FORUM REVIEW ARTICLE

Heme Oxygenase-1 Upregulation: A Novel Approach in the Treatment of Cardiovascular Disease

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Abstract

Significance: Heme oxygenase (HO) plays a pivotal role in both vascular and metabolic functions and is involved in many physiological and pathophysiological processes in vascular endothelial cells (ECs) and adipocytes.

Recent Advances: From the regulation of adipogenesis in adipose tissue to the adaptive response of vascular tissue in the ECs, HO plays a critical role in the capability of the vascular system to respond and adjust to insults in homeostasis. Recent studies show that HO-1 through regulation of adipocyte and adipose tissue functions ultimately aid not only in local but also in systemic maintenance of homeostasis.

Critical Issues: Recent advances have revealed the existence of a cross talk between vascular ECs and adipocytes in adipose tissue. In the pathological state of obesity, this cross talk contributes to the condition's adverse chronic effects, and we propose that specific targeting of the HO-1 gene can restore signaling pathways and improve both vascular and adipose functions.

Future Directions: A complete understanding of the role of HO-1 in regulation of cardiovascular homeostasis is important to comprehend the homeostatic regulation as well as in cardiovascular disease. Efforts are required to highlight the effects and the ability to target the HO-1 gene in models of obesity with an emphasis on the role of pericardial fat on cardiovascular health. *Antioxid. Redox Signal.* 32, 1045–1060.

Keywords: heme oxygenase, obesity, adipocytes, cardiovascular disease, oxidative stress

Introduction

OBESITY AND CARDIOMETABOLIC SYNDROME are on the rise worldwide, and its adverse health effects are insidious. Obesity is a major risk factor for vascular diseases, diabetes type 2, and insulin resistance, as well as fatty liver (2, 8, 11, 43, 50, 87), while increasing inflammatory cytokines (2, 20, 77). The association between obesity and heart failure is evident with a prevalence of 70% in individuals morbidly obese for 20 years and 90% in those morbidly obese for 30 years (10).

The consequences of obesity-mediated adipose tissue dysfunction may be extensive as fluctuations in adipose

tissue-derived paracrine factors, including adipokines and cytokines, may affect the function of other organ systems, especially the cardiovascular system, and can lead to atherosclerosis and cardiovascular disease (CVD) (2, 37, 134). Although obesity and metabolic syndrome can be treated with diet and lifestyle changes, patient compliance is low and long-term success is difficult to maintain for many of these patients. Thus, there is an increasing focus on developing potential new therapeutic strategies to decrease and even reverse the clinical complications of obesity and metabolic syndrome (2, 6, 84, 129).

One enzyme that has emerged as a key player in modulating metabolic disease is heme oxygenase (HO). In humans,

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HO exists as isozymes HO-1, the inducible form, and HO-2, the constitutive form. Both isozymes are alike in terms of mechanism, cofactor and substrate requirements, and in their susceptibility to activation or inhibition by synthetic metalloporphyrins, in which the central iron atom of heme has been replaced by other elements including tin, zinc, cobalt, and chromium (2, 3). HO is responsible for the degradation of heme (a pro-oxidant and also a strong inducer of HO-1), resulting in the formation of equimolar amounts of carbon monoxide (CO), iron, and biliverdin, which is then converted to bilirubin by biliverdin reductase (1, 2, 7, 64). Bilirubin and biliverdin have been found to be potent antioxidants, and induction of HO-1 reduces adiposity and oxidative stress, decreases excessive heme levels, and increases antioxidant enzymes, such as superoxide dismutase and catalase (3, 31, 119, 121, 124). Bilirubin has also been shown to inhibit NADPH oxidase and protein kinase activity (75, 95, 115). In addition, albumin-bound and free bilirubin inhibits oxidation of low-density lipoprotein (LDL), thereby preventing atherosclerosis (44, 95).

HO-1 is induced by a broad range of compounds and is considered the first line of defense against oxidative stress, provided that oxidative stress is a strong inducer of HO-1. Increased expression of HO-1 enables cells to resist injury by heme and reactive oxygen species (ROS). Thus, induction of HO activity is central to the antioxidant defense and exerts cytoprotective effects in the cardiovascular system, contributing to decreased cardiac remodeling (2, 4, 83). This effect is apparent in patients with Gilbert's syndrome, whose higher serum bilirubin is associated with decreased risk for coronary artery disease (27, 51, 95).

Deletion of the human HO-1 gene results in organ damage in the liver, kidney, and vasculature system. This damage is caused by severe heme and iron deposition, which results in increased levels of ROS (65, 67, 88). Similarly, mice deficient in HO-1 have decreased iron utilization, increased heme levels in the systemic circulation, and a defective stress defense system (101, 102). Interestingly, HO-1-knockout mice exhibit sex-dependent effects, and HO-1 expression in adipose tissue has a greater protective role in females compared with males in preventing hyperglycemia and insulinemia (56).

HO-1 Genetic Polymorphisms and Their Impact on CVD

Genetic polymorphism of the *HMOX1* gene indicates a significance of HO-1 expression in the pathogenesis of pulmonary and CVDs (2, 138). It is believed that the length of (GT)_n repeats affect the transcriptional activity of the *HMOX1* gene. Association exists between longer (GT)_n repeats in the *HMOX1* gene promoter and reduced HO-1 inducibility by ROS present in cigarette smoke, thus predisposing to the development of emphysema (2, 138). Diabetic patients with Gilbert's syndrome and elevated levels of bilirubin are partially protected from vascular complications compared with diabetic patients with normal bilirubin levels (2, 60).

In an Asian population, patients carrying longer (≥ 32) (GT)_n repeats and who were diagnosed with type 2 diabetes had elevated oxidative stress levels and an increased risk of the development of coronary artery disease and atherosclerosis (2, 26). Meanwhile, patients with significant obesity-associated risk factors for coronary artery disease (hyperlipidemia,

smoking, and diabetes), but with shorter (<27) (GT)_n repeats had reduced disease risk (2, 63). One cohort study performed to evaluate *HMOX1* gene promoter polymorphisms and the risk for restenosis after percutaneous transluminal angioplasty found that patients with short (<25 GT) dinucleotide repeats in the *HMOX1* gene promoter on either allele had reduced risk of restenosis compared with patients with longer dinucleotide repeats (2, 38). These data indicate that shorter dinucleotide repeats and upregulation of *HMOX1* gene expression may be protective after balloon angioplasty.

However, not all studies support a clinical significance of the genetic polymorphism in the *HMOX1* gene; for example, in a study of 1807 patients with coronary artery disease undergoing coronary stenting, no correlation was found between *HMOX1* promoter polymorphism and ischemic events (2, 127). In support of this finding, no evidence of a beneficial effect of low (GT)_n repeats on survival rate of graft or recipient in clinical renal transplantation was observed (2, 28).

A single nucleotide polymorphism in the *HMOX1* promoter, T(−413)A, has been described that correlate with a reduced incidence of ischemic heart disease (2, 89). A study of 3104 patients with vascular disease observed a correlation between restenosis after percutaneous coronary intervention and an angiotensin II type I receptor (*AGTR1*) 116 A/C polymorphism, but no association with *HMOX1* polymorphism was observed (2, 136). These studies provide evidence for and/or contradict the role of the *HMOX1* gene in genetic polymorphism and cardiovascular health and risk of disease. In a recent study, the role of *HMOX1* genetic polymorphism in CVD was investigated.

The authors focused on the incidence of stroke, myocardial infarction (MI), and vascular disease in patients registered between 1995 and 2010. After investigating more than 800 patients (aged 45–84 years), an association between the *HMOX1* variable number tandem repeat polymorphism and CVD was observed and confined to subjects with a high number of GT repeats on both alleles of the *HMOX1* gene, providing evidence of atherogenesis and decreased antioxidant defense in patients with high risk of vascular complications (2, 92). These studies point to an important role of *HMOX1* gene regulation in atherosclerotic disease processes.

Upregulation of HO-1 Using Gene Targeting and Pharmacological Agents

The protective role of HO-1 has led to a wealth of research investigating the effect of pharmacological induction of HO-1 levels; these include the use of Cobalt protoporphyrin IX (CoPP), heme, apolipoprotein A1 (APOA1) mimetic peptides L-4F or D-4F, epoxyeicosatrienoic acid (EET), and peroxisome proliferator-activated receptor alpha (PPAR α). Nonspecific HO-1 induction in mice has been found to attenuate inflammatory markers, reduce kidney damage, ameliorate fatty liver disease and hypertension (2, 35, 36, 52, 113, 122), and also to increase adiponectin levels and insulin sensitivity (68, 86, 94, 96) [Table 1; reviewed in Abraham and Kappas (3) and Drummond *et al.* (33)].

HO-1 upregulation via nonspecific global gene transfer

As HO-1 gene therapy provides a more stable and prolonged vascular protection than pharmacological induction does, subsequent studies investigated the effect of HO-1

TABLE 1. LIST OF HEME OXYGENASE-1 INDUCERS, INCLUDING BOTH PHARMACOLOGICAL AND GENETIC, AS WELL AS GENERAL AND TISSUE SPECIFIC

<i>General inducers</i>	<i>Tissue-specific inducers</i>
CoPP	VECAD-HO-1 lentiviral vector (endothelial cells)
ApoA-1 mimetic peptides	aP2-HO-1 lentiviral vector (adipocytes/macrophages)
L-4F/D-4F	Adiponectin-HO-1 lentiviral vector (adipocytes)
Heme arginate	Intrarenal medullary interstitial catheter
General gene therapy	TALH-HO-1 lentiviral vector (kidney cells)
EET	
Statins	
Aspirin	
Free and complexed metals	
Prostaglandins	
Niacin	
Paclitaxel	
Rapamycin	
Telmisartan	
Turmeric	
Resveratrol	

aP2, adipocyte protein 2; CoPP, cobalt protoporphyrin IX; EET, epoxyeicosatrienoic acid; HO-1, heme oxygenase-1; TALH, thick ascending loop of Henle; VECAD, vascular endothelial cadherin.

overexpression at the early stage of adipocyte dysfunction following high-fat diet (HFD) intake in mice. Induction of HO-1 gene expression is associated with an increased number of preadipocytes. Conversely, suppression of HO-1 results in increased number of large adipocytes and increased insulin resistance (23).

Pharmacological induction of HO activity

Obesity in general, abdominal obesity in particular, is associated with insulin resistance and the pathogenesis of type 2 diabetes mellitus and hypertension, contributes to high serum levels of LDL and triglycerides and low serum levels of high-density lipoprotein, and leads to the development of atherosclerosis and associated CVD (2, 40, 82, 128). HO-1-mediated decreases of ROS and of LDL were reported in a number of models of diabetes (2, 21, 22). Leptin-deficient mice or mice fed a HFD exhibit a metabolic syndrome-like phenotype, which includes an increase in LDL that is amenable to rescue by increased levels of HO-1 and adiponectin (2, 21). Chronic HO-1 induction increased oxygen consumption and lowered body weight in obese melanocortin-4 receptor-deficient mice with an improvement in vascular function (2, 30, 55). Pharmacological inducers of HO-1 and their effects have been reviewed by Abraham *et al.* (2).

Human microvessel endothelial cells (ECs) were treated with angiotensin II (Ang II) in the presence and absence of CoPP, with or without the inhibitor of HO activity, stannous (or tin) mesoporphyrin IX (SnMP). Conditioned medium (CM) from the ECs was added to cultures of mesenchymal stem cell (MSC)-derived adipocytes. Adipocytes incubated with CM from Ang II-treated ECs showed a marked increase in adipogenesis, whereas adipocytes incubated with CM from Ang II-treated EC concomitantly treated with CoPP attenu-

ated adipogenesis. In contrast, CM from ECs treated with Ang II together with SnMP increased MSC adipogenesis. These results demonstrate that CoPP induces HO-1 and SnMP inhibits HO activity. It further shows that HO activity can prevent the negative effects of Ang II.

The APOA1 mimetic peptide L-4F, a putative antidiabetic drug, improves endothelial function likely through its anti-oxidant and anti-inflammatory properties. In obese mice, L-4F increases adiponectin levels, improves insulin sensitivity, and reduces visceral adiposity (131). L-4F treatment prevented myocardial and coronary functional abnormalities in db/db mice. These effects were associated with increased levels of HO-1 resulting in increased levels of anti-inflammatory, anti-oxidative, and vasodilatory actions through a mechanism involving increased levels of adiponectin, pAMPK, and peNOS (131).

Pharmacological (*e.g.*, L-4F/D-4F, CoPP, heme arginate) or gene-mediated induction of HO-1 (2, 21, 22, 77, 85, 94, 96, 114) correlates with increased plasma adiponectin levels, a concomitant increase in the number of healthy adipocytes, improved insulin sensitivity, a lowering of blood pressure, and a decrease in inflammatory adipokines (2, 22, 68, 74, 84–86, 96). The effect of HO-1 induction on adipocyte morphology was confirmed in Zucker diabetic rats (2, 85) and ob/ob diabetic mice, where increased levels of HO-1 and HO activity prevented weight gain and reduced visceral and subcutaneous fat deposition. In addition to the above-mentioned means of induction, HO-1 can also be induced by a wide range of drugs, and naturally occurring compounds and elements, including niacin, aspirin, statins, eicosanoids such as EET, certain prostaglandins, as well as free and complexed metals (1, 2).

Additionally, upregulation of HO-1 decreases adipogenesis in MSCs and increases adiponectin levels in culture media, effects reversed by the inhibition of HO activity (2, 77, 94), thus confirming an HO-1-adiponectin-EET regulatory module that can be manipulated to reduce the negative impact of obesity, diabetes, and metabolic syndrome. This offers a portal into the therapeutic benefits of HO-1 upregulation and increase in HO activity. Chronic induction of HO-1 lowers body weight *via* increased oxygen consumption, an effect that is independent of the melanocortin-4 system as chronic induction of HO-1 in obese melanocortin-4 receptor-deficient mice still results in the attenuation of obesity and type II diabetes (2, 30, 55). The increased oxygen consumption following HO-1 induction is likely mediated through an elevation of endogenous CO levels as chronic treatment of obese mice with a CO-releasing molecule (CORM) also attenuates obesity and increases oxygen consumption (2, 54).

Tissue-specific targeting of HO-1 gene

Targeting the HO-1 gene with gene therapy to specific tissues has been effective in producing a local upregulation of HO-1:

Vasculature. Specific upregulation of HO-1 at the endothelium is accomplished by targeting the endothelium-specific promoter, vascular endothelial cadherin (VECAD), which has been used to selectively express genes in this tissue (25, 46, 76). Targeting ECs with HO-1 using the VECAD

promoter attenuated hyperglycemia-mediated cell injury and apoptosis (25), and cell injury and apoptosis caused by hyperglycemia (14). Oxidative stress as a result of hyperglycemia has an important role in the development and progression of diabetic vascular complications (14, 73). Augmentation of superoxide formation by Ang II results in the development of hypertension and endothelial and renal dysfunction; this is prevented by VECAD-HO-1 transduction. HO-1 transduction was also associated with elevation of adiponectin levels, activation of the 5' adenosine monophosphate-activated protein kinase (AMPK)-endothelial nitric oxide synthase (eNOS) pathway, and improved endothelial function coincident with decreased levels of inflammatory cytokines and oxidative stress in animals. These results demonstrated a significant vascular and renal protective role for endothelial HO-1 in an Ang II animal model of hypertension (25). The scheme in Figure 1 depicts how induction of HO-1 improves vascular reactivity.

Kidney. Overexpression of HO-1 in cultured thick ascending loop of Henle (TALH) cells and in transgenic mice with TALH-targeted overexpression of HO-1 has demonstrated that HO-1 protects against Ang II-mediated increases in oxidative stress, attenuates the development of Ang II-dependent hypertension (104), and decreases the levels of the sodium-potassium 2 chloride (NKCC2) transporter (104, 124). HO-1 can also help prevent acute kidney injury. This is clear because patients with long (GT)_n repeats in the *HMOX1* gene promoter have 1.58-fold higher odds of developing acute kidney injury after cardiac surgery compared with individuals with the shorter (GT)_n repeats (34).

Myeloid cells. Classically activated macrophages (M1) are proinflammatory, whereas alternatively activated macrophages (M2) have anti-inflammatory properties. Targeting HO-1 expression in myeloid cells has many benefits (142). Myeloid cell-specific upregulation of HO-1 favors M2 macrophage polarization and has protective effects against ischemia-reperfusion injury (IRI), which develops after a

previously blocked artery is reperfused. IRI is characterized by local inflammation and an increase of ROS that is caused by the sudden reintroduction of oxygen into ischemic tissue. HO-1 is upregulated after liver IRI, and HO-1-knockout mice have increased IRI, suggesting that HO-1 has a protective effect (142). In studies of macrophage polarization, a myeloid-specific HO-1 gene was knocked out in mice causing full deletion of HO-1 in macrophages. Bone marrow-derived macrophages were then cultured. The HO-1-deficient macrophages were found to have increased messenger RNA (mRNA) of proinflammatory M1 markers (C-X-C motif chemokine ligand 10 [CXCL10], interleukin-1 β [IL-1 β], and monocyte chemoattractant protein 1 [MCP-1]) and decreased mRNA of anti-inflammatory M2 markers (Arg1 and CD163) compared with controls. The HO-1 knockouts also had decreased M2 markers after treatment with IL-4. This suggests that HO-1 is involved in macrophage polarization, and the deletion of HO-1 favors the M1 reprogramming (142).

Myocardium. Cardiovascular risk factors associated with myocardial dysfunction and oxidative stress is often observed in obesity. One study performed to determine alterations in enzymatic redox patterns in obese patients with myocardial dysfunction found increased levels of ROS and ROS-producing enzymes and reduced levels of antioxidant defense enzymes, including mitochondrial aldehyde dehydrogenase and HO-1 (47). Targeted deletion of HO-1 in cardiomyocytes exposed to ROS impairs mitochondrial quality control (125). IRI and low levels of HO-1 are associated with an increased risk of ventricular fibrillation (VF) (91), and an inverse relationship between HO-1 levels and VF has been demonstrated (17). Induction of HO-1 using recombinant adeno-associated virus reduced post-ischemic inflammation in pigs (53). Moreover, the post-ischemic infiltration of inflammatory cells, including leukocytes, neutrophils, and monocytes into HO-1-deficient hearts, was significantly increased (53). The protective effects of HO-1 overexpression in the heart tissue are further evidenced by prolonged survival of cardiac allografts, and reduced mortality and preserved left ventricular (LV)

FIG. 1. Induction of HO-1 improves vascular reactivity. Schematic depicting improved vascular reactivity in HFD-fed mice in which the HO-1 system has been induced (5, 25). Images of mice modified from the study of Li *et al.* (77), in which HO activity was induced by administration of CoPP. CoPP, cobalt protoporphyrin IX; HFD, high-fat diet; HO-1, heme oxygenase-1. Color images are available online.

function after acute MI (39, 80). Figure 2 summarizes how HO-1 manipulation affects the heart function.

Adipose tissue. Using a lentiviral construct equipped with the adipocyte-targeting promoter for adipocyte protein 2 (aP2) to drive expression of HO-1 was associated with a several-fold increase in HO activity when compared with control vector (23). This effect was long lasting and attenuated adiposity and vascular dysfunction in mice fed a HFD. This offers a portal into therapeutic induction of HO-1.

CYP2J2 Gene Targeting and the Role of EETs

EETs and HO-1 are involved in a cellular mechanism that modulates mitochondrial function and ROS. EETs are arachidonic acid metabolites whose formation is catalyzed by the cytochrome p450 (CYP450) pathway (109). EETs are vasodilators and mediate many signaling cascades that reduce inflammation, apoptosis, and improve insulin sensitivity (109). Regarding adipocyte function specifically, EETs influence adipocyte phenotype in favor of small healthy adipocytes (69, 79, 123, 129, 141). Downregulation of soluble epoxide hydrolase (sEH, an enzyme that converts EET to a less active class of lipids; dihydroxyeicosatrienoic acids) both *in vitro* and *in vivo* increases the number of small healthy adipocytes, increases the expression of thermogenic genes associated with beige fat, and increases adiponectin levels (79). Targeting ECs with CYP2J2 (cytochrome P450 family 2 subfamily J member 2, a member of the CYP450 family responsible for the EET production) using a tyrosine-protein kinase receptor (Tie2)-CYP2J2 transgenic mouse model decreases body weight, subcutaneous and visceral fat accumulation, blood glucose and inflammatory cytokines in mice fed a HFD while improving adiponectin levels and vascular function (5).

These effects of EET on adipocyte differentiation and phenotype are mediated by HO-1 upregulation (69, 123). EET upregulation leads to increased levels of HO-1 (5, 69,

123), and inhibition of HO activity using SnMP mitigates the positive effects of EET (123). Conversely, the activation of HO-1 gene expression increased EET levels (79), suggesting that HO-1 may contribute to the stimulation of EET production or the prevention of its degradation. Thus, EET and HO-1 appear to work in concert to achieve these beneficial effects.

The mechanism by which EET induces HO-1 has been explored. One mechanism that has been suggested is that EET leads to decreased expression of BTB domain and CNC homolog 1 (BACH-1), a transcriptional suppressor of HO-1 (123, 129). More recently, however, the effect of EET on HO-1 was reported to be mediated by peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α). PGC-1 α is a protein initially described as an inducer of thermogenic genes and is now recognized as a major regulation of mitochondrial function and biogenesis (133). PGC-1 α works cooperatively with PPAR δ to induce HO-1 transcription (9). Knockdown of PGC-1 α prevented EET-mediated HO-1 induction (133). Thus, EET, HO-1, and PGC-1 α constitute a cellular module that improves mitochondrial function and protects against inflammation triggered by ROS.

The relationship between HO-1, PGC-1 α , and EET is schematized in Figure 2. Endothelial-specific targeting of the human CYP epoxidase, CYP2J2, using the Tie2 promoter. Tie2-CYP2J2 transgenic mice, fed a HFD, had reduced body weight gain, blood glucose, insulin levels, and inflammatory markers (5). Tie2-CYP2J2 gene targeting restored HFD-mediated decreases in vascular protein expression of HO-1, Cyp2C44 (cytochrome P450, family 2, subfamily c, polypeptide 44), sEH, as well as phosphorylation levels of p-eNOS, pAKT, and pAMPK proteins, thus improving vascular function. These changes translated into decreased inflammation and oxidative stress within adipose tissue and decreased PPAR γ , CCAAT/enhancer binding protein alpha (C/EBP α), mesoderm-specific transcript (MEST), and aP2 expression and increased uncoupling protein (UCP) 1 and 2 expression, reflecting the effect of vascular EET

overproduction on adipogenesis. This study documented a direct link between endothelial-specific EET production and adipogenesis (5), further implicating the EET-HO-1 cross talk as an important cytoprotective mechanism in the amelioration of vascular and adipocyte dysfunction resulting from diet-induced obesity.

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. UCP1 and UCP2 are expressed in adipose tissue that plays an important role in the control of energy expen-

diture by uncoupling respiration from adenosine triphosphate (ATP) synthesis, thereby dissipates energy as heat and affects energy metabolism efficiency (42, 100, 108). In agreement with these reports, results show that CYP2J2 transgenic (Tr) mice have increased adipose UCP1 and 2 levels compared with wild-type mice, and this could be one of the mechanisms involved in decreased adiposity in the CYP2J2 Tr mice. Taken together and summarized in Figure 3, the evidence supports a paracrine effect of endothelial EET production to improve both vascular and adipocyte functions through increased levels of HO-1. Thus, EET and HO-1 appear to form

FIG. 3. Pharmacological or genetic induction of HO-1-EET-PGC-1 α axis. Schematic depicting pharmacological or genetic induction of HO-1-EET-PGC-1 α axis (121, 133), as well as a decrease in HO-1 transcriptional suppressor, BACH-1 (24, 121, 123, 129). HO-1-PGC-1 α -EET feedback loop and some of the important effects observed systemically (reduced oxidative stress and inflammation), and in the vasculature (dilation) and adipose tissues (prevention or reversal of differentiation, and reduced inflammation). Also included are some of the effects observed on a subcellular level, that is, increased mitochondrial thermogenesis and biogenesis. Based on the HO-1-PGC-1 α -EET axis action on adipocytes, the impaired exosomal secretion is likely increased with reversal of adipocyte dysfunction. BACH-1, BTB domain and CNC homolog 1; EET, epoxyeicosatrienoic acid; PGC-1 α , peroxisome proliferator-activated receptor gamma coactivator 1-alpha. Color images are available online.

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. 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. A direct link between endothelial-specific EET production and HO-1 induction to inhibit adipogenesis insults has been established (5). Thus, studies using CYP2J2 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.

Vascular Adipocyte Cross Talk in Obesity

Upregulation of HO-1 in endothelial tissue also improves adipose tissue function. This is because endothelial tissue and adipose tissue are linked *via* a “cross talk” mechanism, where one affects the other. In experiments performed to elucidate this mechanism, EC-specific overexpression of HO-1 reduced adipocyte maturation resulting in smaller, insulin-sensitive adipocytes, and reduced intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), adipocyte-derived MCP-1, and tumor necrosis factor α (TNF α) levels (97). Moreover, administration of SnMP, an inhibitor of HO activity, reduced these effects, thus showing the dependence of HO-1 (97).

The size of the adipocyte is indicative of their function. Smaller adipocytes release larger amounts of the adipokine adiponectin, which is a significant contributor to the insulin sensitivity of these cells (78, 86, 97, 107, 117, 132). Larger “necrotic” adipocytes are insulin resistant and release markedly less adiponectin than their healthy counterparts (15, 97) (Fig. 3). Adipocyte size is also dependent on C/EBP- α and PPAR γ , downstream activators of inflammatory cytokines, such as MCP-1, which stimulates the development of enlarged adipocytes that ultimately undergo inflammatory cell death, that is, necrosis (49, 57, 62, 110).

This highlights the crucial interaction between vascular function and adiposity. As a very active organ, adipose tissue is capable of secreting adipokines that affect the entire vascular system negatively or positively (5, 97). Particularly, adipose tissue surrounding the vasculature, the so-called perivascular adipose tissue, can interact with underlying vascular bed affecting its contractile tone (97, 106, 126). In turn, the vascular endothelium can regulate expansion of surrounding adipose tissues. This adipose tissue–vascular bed homeostatic interaction is impaired in the case of morbid obesity, leading to the secretion of proinflammatory adipokines, as shown in models of obesity in mice (16, 97). The vascular endothelial growth factor (VEGF) led to key revelations about angiogenesis. One of these is that VEGF appears to be intimately involved with wound repair (61), which depends on vascular growth (12, 139).

For adipose tissue to expand, the vasculature needs to grow as well. This requires VEGF, which is readily provided by healthy ECs. In chronic obese mice, the appearance of large unhealthy, necrotic adipocytes was accompanied by in-

creased levels of VCAM, TNF, ICAM, PPAR γ , and C/EBP along with reduced levels of adiponectin and wingless-type MMTV integration site family, member 10B (Wnt10b) (97). These negative effects were improved by upregulation of HO activity (mediated by CoPP treatment) (97). That these effects were dependent on HO-1 upregulation and HO activity was proven with the simultaneous treatment with SnMP, an inhibitor of HO activity, and that negated the observed positive effects (97). In addition, vascular relaxation in response to acetylcholine (ACH) was improved by upregulation of HO-1 (97). Lentivirus-mediated genetic upregulation of HO-1 in mice was more effective in reducing the levels of inflammatory adipokines and had a greater effect on ACH-mediated vascular relaxation than pharmacological induction of HO activity with CoPP (97). By growing adipocytes in CM derived from ECs transfected with either VECAD-HO-1 or a control vector, signaling originating from EC-influenced adipocyte activity including the production of inflammatory or anti-inflammatory adipokines (97).

Studies describing the use of VECAD-HO-1 gene transfer have examined the effect of HO-1 on the expression of nephroblastoma overexpressed (NOV/CCN3) and angiopoietin 1 (Angpt1), a potent growth factor that prevents adipogenesis and improves insulin resistance *in vitro* (93, 105) and also inhibits MSC differentiation into adipocytes (70, 97). NOV is a recently discovered adipocytokine involved in insulin resistance and many other pathophysiological processes, including cell adhesion, migration, proliferation, differentiation, and survival (24, 33, 81, 90).

Elevated NOV levels are associated with increased levels of a number of inflammatory cytokines that negatively impact insulin signaling, with a consequence of adipose tissue expansion and insulin resistance in patients with metabolic disorders (90, 117, 120). In contrast, reduction in the level of NOV is associated with reduced adiposity, lowering of inflammatory cytokine levels, and enhanced insulin sensitivity in obese mice, effects associated with increased PGC-1 α expression in adipose tissue (33, 81, 120). An EET agonist not only increases HO-1 levels but also downregulates NOV in adipose tissue of mice (113, 117). Targeting ECs with HO-1 *via* gene transfer downregulated expression of NOV/CCN3 and resulted in a significant increase of Angpt1 from EC with a concomitant increase in healthier lipid-sensitive adipocytes. The beneficial effect of Angpt1 was confirmed as adipogenesis was reduced in cells treated with Angpt1. Angpt1 also reduced the expression of NOV in adipocytes. These results show further evidence of the cross talk between the vascular tissue and the adipose tissue surrounding it and that targeting ECs with HO-1 *via* gene transfer has important therapeutic implications in the treatment of inflammation and adipocyte dysfunction due to obesity (97).

Direct targeting of EC by HO-1 through gene therapy is an effective way to reduce inflammatory adipocytokines, while also improving vascular function (Fig. 4). That HO-1 upregulation re-establishes the “cross talk” between the vascular system and the surrounding adipose tissue is evidenced by the observed reduction in adipose tissue expansion and improved vascular function. EC-specific HO-1 expression leads to a direct prolonged reduction of adipogenesis and offers future treatment directives for the management of obesity-associated chronic inflammation and vascular dysfunction (97).

FIG. 4. Cross talk between adipocytes and vascular endothelial cells. Scheme illustrating the cross talk between vascular endothelial cells and adipocytes in both healthy and pathological states. Gene therapy targets that have been studied are indicated. Color images are available online.

Cardioprotective and Vascular HO-1-PGC-1 α Signaling Attenuates Cardiovascular Risk in Humans As Well As in Obese Mice Model

Leptin, adiponectin, and resistin are some of the signaling molecules secreted by adipose tissue (41). When homeostasis is disrupted, for instance, during inflammation, enlarged white adipocytes secrete several inflammatory cytokines (98, 117). In addition to the secretion of adipocytokines, adipocytes, as well as other cells in adipose tissues, secrete extracellular vesicles of endosomal origin used for cell-cell communications, including ECs and adipocytes in adipose tissues (29). Evidence shows that these vesicles, termed exosomes, reach the liver and influence glucose and lipid metabolism. The circulating levels of these exosomes correlate with adiponectin levels, and importantly, are inversely correlated with cellular ceramides causative agents of insulin resistance and CVD. Studies have also determined that metabolic disturbances, including obesity, change the effect of these secretory vesicles, based on their content in terms of cytokines, microRNAs (miRNAs), and so on. Exosomes isolated from adipose tissue macrophages of obese mice cause insulin resistance and glucose intolerance when administered to lean mice (140). Conversely, exosomes from adipose tissue macrophages from lean mice improved glucose metabolism when administered to obese mice (140). For review, see the study of Kita *et al.* (71).

Morbid obesity causes changes to the heart that include increased pericardial thickness in direct correlation with a high LV mass, decreased LF function (41, 111), and impaired fractional shortening (FS). Human adipose tissue locales are: beneath the skin (subcutaneous fat), in the larger bones (yellow bone marrow), surrounding internal organs (visceral fat), and in breast and muscular tissues. Surrounding the heart is visceral adipose tissue known as pericardial fat. Pericardial fat, together with the epicardial fat, is located between the

outer wall of the myocardium and the visceral layer of pericardium (58, 59). When these adipose tissue depots become enlarged, they cause, directly or indirectly, significant cardiac remodeling, release ROS, and secrete inflammatory adipokines along with reduced levels of cytoprotective HO-1 and PGC-1 α (24, 83, 116, 120). Please see the study of Iacobellis (58) for an excellent description of the anatomical differences between epicardial and pericardial fat. Increased epicardial fat deposition is associated with cardiomyopathy and cardiac remodeling (45, 48), and epicardial thickness may be a reliable marker for cardiovascular events, at least in females (41).

PGC-1 α plays a pivotal role in thermogenesis due to its regulation of PPAR α in brown, mitochondria-rich adipose tissue (103), as well as maintains cellular energy metabolism and mitochondrial biogenesis and function (137). Somewhat increased levels of PGC-1 α in muscle cells of transgenic mice confer resistance to age-related obesity and diabetes, suggesting that PGC-1 α stimulates the secretion of factors that act globally (135). Deficiency of PGC-1 α specifically in adipocytes caused a decrease in mitochondrial biogenesis and led to impaired glucose metabolism and insulin resistance (72). Activation of PGC-1 α mediates HO-1 expression that can, indirectly, further increase PGC-1 α levels (121). Similar to PGC-1 α , changes in HO-1 levels regulate mitochondrial biogenesis, function, as well as mitochondrial transport carriers (31, 99). In mice, cardiac tissue-specific overexpression of PGC-1 α activated mitochondrial biogenesis and proliferation (13, 112). Conversely, obese mice with adipose tissue-specific knockdown of PGC-1 α develop insulin resistance and have increased levels of circulating lipids (72).

There are several mechanisms that mediate HO-1's ability to reduce adiposity and vascular dysfunction in obese mouse models. One of these mechanisms is an increase in adiponectin expression resulting from products of heme degradation, namely bilirubin and CO, which act to reduce vascular

FIG. 5. Effect of HO-1 and PGC-1 α expression on left ventricular fractional shortening. Gene expression profiling revealing similar expression levels of (A) PGC-1 α and (B) HO-1 in epicardial and visceral fat depots of obese humans and mice. (C) Normalized left ventricular fractional shortening in obese+HO-1-induced mice. *Different from the corresponding control. Color images are available online.

FIG. 6. Schematic depiction of how obesity and increased ROS levels give rise to inflamed dysfunctional visceral (including pericardial) adipose tissue, which altogether drives inflammation of the intraorgan (including epicardial) adipose tissues. There is a significant reduction in HO-1, PGC-1 α , and adiponectin, in visceral and pericardial fat of obese mice, and obese humans with coronary disease when compared with same fat depots of lean mice and age- and gender-matched human controls, respectively. This imbalance causes a shift in the signaling molecule-content of adipocyte-secreted exosomes from supporting homeostasis to disruption thereof. This is accompanied by a reciprocal increase in levels of inflammatory cytokines in intraorgan fat depots, including epicardial fat, that causes organ dysfunction and disruption of homeostasis. ROS, reactive oxygen species. Color images are available online.

dysfunction in models of hypertension (19, 55, 66, 77, 86). Bilirubin also has anti-inflammatory effects on the heart, one of which is an increase in the bioavailability of nitric oxide (18). Bilirubin also decreases both markers of endoplasmic reticulum stress and inflammatory cytokine levels (32). However, HO-1 downregulation is associated with organ damage in both humans and mice resulting from a rise in ROS levels (88, 102).

Our group has previously demonstrated that HO-1 deficiency leads to an increase in white fat as opposed to brown-like fat *via* a reduction in PGC-1 α , which in turn lowers MFN2 levels (117, 130). As noted earlier, PGC-1 α activation also mediates HO-1 expression, which have an indirect positive feedback effect on PGC-1 α expression by enhancing the activity of EET, which is located upstream of PGC-1 α in the signaling pathway. In this way, HO-1, PGC-1 α , and EET influence each other in a positive feed-forward loop that significantly affects cardiovascular function (Figs. 3 and 6).

PGC-1 α inhibition is associated with a reduction in MFN2 and thermogenic genes such as PRDM16 with the result of an overall decrease in mitochondrial biogenesis. This decrease has multiple negative effects, including a reduction in energy expenditure, the conversion of brown fat to white fat, cardiac remodeling, and cardiomyopathy. Induction of the HO-1-PGC-1 α axis has also been found to reverse this process, and increased levels of HO-1 and PGC-1 α are crucial to restoring LV function (24, 116, 120).

The increase of FS in obese mice given an EET agonist has the simultaneous effects of inducing HO-1-PGC-1 α downstream signaling, markedly decreasing NOV protein expression in epicardial fat, and restoring ejection fraction and overall heart function. However, the reduction in HO-1 and PGC-1 α observed in obese mice not given the EET agonist was associated with increased NOV expression. This indicates that NOV is located downstream of either HO-1 or PGC-1 α . Downregulation of NOV is also associated with a reduction in the levels of other inflammatory adipocytokines (24, 116, 120).

In this review, we present recent findings in a report (118) in which they compared expression profile of signaling pathways in adipose tissue isolated from diseased hearts with the expression profile in visceral fat. They compared their results with those obtained from same tissues of obese mice, in which the development of heart failure seems to result from changes in levels of inflammatory mediators and cytoprotective/antioxidant autacoids. Importantly, local adipose tissue seems to exert a more pronounced influence on the myocardium compared with other visceral adipose tissue.

By combining genome-wide and heatmap analyses of adipocyte gene expression, these researchers were able to observe the expression levels of novel regulatory genes, which would help them identify influential epicardial fat adipokines. Their analysis yielded the same pattern in HO-1 and PGC-1 α gene expression in both humans and mice—obese subjects exhibited a higher increase in visceral adipose tissue gene expression relative to epicardial fat tissue by a factor of ~ 2 . This suggests the existence of conserved signaling pathway in these two species (Fig. 5A, B). Furthermore, an increase of HO-1-PGC-1 α was associated with an improvement in LV function in obese mice treated with an inducer of PGC-1 α -HO-1 as evidenced by a normalization of the FS (Fig. 5C) and

reduced inflammation and tissue remodeling (118). Figure 6 depicts a schematic of how obesity and increased ROS levels give rise to inflamed dysfunctional visceral (including pericardial) adipose tissue, which altogether drives inflammation of the intraorgan (including epicardial) adipose tissues and how these changes affect the PGC-1 α -HO-1 levels, and indicates how activation/enhancement of this pathway could lead to a return to cardiac homeostasis (24, 118, 120).

To conclude, this review, and the discussed observations open a door toward further human studies aimed at investigating these pathways that have the potential of leading to the identification of novel targets for the treatment of obesity-associated CVDs.

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Abbreviations Used

ACH = acetylcholine
AKT = AKT serine/threonine kinase 1
Ang II = angiotensin II
Angpt1 = angiopoietin 1
aP2 = adipocyte protein 2
APOA1 = apolipoprotein A1

C/EBP α = CCAAT/enhancer binding protein alpha
CM = conditioned medium
CO = carbon monoxide
CoPP = cobalt protoporphyrin IX
CVD = cardiovascular disease
CYP2J2 = cytochrome P450 family 2 subfamily J member 2
CYP450 = cytochrome p450
ECs = endothelial cells
EET = epoxyeicosatrienoic acid
FS = fractional shortening
HFD = high-fat diet
HO = heme oxygenase
ICAM = intercellular adhesion molecule
IL = interleukin
IRI = ischemia–reperfusion injury
LDL = low-density lipoprotein
LV = left ventricular
M1 = classically activated macrophages
M2 = alternatively activated macrophages
MCP-1 = monocyte chemoattractant protein 1
MEST = mesoderm-specific transcript
MI = myocardial infarction
mRNA = messenger RNA
MSC = mesenchymal stem cell
NOV/CCN3 = nephroblastoma overexpressed
PGC-1 α = peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PPAR = peroxisome proliferator-activated receptor
ROS = reactive oxygen species
sEH = soluble epoxide hydrolase
SnMP = stannous (or tin) mesoporphyrin IX
TALH = thick ascending loop of Henle
Tie2 = tyrosine-protein kinase receptor
TNF = tumor necrosis factor
Tr = transgenic
UCP = uncoupling protein
VCAM = vascular cell adhesion molecule
VECAD = vascular endothelial cadherin
VEGF = vascular endothelial growth factor
VF = ventricular fibrillation