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Translational Significance of Heme Oxygenase in Obesity and Metabolic Syndrome

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Abstract

The global epidemic of obesity continues unabated with sequelae of diabetes and metabolic syndrome. This review reflects the dramatic increase in research on the role of increased expression of heme oxygenase (HO)-1/HO-2, biliverdin reductase, and HO activity on vascular disease. The HO system engages with other systems to mitigate the deleterious effects of oxidative stress in obesity and cardiovascular disease (CVD). Recent reports indicate that HO-1/HO-2 protein expression and HO activity have several important roles in hemostasis and ROS-dependent perturbations associated with metabolic syndrome. HO-1 protects tissue during inflammatory stress in obesity through the degradation of pro-oxidant heme and the production of carbon monoxide (CO) and bilirubin, both of which have anti-inflammatory and anti-apoptotic properties. In contrast, repression of HO-1 is associated with increases of cellular heme and inflammatory conditions including hypertension, stroke and atherosclerosis. HO-1 is a major focus in the development of potential therapeutic strategies to reverse the clinical complications of obesity and metabolic syndrome.

Obesity and Metabolic Syndrome

Obesity and metabolic syndrome have earned the name “the silent disease” because their adverse effects are insidious. Although obesity and metabolic syndrome are theoretically treatable with modern medical and lifestyle management, this is not a trivial undertaking. While a discussion about the long-term success of different therapeutic strategies addressing obesity and metabolic syndrome is beyond the scope of this introduction, it is fair to say that our national and regional battle with this “silent disease” is not going well. Obesity and metabolic syndrome are statistically on the rise in the USA in general and in West Virginia and Mississippi in particular (http://www.cdc.gov/obesity/data/adult.html). Obesity is major risk factor for vascular dysfunction, insulin resistance and vascular diseases including

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obesity/diabetes and hypertension[1–3], while increasing inflammatory cytokines and insulin resistance [4,5]. These consequences of obesity-mediated adipocyte dysfunction may be far-reaching, as changes in adipocyte-derived paracrine factors including adipokines and cytokines may impact the function of other organs and in particular the vasculature [6–8].

Several studies have demonstrated that increases in reactive oxygen species (ROS) *in vitro* and *in vivo*, [9–11] as well as increases in heme, induce the differentiation of pre-adipocytes and increase adipogenesis *in vitro* and *in vivo* [10,12–14]. Interestingly, despite this increased ROS production, HO-1 expression is not increased, further contributing to the development of obesity and metabolic syndrome [13].

Obesity leads to the activation of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) and the angiotensin II system, resulting in the development of diabetes, hypertension and CVD in part due to impairment of adipocyte function [1,15,16]. Obesity-mediated development of hyperglycemia has a direct effect on HO-1 suppression [17–19], and its inactivation by peroxynitrite [11,20,21] increases cellular heme levels [11,20,22]. Heme, a pro-oxidant, acts together with increased ROS to produce vascular and adipocyte dysfunction [14]. This review will examine the role of HO-1 and of the heme degradation products biliverdin/bilirubin and CO in mitigating obesity, as well as their role in the regulation of oxidative stress and cell survival. We discuss these findings in the context of the identification of potential future therapeutic approaches to prevent the development of obesity, diabetes and the detrimental effects associated with the progression of atherosclerotic disease [23,24] (Figure 1).

Heme Oxygenase

HO exists in two forms, HO-1, the inducible form, and HO-2, the constitutive form. Both isozymes degrade heme in an identical stereospecific manner to biliverdin with the concurrent release of CO and iron [25]. In mammals, biliverdin is rapidly reduced by biliverdin reductase to bilirubin [26,27]. HO-1 and HO-2 are alike in terms of mechanism, cofactor and substrate requirements, as well as their susceptibility to inhibition by synthetic metalloporphyrins in which the central iron atom is replaced by other elements including tin, zinc, cobalt and chromium (reviewed in [14]). HO-1 can be induced by an extraordinarily wide variety of drugs and chemical agents including statins, aspirin, niacin, certain prostaglandins, eicosanoids such as epoxyeicosatrienoic (EETs) and free and complexed metals [25]. Iron, bilirubin and CO, the three degradation products of the HO reaction, may have important regulatory functions in cells. Iron is, of course, an essential requirement for the synthesis of hemoglobin and ferritin, and HO-1-deficient mice are known to develop anemia. Reduced stress defense in HO-1 deficient cells is well described [28]. HO-1 deficiency accelerates formation of arterial thrombosis[29] that is rescued by inhaled CO [30]. The discovery that HO-1 is a novel target for modulation of the inflammatory response [31,32] and for diminishing fibrosis [33] increases interest in HO-1 signaling pathways. Yachie et al., [34] reported that a child with congenital HO-1 deficiency, or mice deficient in HO-1 displayed severe growth retardation in addition to microcytic hypochronic anemia, renal endothelial cell injury[34–37]. Similarly, HO-2 deficiency increased oxygen toxicity and iron accumulation in lung [38] and in response, diabetes-induced renal injury [39], an increase in

adiposity and systolic blood pressure [40] and impaired vasodilation to acetylcholine [41]. The constitutive nature of HO-2, however, makes it less attractive as a drug target.

The first therapeutic approach to regulate HO activity began by devising a means to downregulate HO activity by competitive inhibition. This was recognized early [42,43] and achieved clinical fruition in neonatology when a safe, rapidly acting and effective method for transiently blocking bilirubin production in newborns was developed [43,44]. The development of inhibitors of HO activity having a pharmacological profile permitting their use in infants provided the first demonstration of the potential clinical usefulness of agents that can downregulate HO. However, when selecting an inducer or an inhibitor of HO activity, one must consider the mechanism of induction/inhibition and duration of response. Rapid response of HO-1 mRNA by inducers such as cobalt protoporphyrin IX dichloride (CoPP) do not reflect an immediate increase in HO activity [45], suggesting that an increase of HO-1 mRNA is meaningless unless HO activity is also determined. Induction of HO-1 by heme or CoPP has a differential effect on the timing of an increase in HO activity, with heme resulting in a maximum increase in HO activity at 16 hr, whereas with CoPP maximal HO activity is attained at 3 days and lasts for extended periods of time up to 14 days [25]. In metabolic syndrome and vascular disease, elevation of inducible nitric oxide synthase (iNOS) and peroxynitrite resulted in inactivation of HO-1 protein [20,21] and decreased HO activity. These considerations regarding the time frame of induction or inhibition of HO activity, not just HO-1 expression levels, must be taken into account in the development of new therapeutic strategies targeting HO-1 for the treatment of obesity-hypertension and metabolic syndrome as has been suggested in numerous publications. As an alternative strategy to drugs, gene therapy has been identified as a long lasting (1 year) and effective way to induce HO-1 expression to prevent against CVD [20,46–51].

Both biliverdin and bilirubin are potent antioxidants and may exert cellular protective effects against injurious stimuli *in vivo* and *in vitro* [52,53]. CO has been identified as a second messenger in the central nervous system (CNS) [54] and suppresses endothelial cell apoptosis through activation of p38 MAPK [55,56]. The gas has a multitude of functions in biology and medicine which are described in series of articles [57–59] and include acting as a vasodilator via stimulation of soluble guanylate cyclase (sGC).

Biological Action of Bilirubin/Biliverdin

The biologic actions of HO-1-derived bilirubin may be especially relevant to the prevention of oxidant-mediated cell death [10,11,60] (Figure 2). Bilirubin, at a low concentration, scavenges ROS, thereby reducing oxidant-mediated cellular damage and attenuating oxidant stress *in vivo* [52]. The roles of biliverdin and bilirubin in counteracting oxidative and nitrosative stress have been extensively reviewed [32]. Administration of the degradation products of heme confers protection against various types of pathological injuries. Biliverdin therapy protects the liver of rats from ischemia and reperfusion injury, while bilirubin was reported to increase tolerance to islet allograft by both its anti-inflammatory and anti-oxidative properties[61]. Bilirubin increased tolerance to islet allograft by both its anti-inflammatory and anti-oxidative properties [62,63]. Additionally, biliverdin reductase acts as transcriptional factor and increases HO-1/HO-2 levels and HO activity [26] and protects

against glucose intolerance (reviewed in [64]) through a mechanism involving activation of phosphatidylinositol 3-kinase (P13K) [65]. Unconjugated bilirubin mediated HO-1-induced vascular benefit in diabetes [66] and administration of mesobiliverdin analogues of biliverdin to the pancreas lowered blood glucose and increased insulin function [67].

The potential beneficial and protective role of bilirubin in obesity and metabolic syndrome as well as the resulting cardiovascular disease has been highlighted in recent years. First, bilirubin was reported to increase insulin sensitivity and ameliorated obesity in leptin-receptor deficient and suppression of chronic inflammation [68]. Bilirubin also displays cytoprotective properties in the cardiovascular system [69,70], as elevated serum bilirubin levels are associated with a decreased risk for coronary artery disease in humans (reviewed in [71,72]). Additional studies have demonstrated that both free and albumin-bound bilirubin can inhibit the oxidation of low density lipoproteins (LDL) [73]. The roles of bilirubin and biliverdin reductase in obesity and CVD have been recently reviewed [64,72].

Biological Action of Carbon Monoxide

The function of CO is unclear. It is generally accepted that CO is toxic to cells, but less is known about pico-levels of CO in physiological function (reviewed in [74]). A dose-dependent toxic effect of CO on BSC-1 cells established that this was due to stimulation of iNOS and production of nitrate NOO^-, which is known to cause cellular injury [75,76]. Although CO, like NO, functions as a vasorelaxant via stimulation of sGC; the relative potency of CO is miniscule compared to NO, however, the potency of CO mediated increase in cGMP levels may be increased by other agents, [77] including the benzyloid derivative such as YC-1, which increases CO-mediated cGMP formation to a level similar to that of NO. HO-1-derived CO was shown to relax vascular tissue of aorta [78], hepatic vein, piglet mesenteric artery, pial arterioles [79], and pulmonary artery.

While the vascular effects of CO have been established for some time now, the regulation of metabolism by CO is an emerging area. Chronic CO treatment through the use of a CO-releasing molecule provide vascular protection [80,81]. CORM-A1-derived CO given to mice fed a high fat diet resulted in decreased levels of fasting blood glucose, insulin and body fat and increased oxygen consumption and heat production compared to controls [82]. Chronic CORM-A1 treatment was found to reverse obesity in mice chronically fed a high fat diet through similar changes in adipocyte phenotype as well as significant reductions in pro-inflammatory adipokines such as high mobility group box (HMGB) protein-1[83].

HO-1 and HO-2-derived CO increases insulin secretion, and this function involves Ca+ signaling, both of which are prevented by inhibition of HO activity [84]. This may also be due to a CO-mediated anti-inflammatory effect [59,85]. The beneficial effects of chronic exposure to CO as a direct result of heme degradation have been recently reviewed 57,57,58,86.

Biological Action of Ferritin

Heme metabolism results in the liberation of iron, and tissues rely on storage proteins to regulate "free" levels of this metal. Free iron is well known to lead to the generation of ROS, potentially leading to damage of various cellular components. Iron can become integrated

into the phospholipid bilayer, and act to oxidize cell membrane constituents and/or participate in reactions leading to the generation of ROS [87,88]. The link between iron, metabolic syndrome and Alzheimer's disease has been recently reviewed [89]. Plasma iron is bound to transferrin, which can transfer iron to the intracellular milieu of endothelial cells via cell surface receptor binding. Ferritin is a high capacity, low affinity protein responsible for binding most intracellular iron, and its synthesis is rapidly upregulated when free iron is present [90]. Under normal conditions, very minute amounts of iron exist in the free metal form; thus the concentration of ferritin is an efficient indicator of intracellular iron levels [91]. The most abundant source of iron is heme, which can release iron during metabolism by HO or via oxidative degradation [92]. While this transition metal is essential to biological processes, iron can be extremely toxic if intracellular concentrations are not tightly regulated; thus, maintenance of cellular homeostasis relies on upregulation of ferritin when HO-1 transcription is increased [93–95]. Iron-mediated oxidative damage increases heme with a concomitant increase in ferritin [96–98]. A cytoprotective effect of ferritin is related to the ferritin H chain, which controls the pro-oxidant effect of iron released during heme degradation by HO-1 [99,100]. Induction of HO-1 is associated with an increase of ferritin synthesis and the subsequent chelation of iron by ferritin to prevent iron toxicity [94,96]. Recently, H-ferritin feroxidate was shown to induce cytoprotection in sickle cell mice. Mice lacking ferritin are more sensitive to doxorubicin induced cellular injury [101]. A clear relationship exists between HO-1 and an increase in ferritin and the prevention of inflammation involving janus kinase (JNK) activation [99]. HO-1 and hemoglobin scavenger receptors play a role as anti-inflammatory agents in human coronary stable and unstable plaques resulting in an atheroprotection effect [102].

Impact of Heme on Adipocyte Differentiation-Adipogenesis and Obesity

The discovery by London's group in 1981 that heme is essential for the increase in pre-adipocyte differentiation and adipogenesis [12] was followed by the elegant work of the Burris group showing that synthesis and an increase of heme is associated with recruitment of REV-ERB ligands and an increase in adipogenesis [13]. This suggests that heme metabolism plays an important role in adiposity and the discovery of new targets in HO-1 signaling may prove useful for the treatment of metabolic disease (Figure 3). More recently, the heme-mediated increase of 3T3- cell differentiation was shown to be dependent on suppression of sirtuin 1 (SIRT1) [103]. The effect of heme on cell differentiation was not limited to 3T3 cells as adipocyte as heme is indispensable for hematopoietic stem cells differentiation to myeloid and erythroid cell linages [104,105]. While the decrease of heme by the increase in heme degradation by HO-1 decreases adipogenesis, it increases osteoblast differentiation [106,107]. These unique findings opened a new avenue of investigation on the effect of heme and HO-1 expression on metabolic diseases. Similar to HO-1 overexpression, EET was shown to increase osteoblast differentiation but to decrease adipocyte differentiation [107–110]. This finding was strengthened by the observation that administration of CoPP both affected adipocyte differentiation in adult rats and resulted in prompt weight loss, without a decrease in food consumption as measured by pair fed analysis [111]. The beneficial effect of CoPP to increase HO-1 levels and reduce adiposity is shared by other pharmacological agents including hemin, Apo-A1 mimetic peptide L-4F and D-4F, EET and peroxisome

proliferators-activated receptors alpha. HO-1 expression is also transcriptionally regulated by PPARα and PPARγ, indicating a mechanism of antiinflammatory and antiproliferative action of PPAR ligands involved upregulation of HO-1[112]. Hemin, EET, and L-4F are also associated with a decrease in visceral subcutaneous fat and an increase in insulin sensitivity [24,112–115], as well as a decrease in the number of large adipocytes (differentiated adipocytes) and an increase in the number of smaller healthy adipocytes [51,109,110,116]. CoPP or an analogue may have cardiovascular benefit as CoPP-mediated induction of HO-1 attenuates inflammatory markers and hypertension [117] and reduces glomerular injury [118] and obstructive nephropathy [119]. In addition, CoPP induction of HO-1 protects skeletal muscle and ameliorates high fat diet induced liver injury [120–122] in an SIRT-1-dependent manner. Other reports establish that induction of HO-1 by CoPP decreased circulating free fatty acids and C-reactive protein and increased adiponectin through the activation of the AMPK-P13K-eNOS pathway [51,113,115,116], toll-like receptor 4 signaling [123], and adiponectin- downstream signaling pAMPK-AKT and peNOS pathways [14,115] . CoPP-mediated induction of HO-1 provided renal and vascular protection[6,124,125]. It should be emphasized that HO-1 does not directly increase adiponectin per se; the HO-1-mediated antioxidant mechanism and decreases in heme associated with an increase in the levels of glutathione and superoxide dismutase decreased levels of ROS results in increased levels of adiponectin [10,24,113,114,126,127].

HO, Inflammation and Vascular Disease

The protective role of HO-1 and CO on inflammation occurs in many different disease models including ethanol-induced liver cell death [128]. Resveratrol both *in vitro* and *in vivo* upregulates HO-1 expression, NAD(P)H; quinone oxidoreductase 1, gamma glutamylcysteine synthetase via activation of nuclear factor (erythroid-derived)-like 2 (Nrf2) target genes. Resveratrol administered to mice fed a high fat diet attenuated oxidative stress and improved acetylcholine-induced vasodilation. The beneficial effects of resveratrol were attenuated in Nrf(−/−) mice fed a high-fat diet, indicating that the endothelial protective function of resveratrol is mediated by the activation of Nrf2 [129]. Resveratrol as well as other natural HO-1 inducers are reported to prevent CVD [130]. The beneficial effect of HO-1 induction along with a subsequent increase in adiponectin and EET production is not limited to obesity. The HO-1-TTP signaling pathway has been shown to be effective in: i) treatment of inflammatory diseases [131]; ii) induction of mitochondrial biogenesis [132]; iii) preservation of cardiac function [133]; iv) down regulation of inflammatory response to osteoarthritis [134]; v) decreasing LPS-induced vascular inflammation triggered by bacterial infection; vi) suppression of macrophages migration [135] and v) decreasing contact hypersensitivity [136] and regulators of pregnancy and preeclampsia, meaing it may serve as a valuable therapeutic tool in the management of a momentous health problem [137]. Several studies have indicated that the absence of HO-1 exacerbated ventricle dilation in hypoxia [138], atherosclerosis and vascular remodeling [138]. CoPP-mediated vascular and cardioprotection is well described [139,140] and includes the effect of CO releasing molecules that induce cardioprotection [141]. In HO-1 deficient mice there is a reduction of the proliferative response to vascular injury, while an increase of HO-1 expression inhibited lesion formation [30],

Taken together, these results demonstrate that HO-1 levels determine atherosclerotic lesion progression [142] and that the induction of the HO-1 pathway provides an important adaptive mechanism to reduce the severity of vascular dysfunction, thus representing a potential therapeutic target for vascular diseases. Li et al [143] discovered a previously unrecognized EET pathway to affect or increase hematopoietic stem cell transplant associated with activation of transcriptional factor AP-1. Since AP-1 is present in humans in the HO-1 promoter; this pathway activates HO-1 gene expression [144,145]. HO-1 expression is essential for stem cell differentiation to the osteoblast lineage, consistent with the role of heme and HO-1 in differentiation of hematopoietic stem cell [104–107]. HETE and EET modulate adherent stromal stem cells differentiation [146].

Impact of HO-1 /HO-2 on Hypertension

The biological action of HO-1 and HO-2 gene expression suggests their capacity to participate in the regulation of renal function and blood pressure [147–152]. HO-2 deficiency contributes to a diabetes-mediated increase in superoxide anion and renal dysfunction [39]. Inhibitors of HO activity exacerbate salt-sensitive hypertension in Dahl salt-sensitive rats via inhibition of the pressure-natriuretic response [153]. Inhibition of HO activity can blunt pressure-natriuresis via two mechanisms, with the first being a decrease in renal blood flow, implying that the renal HO system supports renal circulation via formation of CO [154–156]. This hypothesis is supported by the observation that upregulation of HO-1 expression by both CoPP [157] and $SnCl_2$ increases mesenteric artery relaxation in spontaneously hypertensive rats (SHR) and decreases the CYP4A-mediated generation of vasoconstrictors by 20-HETE, and that HO-1-derived CO counterbalances 20-HETE mediated vasoconstriction [158]. HO-1 can also regulate renal tubular function by regulating ROS production in renal tubules and by regulation of renal sodium transporters such as the NKCC2 channel of the thick ascending loop of Henle[159].

HO and Regulation of Lipid-mediators Signaling in Hypertension and Obesity

The cytochrome P450 (CYP) monooxygenases/epoxygenases family is responsible for formation of 20-HETE and EETs [160,161]. Upon formation, EETs are subjected to rapid hydrolysis by epoxide hydrolases and ROS (preventable by HO-1 induction) to their respective dihydroxyepoxytrienoic acids (DHETs), as well as to esterification primarily to glycerophospholipids. Vasodilatory, anti-inflammatory and anti-apoptotic actions of EETs are well established and it is well documented that sEH inhibition significantly increases cellular and circulating EET levels [162,163]. An effect of HETE and EET on stromal adherent stem cells differentiation (i.e., mesenchymal stem cells), was first reported in 1991 [146]. In contrast EET increases adipocyte proliferation but may inhibit differentiation and hypertrophy *in vitro* and *in vivo* [40,109,110,116,164] by reprogramming adipocyte phenotype to increase uncoupling proteins (UCP1) and (UCP2) expression and decrease PPARγ and mesoderm-specific transcript (MEST) [40,109,110,116,164,165]. UCP1 and UCP2 are expressed in adipose tissue that plays 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 [166]. Thus, EET and HO-1 appear 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 [109,110,116]. EETs is the first lipid-mediator derived from lipid metabolism via the CYP system shown to regulate insulin sensitivity and abate obesity associated adipose tissue and vascular dysfunction; an effect which is reversed by the inhibition of HO activity [165].

The mechanism by which EET increases HO-1 could be related to an EET-mediated decrease in Bach1, which is a known supressor of HO-1 gene expression. It is also possible that EETs act as a transcriptional regulator of the HO-1 promoter through glucocorticoid and AP-1 binding sites which are present on the human promoter [144]. These binding sites can activate HO-1 gene expression [144] and subsequently increase HO activity. Recently, a novel EET isomer, 11,12-EET, was reported to promote hematopoietic stem cell transplant by activating a unique activation protein 1 and activation of P13K pathway [143]. This novel pathway of EET activation of HO-1 may lead to clinical application for EETs and drug development.

It is clear that the pleiotropic effect of the HO system and its subsequent signalling mechanisms leads to increases in EETs, adiponectin and NO bioavailability. Activation of EETs can also increase HO activity as outlined above. The antioxidant action of HO metabolites is associated with expansion of small adipocytes which are associated with increased adiponectin and downstream signals that include phosphorylated liver kinase B1 (pLKB1), pAMPK, phosphorylated endothelial nitric oxide synthase (peNOS) and an increase in NO bioavailability [108,110,113,165,167]. Upregulation of these pathways is associated with improvement of vascular function and attenuation of hypertension. It is evident that the pleiotropic effect of the HO system and signalling mechanism [11,51,71,165,168] and increase in biliverdin leads to increases in the protection of EET from degradation by ROS and adiponectin. These results establish the interdependence of four protective pathways, namely HO, EETs, bilirubin and adiponectin, all of which are affected by HO activity and result in the prevention of obesity, hypertension and insulin resistance. Activation of these pathways also protects the vasculature from injury which is known to increase organ dysfunction and vascular diseases (Figure 1 and 4).

HO-1/HO-2 and Health Impact

Obesity is a critical risk factor for endothelial dysfunction and the subsequent development of diabetes mellitus and vascular diseases including hypertension. Abdominal obesity is associated with insulin resistance and the pathogenesis of T2DM and hypertension, contributes to high serum levels of LDL and triglycerides but low serum levels of HDL, and leads to the development of atherosclerotic CVD [169–171]. HO-1-mediated decreases ROS and of LDL were reported in a number of diabetes models [113,172]. Leptin-deficient mice or mice fed a high-fat diet exhibit a metabolic syndrome-like phenotype which includes an increase in LDL which is amenable to rescue by increases in HO-1 and adiponectin [113]. Chronic HO-1 induction increased oxygen consumption, and lowered body weight in obese melanocortin-4 receptor deficient mice with an improvement in vascular function [86,173]. Induction of HO-1 by several inducers such L-4F, CoPP, heme, or by gene

transfer [4,113,114,172,174–176] is associated with an increased number of healthy adipocytes, a concomitant increase of plasma adiponectin levels, improved insulin sensitivity and a decrease in inflammatory adipokines and blood pressure [20,24,115,172,174,175,177]. This effect of HO-1 induction on adipocyte morphology was confirmed in Zucker diabetic rats [175] and extended to ob/ob diabetic mice, where increased levels of HO-1 and HO activity prevented weight gain and decreased visceral and subcutaneous fat levels. Additional studies have reported that upregulation of HO-1 decreases adipogenesis in mesenchymal stem cells (MSCs) and increases adiponectin levels in culture media, which is reversed by the inhibition of HO activity [4,114]. These studies confirm the existence of an HO-1-adiponectin-EET regulatory module that can be manipulated to ameliorate the deleterious effects of obesity, diabetes and metabolic syndrome. This offers a portal into the therapeutic benefits of the upregulation of HO-1 and increase in HO activity. Chronic HO-1 induction also increases oxygen consumption, which is another mechanism by which HO-1 induction lowers body weight. This effect on oxygen consumption is independent of the melanocortin-4 system as chronic treatment of obese melanocortin-4 receptor deficient mice results in the attenuation of obesity and type II diabetes [86,173]. The effect of HO-1 induction of oxygen consumption is likely mediated through increases in CO release as chronic treatment with a CORM increases oxygen consumption and attenuates obesity in mice fed a high fat diet [82].

While the general induction of HO-1 has been reported to result in improved insulin sensitivity, downregulation of the peripheral endocannabinoid system, and a reduction in adipose tissue volume and adipose tissue remodeling, there have been some sex dependent differences reported [113,115]. Adipocyte HO-1 induction by CoPP, L-4F or VECAD-HO-1 attenuated metabolic syndrome in both obese male and female mice although the rate of weight gain was slowed only in obese male. [113,114,178]. These results emphasize that gender differences must be considered in the development of therapeutic approaches targeting induction of HO-1 for the treatment of obesity and diabetes [113].

Another approach which may be beneficial for the treatment of obesity is adipose-specific induction of HO-1. Recently, it was reported that induction of HO-1 in adipocytes was able to reverse the detrimental metabolic consequences of obesity, including insulin resistance and dyslipidemia as well as decreasing blood pressure in a mouse model of obesity [113]. These studies further highlight the protective cardiovascular role of the HO-1-adiponectin axis in hypertensive animals [172]. While adipose specific targeting of the HO-1 gene was successful in attenuating adiposity, vascular dysfunction and hypertension in mice fed a high-fat diet, others reported that HO-1 overexpression in adipocytes does not protect against a high-fat diet induced obesity and the development of insulin resistance [179] These differences in phenotypes reported between these two studies are not clear, although the specific activity of HO in the adipose tissue of the transgenic model was not reported. The importance of measuring not only HO-1 protein levels but also HO activity was highlighted in a study in ZDF [20,21]. In this study, the onset of diabetes coincided with an increase of HO-1 protein and a paradoxal decrease in HO activity [20], most likely due to an increase of peroxynitrite [20,21] which results in an inactivation of HO activity.

Recently, HO-1 overexpression has been shown to ameliorate the development of non-alcoholic fatty liver disease in obese leptin deficient mice via a decrease in hepatic heme [22]. In addition, HO-1 induction decreased hepatic lipid droplet size, fatty acid synthase levels, PPARα and glucose transporter 1 and all of these beneficial effects were reversed by inhibition of HO activity indicating that low levels of HO-1 and HO activity exacerbate the development of obesity induced fatty liver [22]. As in 3T3 adipocyte cells, an increase in heme increases adipogenesis [12,13] without upregulation of HO-1 [13]; however, chronic induction of HO-1 decreases adipocyte heme which in turn decreases adipogenesis [110]. This decrease in adipogenesis is also associated with an increase in the levels of CYP-epoxygenase-derived EETs and adiponectin [164].

A number of clinical studies have examined the relationship between HO-1 and obesity. CD163 expression was upregulated in human adipose tissue and soluble CD163 concentration was elevated in obese (BMI > 40 Kg m^{-2}) compared to lean subjects (BMI < 30 Kg M^{-2}). The HO-1 gene was upregulated in adipose tissue and expressed predominantly in macrophages [180,181] and in fat tissues [164]. Similarly, diminished upregulation of visceral adipose HO-1 correlates with waist-to-hip ratio and insulin resistance [182]. Visceral adipose tissue expression of HMOX1 negatively correlated with insulin resistance [182]. Morbid obesity is associated with thrombophilia. Adipocytes obtained from obese patients have increased HO activity as nonsmoking bariatric patients increased COHb concentrations, indicative of HO-1 upregulation [183]. Assessment of HO activity by measuring CO production may yield conflicting result unless adequate steps are taken to differentiate between HO-dependent and HO-independent CO generation independent [74,92,184]. Rodgers et al [184], described increased CO formation in an HO-independent fashion as a result of photo-oxidation most commonly observed via the peroxidation of lipids along with the auto-oxidation of organic molecules such as phenols and flavenoids as a result of severe stress [184]. These differences in HO dependent versus HO independent CO generation should be carefully taken into consideration when interpreting the results of studies in which CO production is measured as an index of HO activity.

Therapeutic Potential of HO-1 and Signaling Pathways

HO-1, as the only enzyme that degrades the pro-oxidant heme and generates antioxidant products, may be a beneficial target to limit the pathogenesis of obesity and diabetes and their complications (Figure 5). In diabetes, increased levels of HO-1 provide vascular cytoprotection against oxidative stress via mechanism(s) that involve improvements in mitochondrial biogenesis and function [127,185]. Cardiac mitochondrial damage, such as that seen in type 1 diabetes mellitus (TD1M), is the result of a decrease in glutathione levels which negatively impact the mitochondrial respiration system [186]. A deficiency in the deoxynucleotide carrier has been associated with abnormal brain growth, and a deficiency in carnitine-acylcarnitine linked to muscle weakness and cardiomyopathy in diabetes. Diabetic complications have also been related to abnormalities in mitochondrial function [186,187] as well as increased endothelial cell death and detachment [188]. Furthermore, translocation of HO-1 into mitochondria [185,189,190] suggests that targeting of HO-1 specifically may prove to be essential in the modulation of the redox state in favor of antioxidants, enhancing mitochondrial transport of substrates and metabolites and restoring mitochondrial function

in diabetes [185]. Induction of HO-1 has been reported to result in the restoration of six mitochondrial carriers, i.e., carnitine, citrate, phosphate, deoxynucleotide, ATP and dicarboxylate in diabetes [185]. An increase in AKT phosphorylation is also critical to cell survival in diabetes [191]. The alteration in mitochondrial function *in vitro* and *in vivo* has been correlated with the levels of activation of AKT and the BcL-2 family of proteins [192,193]. A decrease in BcL-2 family members has been suggested to contribute to apoptosis and the translocation of cytochrome c from the mitochondria to cytosol [191,192,194]. Activation of AKT has been reported to augment ATP synthesis [195] and promote association of hexokinase with the voltage-dependent anion channel (VDAC) channel and, in so doing, results in VDAC closure which blocks release of cytochrome c. The lack of HO-1 or HO-2 results in increased apoptotic cell death [19,38,196]. While these results suggest that increases in mitochondrial HO-1 may favorably modulate the balance between pro-and anti-apoptotic mechanisms, the clinical applicability of targeting either HO-1 or its metabolites, bilirubin and CO, specifically to the mitochondria has not been tested as therapeutic approach for the treatment of diabetes, although pre-clinical results support such a clinical application [197].

It is of interest that beta cell destruction caused by elevated intracellular levels of ROS, including superoxide radicals, hydrogen peroxide and nitric oxide, is a process that occurs through both apoptotic and necrotic mechanisms [198]. T cell-mediated infiltration of the pancreas leads to ROS generation and proinflammatory cytokines. The HO system regulates T cell proliferation and immune response [199,200]. $CD4^+$ T cells express HO-1 in response to CoPP, and that the lack of HO-1 modulates T cell proliferation and maturation [201,202]. An increase in HO-1 activity resulted in a decrease in infiltrated CD11c+ dendritic cells, and suggested that induction of HO-1 and increased HO activity can prevent the development and/or moderate the diabetic state [14]. HO-1 upregulation has proven capable of providing cytoprotection to pancreatic beta cells *in vivo* [203,204]. An increase in HO-1 levels has a salutary effect, modulating the pancreas phenotype and rendering beta cells resistant to oxidant stress and, hence, preventing the development of type 1 diabetes.

A protective effect is also seen in diabetes where insulin induces HO-1 through the pI3K/Akt pathway and the Nrf2 transcription factor in renal cells [205]. HO-2 deficiency in diabetic HO-2 knockout mice caused major renal morphological injury and impaired renal function that was rescued by upregulation of HO-1 in the STZ animal model of diabetes [39].

The significant role that HO-1 plays in obesity/diabetes stems from the presence of binding sites for several transcriptional factors including CRE B, OKT1, STATS and glucocorticoid-response elements that are expressed on the human HO-1 promoter [144,145]. Targeting HO-1 or the products of heme degradation stems from the finding that induction of HO-1 increases oxygen consumption, heat production and lowers body weight [173]. While upregulation of HO-1 reduces body weight in obese animals, it also is associated with a decrease in the levels of adipokines including TNF, IL-6, MCP-1 and an increase of adipocyte secretion of adiponectin. Adiponectin is secreted only by adipose tissue and is associated with decreased body weight [206–209], suggesting that the HO-1-mediated increase bilirubin and the number of smaller healthy adipocyte capable of secreting adiponectin is related to the effect of adiponectin on body weight loss and attenuation of inflammatory process [206–209].

HO-1 Genetic Polymorphism and its Impact on Atherosclerosis and CVD

Genetic polymorphism of the HO-1 gene indicates the potential importance of HO-1 in the pathogenesis of cardiovascular and pulmonary diseases [210]. The larger the size of the (GT)n repeats in the HO-1 gene promoter, the greater the chance of reducing HO-1 indelibility by ROS in cigarette smoke and reducing bilirubin, resulting in the development of emphysema [210]. Patients with short (<25 GT) dinucleotide repeats in the HO-1 gene promoter on either allele had restenosis significantly less often that patients with longer (≥25 GT) dinucleotide repeats [211]. Diabetic patients who have Gilbert syndrome, elevated levels of bilirubin, have a lower rate of vascular complications, compared to normal bilirubin levels seen in diabetes [212]. It is believed that shorter (GT)n repeats, compared to longer (GT)n repeats, have a higher transcriptional activity and thus higher HO-1 expression levels. In an Asian population, type-2 diabetics carrying longer (≥32) (GT)n repeats had higher oxidative stress and increased susceptibility to the development of coronary artery disease and atherosclerosis [213]. Patients with significant risk factors (hyperlipidemia, diabetes, and smoking) for coronary artery disease and who possessed shorter (<27) (GT)n repeats were associated with less disease [214]. A cohort study in patients to evaluate HO-1 gene promoter polymorphisms and the risk for restenosis after percutaneous transluminal angioplasty found that patients with short (<25 GT) dinucleotide repeats in the HO-1 gene promoter on either allele had restenosis significantly less often that patients with longer (≥25 GT) dinucleotide repeats [215]. These data imply that up-regulation of HO-1, associated with shorter dinucleotide repeats, may be protective after balloon angioplasty. However, not all studies support the clinical effect of genetic polymorphism, for example, in a study of 1807 patients with coronary artery disease, no clinically relevant association of a HO-promoter polymorphism and ischemic events after coronary stenting was reported [216]. In support of this finding, no evidence of a protective effect for short alleles, i.e., low (GT)n repeat, for graft or recipient survival in clinical renal transplant was seen [217]. In addition to (GT)n dinucleotide-length polymorphism, a single nucleotide polymorphism in the HO-1 promoter, T(-413)A, correlated with a reduced incidence of ischemic heart disease [218]. In a study of 3,104 patients with vascular disease, restenosis after percutaneous coronary intervention was associated with angiotensin II-type l receptor 116 A/C polymorphism but was not associated with polymorphism of HO-1 [219]. These studies both advocate and/or contradict the role of the HO-1 gene in genetic polymorphism and atherosclerotic processes. Recently, in an elegant well designed study to investigate the role of genetic polymorphism of HO-1 in CVD, the authors focused on the incidence of stroke, MI and vascular death of patients registered between 1995 and 2010. In more than 800 patients aged between 45–84 years [220], there was an association between the HO-1 variable number tandem repeat polymorphism and CVD confined to subjects with a high number of repeats on both HO-1 alleles, providing evidence of atherogenesis and decreased antioxidant defense system in vascular high risk subjects. These studies support an important role of HO-1 gene regulation in the atherosclerotic disease processes.

A new approach would be to examine whether increasing HO-1 expression via either pharmaceutical or genetic agents has the potential to correct for the GT repeat leading to low expression, or whether introducing anti-oxidative agents may correct for the increased

oxidative stress caused by low HO-1 expression. Genetic testing may also have a role in identifying patients with HO-1 polymorphisms for which HO-1 based therapies could have a corrective effect.

Concluding Remarks

The pharmaceutical industry may be interested in finding novel drugs with the potential to attenuate adiposity and reprogram adipocyte stem cells to new phenotypes to express HO-1 or HO-1 downstream targets (see Outstanding Questions). A potential inducer of bilirubin and HO-1 is the peptide derived from the human biliverdin reductase protein [221]. This peptide, as well as the L-4F (ApoA-1 mimetic) peptide, could have a powerful effect on the induction of HO-1, with a reduction of fatty liver insulin resistance and adiposity. Selective expression of the HO-1 gene has also been identified by numerous sources as a viable method of stimulating HO-1 gene expression [10,47,141,152,178,222–224]. Another novel dimension of targeting the bilirubin/biliverdin pathway includes the development of meso-biliverdin as an anti-obesity and anti-vascular disease agent [67,225], as well as the development of a CO-releasing molecule for preconditioning the heart in MI or in the protection of pancreatic function [67]. Niacin has additionally been shown to induce the HO-1 gene to counter cardiovascular disease [226] and may have a beneficial effect on obesity-induced hypertension. Further, certain statins have activated the HO-1 promoter in mouse pre-adipocytes, preventing adipocyte differentiation, suggesting this class of drugs can combat adiposity and adipogenesis [227]. Additionally, analogs of EET targeting β-catenin/SIRT1 will represent novel small molecules enhancing bilirubin downstream signaling to prevent pre-adipocyte full differentiation and inflammation and protect against obesity and diabetes.

The wide spectrum of inducers of HO-1 highlights the pivotal role this enzyme plays in providing protection against metabolic insults in humans. This offers an obvious target for designing compounds with clinical application in multiple human disease states.

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Trend Box

- Obesity is a major risk factor in the development of diabetes, hypertension, fatty liver and CVD.
- HO-1/HO-2 catalyzes the breakdown of heme, a potentially harmful pro-oxidant, into potent anti-oxidants such as biliverdin/bilirubin and CO, with an anti-inflammatory effect.
- This is the first review to discuss translational research that summarizes human genetic polymorphism of HO-1 and the effectiveness of bilirubin to ameliorate CVD.
- This review uncovers a mechanistic link between obesity and the vascular system and provides a conceptual basis for developing new drugs for the management of metabolic syndrome.
- We provide a conceptual basis for the development of new therapeutic strategies that target HO-1 and biliverdin to ameliorate obesity, adipocyte (fat stem cell) dysfunction and, vascular dysfunction associated with the metabolic syndrome.

Outstanding Questions

- Is obesity is the hallmark of vascular disease?
- Can drugs correct HO-1 genetic polymorphisms resulting in low expression?
- Do HO-1 inducing drugs affect people uniformly?
- Can genetic testing identify individuals that would be helped by HO-1 inducing drugs?
- Are there side effects to HO-1 inducing drugs?

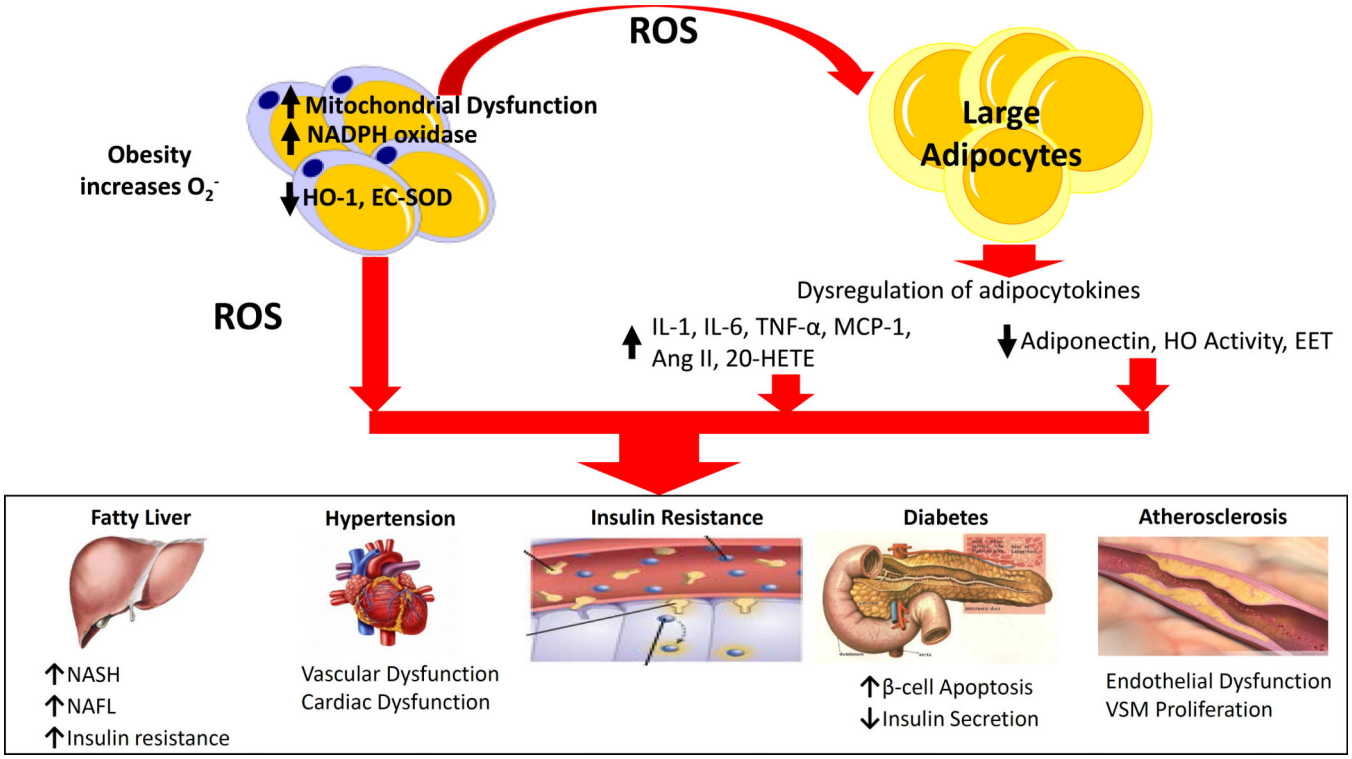

Figure 1.

Obesity increases risk for cardiovascular disease. Obesity leads to an increase in ROS within adipocytes, accomplished by increasing NADPH oxidase activity, mitochondrial ROS production, and heme levels while repressing antioxidative enzymes such as HO-1 and SOD. This increase in adipocyte ROS and heme leads to increased adipocyte differentiation, maturation, resulting in increased production of proinflammatory compounds such as cytokines and decreased production of antioxidative compounds and compounds preventing adipocyte growth and differentiation. The consequences of obesity-mediated adipocyte dysfunction may lead to vascular dysfunction which is a prelude to vascular disease and hypertension.

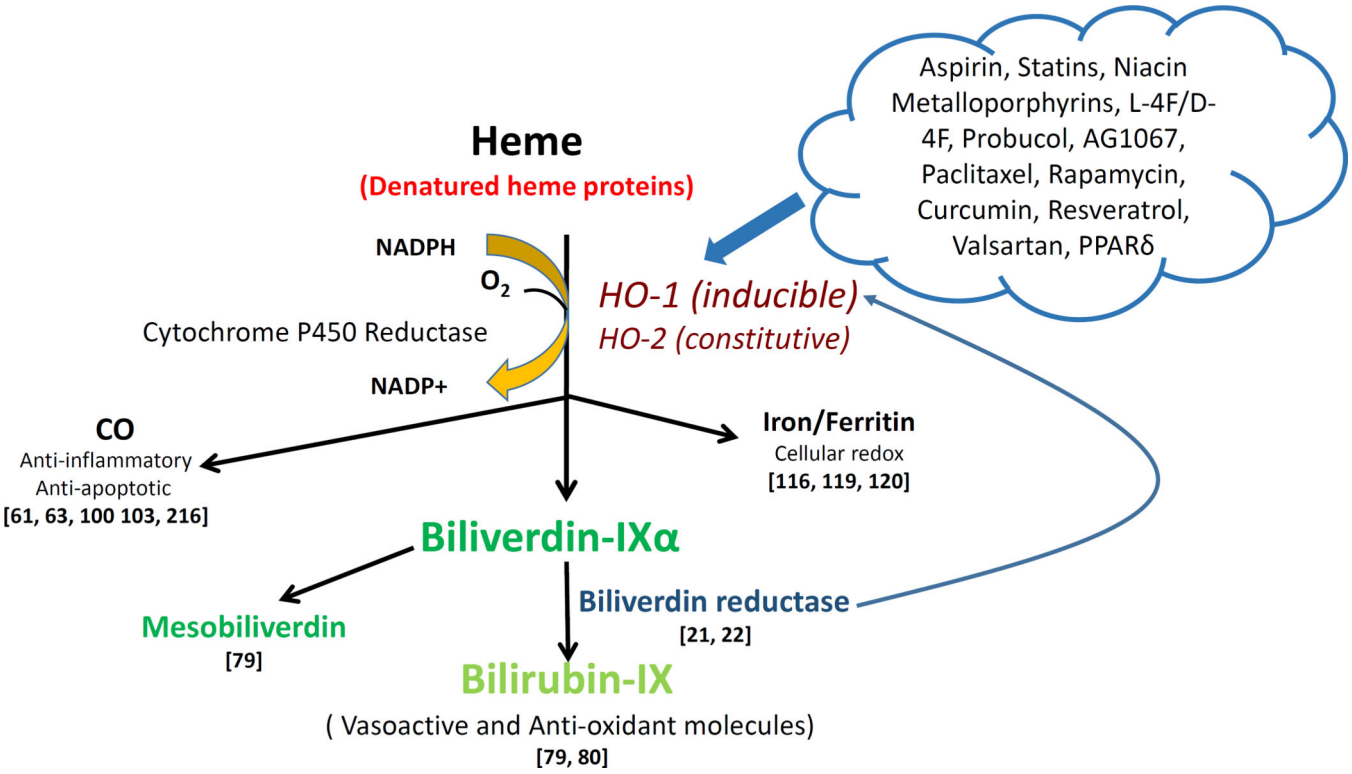

Figure 2.

Drug actions in the heme degradation pathway. HO-1 (inducible) and HO-2 (constitutive) cleave free heme or denatured heme proteins to generate CO, ferritin, and biliverdin, which is subsequently converted to bilirubin by biliverdin reductase. CO has both anti-inflammatory and anti-apoptotic properties [55,57,57,59,171] Ferritin is essential for cellular redox reactions [99,100,102] Serum bilirubin levels are positively linked with a decreased risk of CVD and protection against diabetes and vascular dysfunction [67,68]. Drugs focused on the heme degradation pathway predominantly induce HO-1 activity, possibly by interacting with the gene promoter. Biliverdin reductase functions via a direct increase of HO-1 or an increase in bilirubin levels to promote a reduction in oxidative species [26,27]. And mesobiliverdin enhances β-cell function in the pancreas through its antioxidant properties [67] This pathway provides the basis for multiple pharmaceutical and genetic agents that can protect against CVD by increasing HO-1 expression.

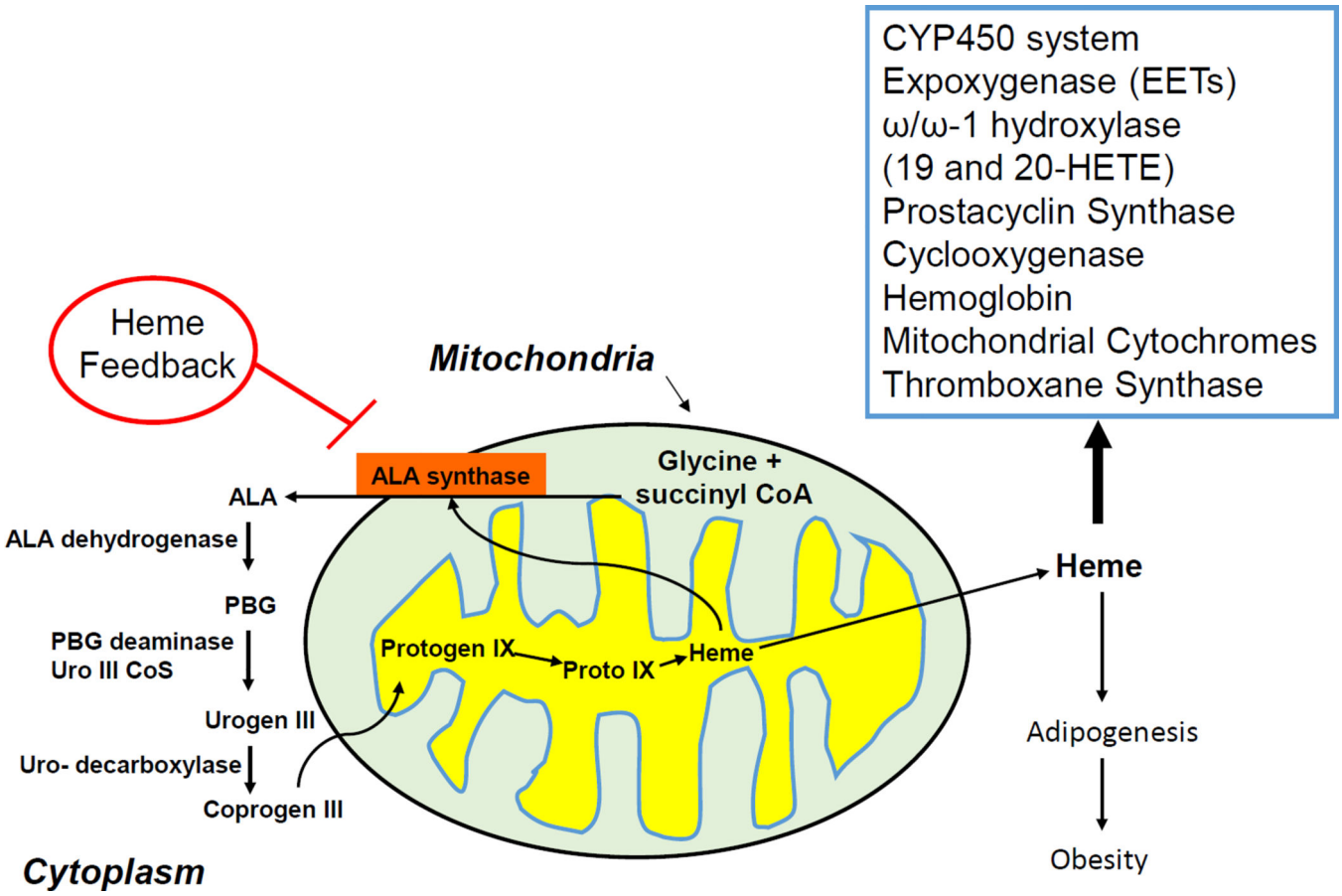

Figure 3.

Heme synthethic pathway. The rate-limiting synthetic enzymes are believed to be ALA synthase and, in part, porphobilinogen deaminase (PBGD). Both enzymes exist in adipocytes and in erythroid and non-erythroid forms. In non-erythroid cells such as liver, kidney, heart, ALA synthase essentially plays a housekeeping role, maintaining intracellular heme levels. High levels of heme thus repress the synthesis of ALA synthase while stimulating heme degradation through the induction of HO-1 [12,228]. In the origin of hematopoietic-derived cells such as adipocyte and erythroid cells, heme is essential for cellular proliferation and differentiation [12,14,14,228] and increase ALA synthase mRNA levels and enzyme activity. Further, excess heme enhances the synthesis of globin mRNA [14] An iron-binding element has been located on the 5' untranslated region of the erythroid type cells ALA synthase, so it is possible that the enzyme is actually regulated by intracellular levels of iron. Thus, an increase in heme may induce HO, increasing the levels of free iron which in turn stimulate the formation of adipocyte ALA synthase mRNA and adipocyte during differentiations.

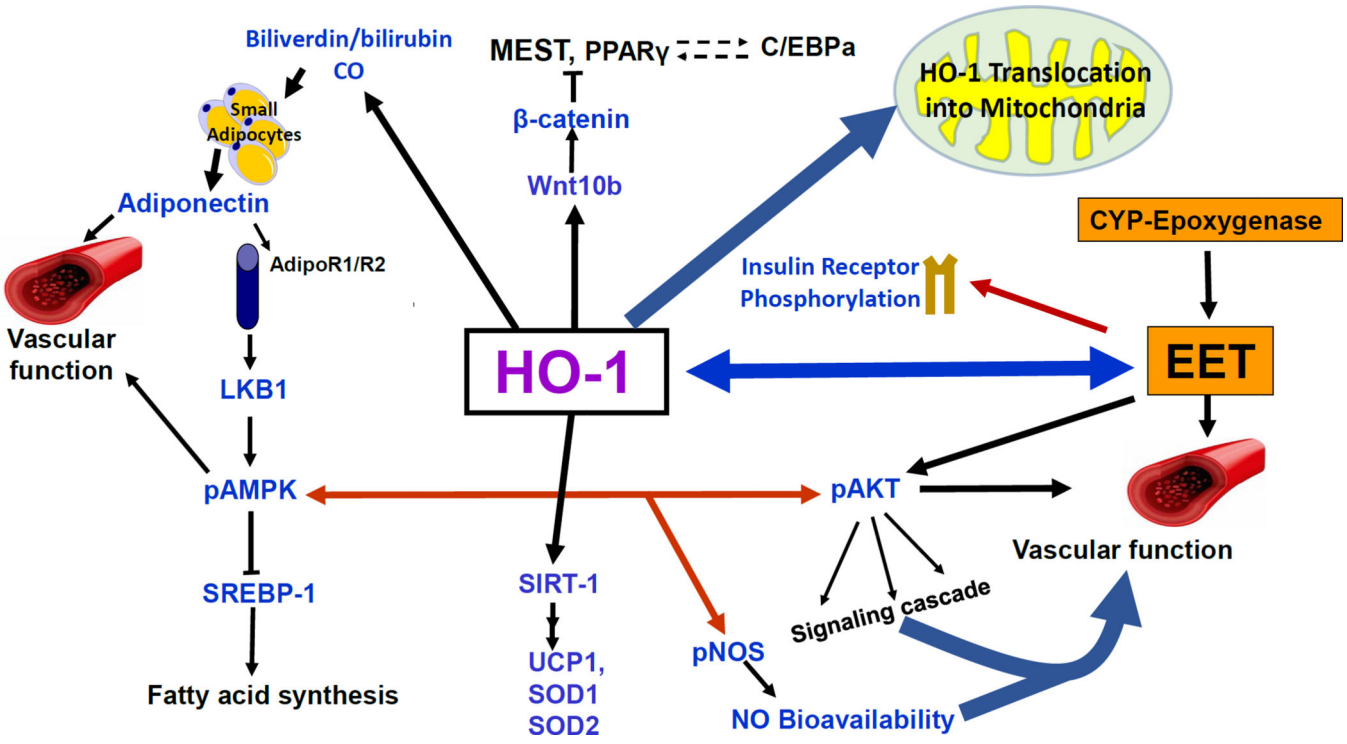

Figure 4.

Schematic representation of the potential mechanism of HO-1 signaling pathways. HO-1 signaling pathways act to improve vascular function and attenuate adiposity adipocyte differentiation. Some of these signaling targets are insulin receptors, adiponectin, via an increase in small adipocytes, EET, SIRT-1, Wnt10b, and β-catenin. The decrease in ROS as a result of an increase of HO-1 and HO-1 derived biliverdin/bilirubin provides stability to EET, leading to an enhancement of insulin sensitivity and an increase in vascular function. HO-1 also translocates into the mitochondria, increasing mitochondrial biogenesis and transport carriers and decreasing mitochondrial ROS [185,189,190].

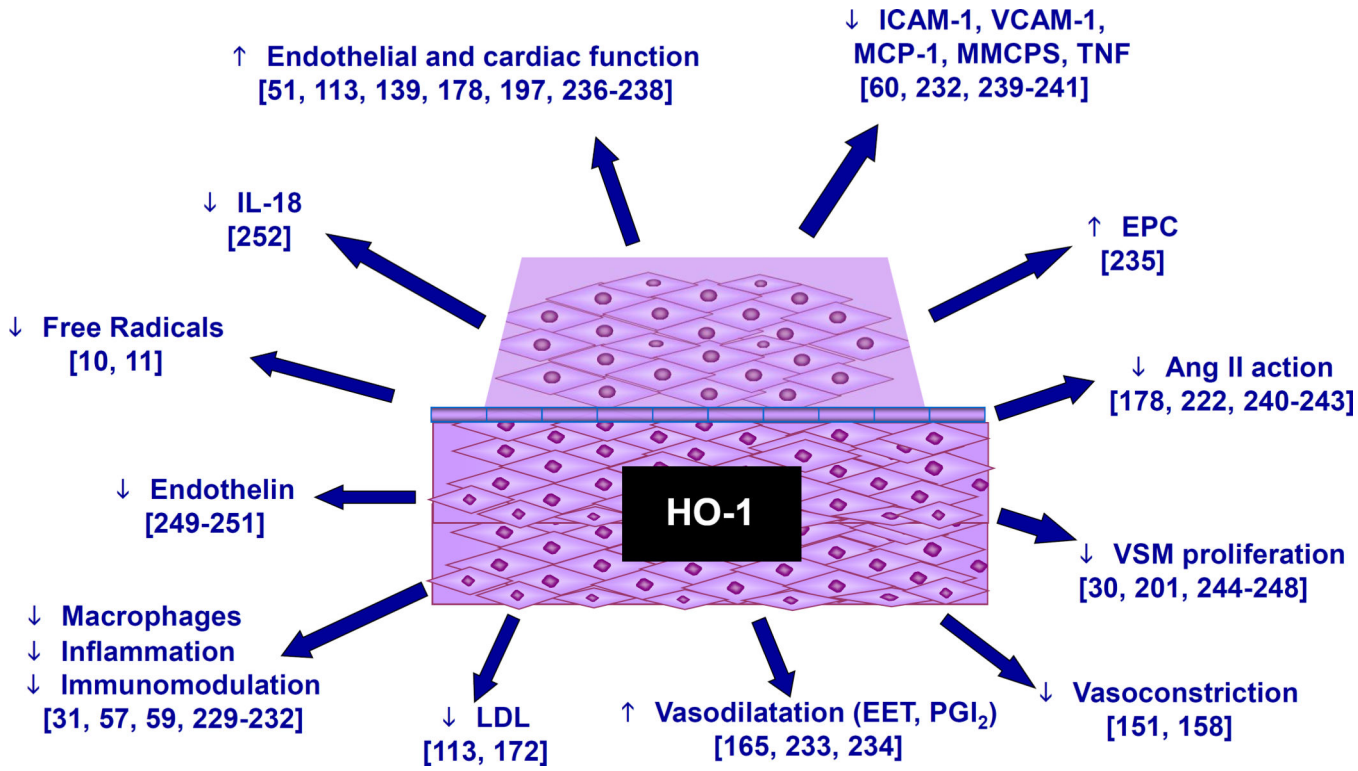

Figure 5.

Pleiotropic effect of HO-1 on obesity and CVD. HO-1 has a diverse range of actions on blood vessel endothelial tissue, decreasing inflammation [31,57,59,229–232] and vasoconstriction [151,158] while increasing vasodilation [165,233,234], endothelial progenitor cells [235] and endothelial and cardiac cell function [51,113,139,178,197,236–238]. As seen on the scheme, HO-1, bilirubin, and CO decrease pro-inflammatory molecules [60,232,239–241], angiotensin II [178,222,240–243], free radicals [10,11], VSM proliferation [30,201,244–248], LDL [113,172], endothelin [249–251], and IL-18 [252]. The overall effect is the amelioration of cardiovascular disease and protection against the development of a disease state.