulating glucose levels, such as those obtained with an HF diet, result in elevated ATP and a subsequent decrease in AMPK activation. The ability of Tie2-driven endothelial CYP2J2 expression to restore AMPK activation in the presence of an HF diet suggests that endothelial EET production improves adipocyte function despite the presence of high levels of glucose.

The relationship between EETs and AMPK has yet to be clarified. Others have shown in diabetic mice and hypertensive rats that CYP2J3 gene delivery increased EET generation and adiponectin levels, reduced blood pressure and improved insulin sensitivity through AMPK activation. We showed that administration of an EET agonist to animals on an HF diet restored the decreased adipocyte AMPK activation. The increase in adiponectin levels with Tie2-driven endothelial CYP2J2 expression supports a mechanistic link between EETs and AMPK, as other studies demonstrate that adiponectin provides vascular and renal protection. These data illustrate potential autocrine regulation of adipocyte AMPK activity, via adiponectin synthesis. In addition, increased HO-1 protein levels are associated with increases in the AMPK and AKT signaling pathway, supporting the role of HO-1 in mediating EET-AMPK signaling.

We further demonstrate that changes in vascular signaling are associated with changes in adipogenic proteins.
Increased expression of CYP2J2 resulted in decreased levels of Mest, aP2, and PPARy in accordance with previous studies demonstrating the EET-mediated abrogation of adipogenesis.5 Furthermore, the role of HO-1 in improving adipocyte function in the current model is substantiated by previous work showing reduced adipogenesis via targeting HO-1 to adipocytes. UCPI and 2 are expressed in adipose tissue and play an important role in the control of energy expenditure by uncoupling respiration from ATP synthesis, thereby dissipating energy as heat and affecting energy metabolism efficiency.51,52 In agreement with these reports, our results show that CYP2J2-Tr mice have increased adipose UCP1 and 2 levels compared with WT mice and this could be one of the mechanisms involved in decreased adiposity in CYP2J2-Tr mice. Taken together and summarized in Figure 7, we provide evidence to support the paracrine effect of endothelial EET production to improve both vascular and adipocyte function through increased levels of HO-1. Thus, EET and HO-1 seem to form a module that serves as a molecular switch to genetically reprogram the adipocyte phenotype to express lower levels of Mest and prevent hypoadiponectinemia.

One of the consequences of obesity-mediated adipocyte dysfunction is vascular dysfunction, which is a prelude to vascular disease and hypertension.53 With the growing prevalence of obesity and impaired glycemic control, the correlation between these conditions, and an elevated predisposition for the development of vascular disease, research emphasis is increasingly being targeted to the mechanistic basis and functional outcomes of these relationships. The current study is the first to show a direct link between endothelial-specific EET production and HO-1 induction to inhibit adipogenesis. Thus, our studies offer a portal to a role of HO-1-EET interplay in adipocyte-derived regulation of inflammation that is directly related to vascular dysfunction in obesity.

Perspectives

The global epidemic of obesity continues unabated with sequelae of type 2 diabetes mellitus and metabolic syndrome, and its consequent and severe vascular disease that ranges from early endothelial dysfunction and hypertension to end-stage cardiac, cerebral, and renal disease. Because levels of vascular EET are decreased in obesity and EET analogues attenuate adiposity, we hypothesized that the vascular protective properties of EETs are an integral component of the adipocyte HO-1-adiponectin axis that controls adipocyte-vascular interactions in obesity-mediated metabolic and vascular complications. Indeed, specific targeting of CYP2J2, a major EET-producing enzyme, to the vascular endothelium affected not only the vasculature, attenuating HF-induced endothelial dysfunction, but also adipose tissue, changing the phenotype of adipocytes and ameliorating the HF diet-induced metabolic conditions including insulin resistance and adiposity by a mechanism that increases the HO-1-adiponectin signaling pathway. EET and HO-1 seem to form a module that serves as a molecular switch to genetically reprogram the adipocyte phenotype to express lower levels of Mest and prevent hypoadiponectinemia. Therefore, the CYP2J2 gene targeting–mediated sustained increase of EET levels merits further investigation to elucidate whether this new and different approach can have clinical value in treating obesity-related metabolic diseases, including diabetes mellitus, atherosclerosis, and vascular disease. A deeper understanding of the mechanisms involved will provide new approaches for the treatment of obesity, diabetes mellitus, and atherosclerosis and improve the effectiveness of cell-based treatment of vascular diseases.

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We had full access to the data and take responsibility for its integrity. We have read and agreed with the article as written.

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Disclosures

None.

References


**Novelty and Significance**

**What Is New?**

- We provide evidence supporting the paracrine effect of endothelial epoxycosatrienoic acid (EET) production to improve both vascular and adipocyte function through increased levels of heme oxygenase (HO)-1.
- Endothelial-targeted EET overexpression reprograms adipocyte phenotype; it restores AMPK activation and adiponectin production in the presence of a high-fat diet.
- The targeting of CYP2J2 to the vascular endothelium results in increased production of EETs in vascular beds. This in situ vascular endothelial-specific CYP2J2 expression and amplification of EET production improved adipocyte function and led to decreased body weight gain and enhanced insulin sensitivity.
- This report uncovers a mechanistic link between the vascular system and adipose tissue whereby improvement of vascular function by the EET-HO-1 axis positively affects adipocyte function.
- An experimental and conceptual basis for the development of new therapeutic strategies that target the EET-HO-1 module to ameliorate adipocyte and vascular dysfunction associated with the metabolic syndrome.

**What Is Relevant?**

- The consequences of obesity-mediated adipocyte dysfunction may lead to vascular dysfunction, which is a prelude to vascular disease and hypertension.
- The existence of a close relationship between the vascular system and adipocytes whereby improvement of vascular function by the EET-HO-1 axis positively affects adipocyte function.

**Summary**

This is the first study to demonstrate that targeting the vascular endothelium with human CYP2J2 in mice fed a high-fat diet decreased adiposity and vascular dysfunction, improved metabolic parameters, and increased levels of HO-1 expression in both the vasculature and in the adipose tissue. Furthermore, this study clarifies the relationship between EET-HO-1 and their ability to increase adiponectin and decrease mesoderm-specific transcript during adipogenesis and demonstrates how they influence the cellular/vascular homeostasis responses in an obese animal model. Finally, this report provides a portal into the mechanisms involved in obesity related to the development of vascular dysfunction and hypertension as well as potential therapeutic targets for the treatment of the metabolic syndrome and cardiovascular disease.
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MATERIALS AND METHODS

Animal experimentation

All experimental protocols were performed following an institutionally approved protocol in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Tie2-CYP2J2 transgenic mice, which exhibit endothelial-specific expression of the human CYP2J2 and increased vascular and circulating EET levels1-3, were provided by Dr. Darryl C. Zeldin. Wild type C57BL/6 controls and Tie2-CYP2J2 transgenic mice (6-7-week-old) were fed ad libitum either a normal or a HF diet (n=10 per group) for 18 weeks in order to quantify any differences between the animals with regard to weight gain4,5. The normal diet contained 11% fat, 62% carbohydrate, and 27.0% protein with total calories of 12.6 KJ/g4,5. The HF diet contained 58% fat from lard, 25.6% carbohydrate, and 16.4% protein with total calories of 23.4KJ/g (bioSERV, Frenchtown, NJ). Body weight and blood pressure, measured by the tail cuff method, were monitored weekly. For blood pressure measurements, mice were acclimatized to the tail-cuff measurement for seven days prior to the start of experiments. In brief, mice were placed on a far infrared heating pad for 7-10 minutes. Systolic blood pressure measurements (CODA non-invasive blood pressure system, Kent Scientific, Torrington CT) were recorded after five cycles of acclimatization. All measurements were taken at the same time of day for all mice. “The tail cuff plethysmography method was chosen to obtain repeated measurements of systolic blood pressure because of the long durations of the experimental protocol (18 weeks) and in accordance to published recommendation for blood pressure measurements in experimental animals6. The differences in systolic blood pressure within each group and between groups (n=10 for each group) were substantial and amounted to 10-20 mmHg. At the end of the 18-week period, mice were placed on a 12-hour fast, anesthetized with sodium pentobarbital (65 mg/kg, i.p.) and blood was obtained from the tail vein for measurement of glucose using a glucometer (Lifescan Inc., Miligitas, CA) and plasma insulin levels using an ELISA kit (Linco Research, St. Charles, MO, USA). At the time of sacrifice, the body weight and visceral and subcutaneous fat content were measured. Adipose tissue and aorta were collected as previously described7,8. Blood samples were collected in K3EDTA tubes at sacrifice and the plasma was separated, flash frozen in liquid nitrogen and maintained at -80°C until analyzed. Adiponectin and IL-6 were determined in serum using an ELISA assay (Pierce Biotechnology Inc. Woburn, MA) as previously described8,9.

Assessment of vascular reactivity

The interlobar artery (ILA) was removed, cleaned of fat and loose connective tissue, placed in cold Krebs-bicarbonate solution, and sectioned into 3-mm-long rings. Vasorelaxation responses of phenylephrine-constricted arteries to increasing concentrations of acetylcholine (10^-9 to 10^-3 mol/L) were examined in the presence of indomethacin (10 µmol/L) as previously described9.

Western blot analysis

Frozen adipose and vascular tissues were ground under liquid nitrogen and resuspended in homogenization buffer (mmol/l: 10 phosphate buffer, 250 sucrose, 1.0 EDTA, 0.1 PMSF, and 0.1% v/v tergitol, pH 7.5). Immunoblotting for HO-1,
Cyp2C44, sEH, AMPK, AKT, peNOS, MEST, aP2, PPARγ, UCP2 and actin was performed in aorta and adipose tissue as previously described.⁸,⁹

**HO-1 and CD68 immunostaining**

Alternate sections were deparaffinized, rehydrated and then incubated in 1% bovine serum albumin; subsequently, sections were incubated with either polyclonal rabbit antibody against HO-1 (diluted 1:500; Stressgene Bioreagents, Victoria, BC, Canada) or with rat monoclonal antibody against CD68 (diluted 1:250; AbCam, Cambridge, UK) for 1h at room temperature and overnight at 4°C. Then, sections were labelled using anti-rabbit Alexa Fluor 488 and anti-rat Alexa Fluor 488 conjugated secondary antibodies. Finally, the samples were counter-stained with DAPI, mounted and observed with a confocal microscope (LSM 510 Zeiss, Germany) at a final magnification of 400X. The immunofluorescent control was performed by omitting the primary antibody and in the presence of isotype matched IgGs. HO-1 and CD68 staining intensity in adipocytes was computed as integrated optical density (IOD). Digitally fixed images of the slices (n=5 per animal) at 200X magnification were analysed using an optical microscope (Olympus, Germany) equipped with an image analyzer (Image Pro Plus, Immagine Computer, Milan, Italy). For quantitative analysis, IOD was calculated for arbitrary areas, measuring three fields with the same area for each section.

**Statistical analysis**

Data are expressed as means ± S.E.M. Significance of difference in mean values was determined using one-way analysis of variance followed by the Tukey-Kramer post hoc test. P < 0.05 was considered to be significant.
RESULTS

Effect of endothelial CYP2J2 gene targeting on expression of gp91phox in aortic and adipose tissue

To study the effect of a HF diet, coupled with Tie2-driven endothelial CYP2J2 expression, on oxidative stress, we measured gp91phox expression in aortic and adipose tissues (S1A and B). Our results showed an increase in gp91phox expression in aortic (S1A; p<0.05) and in adipose (S1B; p<0.05) tissues in WT-HF mice as compared to WT controls. Tie2-CYP2J2-Tr mice fed a normal diet displayed significantly lower expression of gp91phox compared to corresponding WT controls in both aortic and adipose tissue (p<0.05). A HF increased gp91phox levels in aortic and adipose tissues of Tie2-CYP2J2-Tr mice (p<0.05); however, these levels remained more than 2-fold lower than those of WT-HF mice (S1A and B respectively, p<0.05).

Effect of endothelial CYP2J2 gene targeting on UCP1 gene expression in adipose tissues

We examined gene expression of uncoupling protein 1 (UCP1) in adipose tissue, one of the potent markers of energy metabolism. Our results demonstrated that WT mice fed a HF diet exhibited a decreased expression of UCP1 in adipose tissue in comparison to WT mice fed a normal diet (S2). Levels of UCP1 in adipocytes from Tie2-CYP2J2-Tr mice were significantly higher relative to WT mice (p<0.05) and although a HF diet decreased its level, it remained higher than in the corresponding WT mice (S2).
REFERENCES


**Figure S1.** Western blot and densitometry analyses of A) vascular gp91phox gene expression, B) adipose gp91phox gene expression in mice fed a normal diet (WT and CYP2J2) and mice fed a HF diet (WT-HF and CYP2J2-HF). Results are mean±SE; Data are shown as mean band density normalized to β-actin, n=4; *p<0.05 vs. WT; #p<0.05 vs. WT HF; †p<0.05 vs. CYP2J2.
**Figure S2:** Western blot and densitometry analyses of adipose UCP1 proteins in mice fed a normal diet (WT and CYP2J2) and mice fed a HF diet (WT-HF and CYP2J2-HF). Results are mean±SE; Data are shown as mean band density normalized to β-actin, n=4; *p<0.05 vs. WT; #p<0.05 vs. WT-HF; *p<0.05 vs. CYP2J2.