

Heme Oxygenase: A Target Gene for Anti-Diabetic and Obesity

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Abstract: Heme oxygenase-1 (HO-1) is central to the regulation of oxidative injury. The role of increased HO-1 expression and Heme oxygenase (HO) activity in mitigating the detrimental side effect of diabetes is examined. A review of the mechanism(s) of action is included. This may lead to the development of pharmacological and genetic approaches to mitigate the clinical complications associated with the progression of diabetes and obesity.

Key Words: Diabetes, Carbon monoxide, bilirubin, HO-1, Inflammation, TNF, IL-1, adiponectin.

INTRODUCTION

Hyperglycemia, a major cause of kidney disease, results in hypertension and the risk of developing diabetic neuropathy. Hyperglycemia, defined as elevated levels of serum glucose, produces oxidative stress through the increased generation of reactive oxygen species (ROS) leading to the derangement of cell physiology in diabetes. In addition it plays a critical role in the pathogenesis of diabetic complications including cell survival in animal models of diabetes. Impairment of vascular responses due to the formation of superoxide anion radical (O_2^-) represents the major contributor to vascular injury and the clinical complications of diabetes [1]. The perturbations in heme metabolism resulting in increased expression of heme oxygenase(HO)-1 and increased production of carbon monoxide (CO), iron and biliverdin/bilirubin that occur and their role in the regulation of oxidative stress and cell survival will be examined in detail and its relation to obesity and metabolic syndrome.

In Type 1 diabetes, insulin deficiency provokes high blood glucose levels and alterations in lipid metabolism. The evolution of this disease may be associated with the development of premature micro- and macrovascular complications; the pathogenesis of which may be linked to oxidative stress [2-5]. Increased ROS generation may contribute to beta cell damage and vascular dysfunction through various mechanisms [5-7]. In diabetic children, puberty may trigger microvascular complications that may later be the major cause of tissue damage, disability and death. Although the mechanism of glucose toxicity is unknown, recent *in vitro* and whole animal studies have implicated ROS, which promote the formation of cytotoxic lipid peroxides [8-11]. The beta cell destruction by ROS, whether induced by oxidants given exogenously or elicited by cytokines, is a process that occurs through changes in the apoptotic and antiapoptotic balance [12-16]. There appears to be an intrinsic cardiovascular sensitivity to oxidative stress in diabetic rats and in nonobese diabetic (NOD) mice, a property that may extend to human patients.

Type 2 diabetes mellitus is a common disorder, characterized by hyperglycemia, insulin resistance and relative impairment in insulin secretion. Over seven percent of adults in the United States are known to have diabetes and the number continues to rise every year [17]. The spectacular increase in prevalence of type 2 diabetes in the past decade, in large part is linked to the trends in obesity and physical inactivity [18]. Abdominal obesity, in particular, is associated with resistance to the effects of insulin on peripheral glucose and fatty acid utilization. Insulin resistance plays a major role in the pathogenesis of type 2 diabetes and is often accompanied by other

conditions, including hypertension, high serum low-density-lipoprotein (LDL), low serum high-density-lipoprotein (HDL) and high serum triglyceride levels, which promote the development of atherosclerotic cardiovascular disease [19]. The majority of patients with diabetes die of cardiovascular events and controlling risk factors leading to atherosclerosis is a major challenge in clinical practice [20].

Oxidative stress has been implicated in the pathogenesis of insulin resistance, type 2 diabetes and its cardiovascular complications [21,22]. Excessive generation of ROS is the underlying mechanism of endothelium injury, resulting in an accelerated rate of apoptosis and endothelial cell sloughing [23,24]. Overproduction of ROS in the vascular wall enhances endothelial nitric oxide (NO) and endothelial nitric oxide synthase (eNOS) degradation and promotes peroxynitrite formation which results in a preferential increase in O_2^- production. This results in platelet aggregation, release of growth factors in the vessel wall, vasoconstriction and endothelial dysfunction [25,26].

Adipose tissue plays an important role in insulin resistance through the production and secretion of a variety of proteins, including tumor necrosis factor-alpha (TNF- α), interleukin(IL)-6, plasminogen activator inhibitor-1, monocyte chemoattractant protein-1, resistin, proteins of the renin-angiotensin system, leptin and adiponectin [27]. Of these proteins, adiponectin has recently attracted much attention, as it has insulin-sensitizing properties that enhance fatty acid oxidation and glucose uptake in muscle [28] [29]. Adiponectin is exclusively secreted from adipose tissue and its expression is higher in subcutaneous than visceral adipose tissue [30]. It circulates in the blood at very high concentrations and is found as low-molecular weight (LMW) oligomers, and high-molecular weight (HMW) multimers [31]. Several studies suggest that HMW adiponectin is more active and correlates more significantly with glucose and insulin levels than LMW and even total adiponectin [32]. Low plasma HMW adiponectin levels have been consistently associated with obesity, insulin resistance, type 2 diabetes and coronary artery disease [33,34]. Recent data have revealed that adiponectin possesses a vascular protective role, preserving endothelial cell function in diabetic and non-diabetic patients with the metabolic syndrome [35-37]. In addition to modulating atherogenesis through its insulin-sensitizing actions, it also has direct anti-atherogenic effects on the arterial wall. These effects are mediated through inhibition of TNF- α -mediated adhesion of monocytes and vascular cell adhesion molecules to the endothelium, modulating the endothelial inflammatory response. Furthermore, adiponectin decreases cytokine production in macrophages through blocking the nuclear factor κ B (NF- κ B) pathway resulting in decreased transformation of macrophages into foam cells [38-40] and it also improves the beneficial effects of antihypertensive agents in

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hypertensive patients [41]. Adiponectin increases NO production in cultured endothelial cells *in vitro* and *in vivo* [37,42].

Adiponectin could be a novel therapeutic tool for diabetes, the metabolic syndrome and cardiovascular disease. Upregulation of plasma adiponectin is a partial mechanism of the thiazolidinedione (TZD) class of antidiabetic drugs, which have insulin-sensitizing actions [29]. TZDs increase glucose disposal in skeletal muscle and decrease hepatic gluconeogenesis. They are widely used for the treatment of type 2 diabetes. TZDs are presumed to directly affect adipose tissue and increase adiponectin levels. However, it is not clear to what extent the efficacy of these drugs is attributed to adiponectin. TZDs are potent peroxisome proliferators-activated receptor (PPAR) – γ agonists. They bind PPAR γ in adipose tissue, thus promoting adipocyte differentiation and increasing the number of small insulin-sensitive adipocytes, which abundantly express and secrete adiponectin and decrease serum free fatty acid levels and TNF- α expression [28, 29]. The PPAR γ response element found on the promoter sequence for the adiponectin gene, forms a functional heterodimer with retinoid X receptor and bind to the adiponectin promoter region, resulting in promoter activity, increasing plasma adiponectin levels [43]. PPAR γ receptors also regulate the expression of HO-1 in human vascular cells [44].

The heme oxygenase system provides antioxidant and anti-apoptotic properties due to its product activity, bilirubin/biliverdin and carbon monoxide [45]. HO-1 is induced by oxidant stress and plays a crucial role in protection against oxidative insult in diabetes and cardiovascular disease [1]. Upregulation of HO-1 gene expression prevents vascular dysfunction and endothelial cell death and increased ROS levels [46,47]. Recently, L'Abbate *et al.* have shown that induction of HO-1 was associated with a parallel increase in the serum levels of adiponectin, which has anti-inflammatory properties [48]. Adiponectin has been ascribed antioxidant properties [49]; it also improves endothelial function in non-diabetic patients with the metabolic syndrome [36]. These observations serve to define some of the key mechanisms by which HO-1 is involved in diabetes and the metabolic syndrome association with increased insulin resistance and inflammatory cytokines release (Fig. 1). These could offer a possible approach as to how these mechanisms might be therapeutically manipulated.

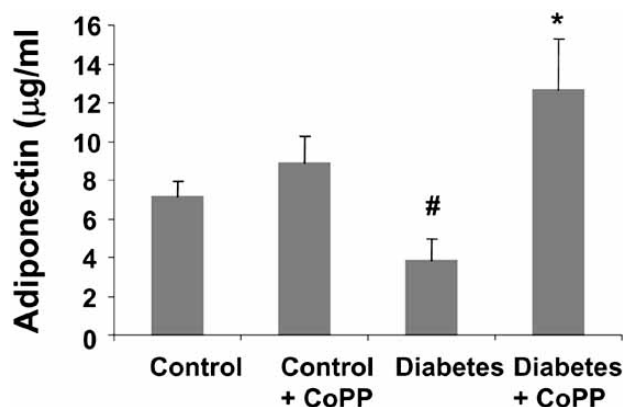


Fig. (1). Measurement of serum adiponectin levels in control and diabetic animals before and after CoPP treatment. Results are mean \pm SE; n=4. #= p <0.05 diabetic vs. control rats; *= p <0.01 Diabetic-CoPP vs. untreated diabetic animals [48].

Levels of HO-1 expression were measured in retinal pigment epithelium (RPE) cells of eyes from both normal and diabetic patients and a decreased expression of HO-1 in the RPE of diabetic patients compared to controls was reported leading the authors to conclude that this could contribute to the vulnerability of the neuroretina to the significant metabolic insult that is encountered in the diabetic state [50]. Bruce *et al.* [51] have reported a decrease in

HO-1 expression in type 2 diabetes. This has been confirmed in a clinical study that examined the leukocytes of patients with type 2 diabetes and reported a decrease in HO-1 gene expression. This was associated with increased NADPH oxidase expression and activity [52]. The psychosocial, neuroendocrine and socioeconomic aspects of type 2 diabetes have been summarized by Abraham *et al.* [53].

In a streptozotocin (STZ) model of type 1 diabetes, HO activity and HO-1 were decreased in the aorta of diabetic animals compared to controls. High glucose levels decreased HO-1 promoter activity. In addition hyperglycemia was augmented by HO inhibition and decreased by upregulation of HO-1 with cobalt protoporphyrin IX dichloride (CoPP). Circulating endothelial cells were higher in diabetes and could be decreased by manipulating HO activity with the use of CoPP and tin mesoporphyrin IX dichloride (SnMP), respectively [54]. These results were confirmed by the demonstration that overexpression of the human HO-1 gene attenuated endothelial cell sloughing in the STZ animal model [47]. This work was extended to show that the inducers of HO-1, apolipoprotein mimetic peptide (D-4F) and CoPP, decreased endothelial sloughing in animal models of diabetes. This was associated with the restoration of extracellular superoxide dismutase (EC-SOD) levels by D-4F, levels that are low in diabetes [24]. With CoPP treatment the increase in EC-SOD was confirmed and, in addition, catalase and eNOS levels were increased (Fig. 2) with a concomitant increase in endothelial relaxation and a decrease in O_2^- [55]. Recently it has been reported that increased HO-1 gene expression increases both vascular relaxation and inducible nitric oxide synthase (iNOS) in diabetic animals [56] and that increased levels of CO resulting from the use of carbon monoxide releasing molecule (CORM) CORM-3 improve vascular reactivity [57]. These results indicate the crucial role of HO-1 in protecting against the deleterious effects of hyperglycemia.

The experimental basis for chronic oxidative stress as an underlying mechanism for glucose toxicity in beta cells has been examined and shown to be a major problem in diabetes [21,58]. Hyperglycemia-mediated local formation of ROS is considered to be a major contributing factor to endothelial dysfunction, including endothelial cell apoptosis abnormalities in cell cycling due to lack of HO-1 induction and increase in O_2^- formation [59] and delayed replication [60]. Some of these dysfunctions can be reversed by antioxidant agents or by increased expression of antioxidant enzymes [61]. A reduction in antioxidant reserves has been related to endothelial cell dysfunction in diabetes [6,60]. Increased levels of HO-1 as a result of gene transfer in hyperglycemic rats results in a decrease of endothelial cell sloughing [47]. Sacerdoti *et al.* [62] have shown that delivery of the human HO-1 gene to endothelial cells attenuated glucose-mediated oxidative stress, DNA damage and cell death. The ability of increased levels of HO activity to attenuate the production of ROS is attributed to its ability to degrade heme to bilirubin and CO. Increased HO-1 activity was shown to attenuate endothelial cell apoptosis and decrease O_2^- formation in experimental diabetes [55]. HO-1 induction has been shown to provide vascular cytoprotection against oxidative stress *via* mechanism(s) that involve an increase in mitochondrial function [55,63]. The HO-1 mediated increase in EC-SOD converts O_2^- to hydrogen peroxide which is subsequently detoxified by glutathione peroxidase [64]. The finding that HO-1 increases EC-SOD is seminal, as a decrease in superoxide will limit the formation of peroxynitrite and inactivation of eNOS, which leads to an increase in NO bioavailability [24,55,65,66]. Hyperglycemia is also known to increase the levels of cellular heme and O_2^- as a result of a decrease in HO activity (for review, see Abraham and Kappas [1]). Thus HO-1 plays a pivotal role in mitigating the detrimental effects of hyperglycemia. It should be noted however that, using hyperglycemic heme oxygenase isozyme 2 (HO-2) (+/+) mice, HO-2 deficiency can contribute to a diabetes-mediated increase in superoxide anion levels and renal dysfunction. HO-2 deficiency causes major renal morphological injury and impairs renal function [67] sug-

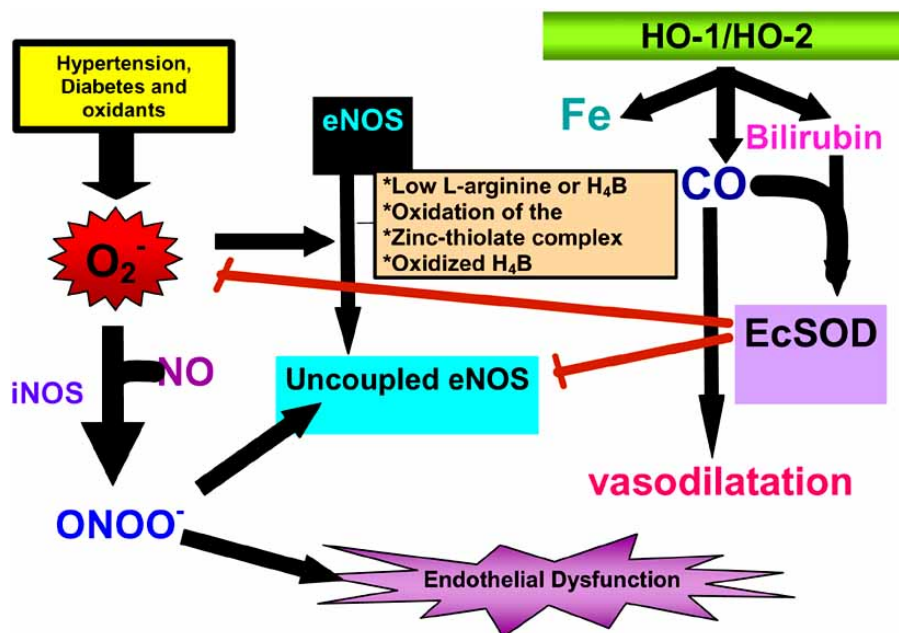


Fig. (2). Schematic representation of HO-1-derived CO and bilirubin in the regulation of oxidative stress and cell survival. Upregulation of HO-1 by pharmacological agents or by gene transfer or as a result of stress leads to an increase in heme degradation and the generation of CO and bilirubin. This process increases heme turnover with a resultant effect of decreasing inducible enzymes such as iNOS, but not eNOS. Simultaneously, CO or bilirubin or both enhance anti-apoptotic, antioxidant and signaling molecules. HO-1-derived CO or bilirubin may directly increase EC-SOD or, *via* their activation, transcriptional factors. EC-SOD scavenges O_2^- , decreases formation of peroxynitrite (ONOO $^-$), and limits oxidative and nitrative stress.

gesting that diabetes-induced renal impairment is markedly attenuated in HO-2 (-/-) mice, and with the use of STZ, is toxic to tubular necrosis. There are several mechanisms by which upregulation of HO-1 compensates for HO-2 deficiency. The upregulation of HO-1 inhibits the inflammatory response. Consistent with these observations, animals and humans deficient in HO-1 suffer from progressive chronic inflammation and are sensitive to stressful injury, presumably due to elevated heme iron levels [1].

Cardiac mitochondrial damage, such as that seen in Type I diabetes, is the result of a decrease in reduced glutathione and decreased signal transducer and activator of transcription-3 of the mitochondrial respiration system [68]. A deficiency in the deoxynucleotide carrier has been associated with abnormal brain growth [69], and a deficiency in carnitine-acylcarnitine was shown to cause muscle weakness and cardiomyopathy [70]. Diabetic complications have been related to abnormalities in mitochondrial function [68,71,72] as well as to increased endothelial cell death and detachment [73]. Therefore, upregulation of HO-1 in the mitochondria or in the vicinity of mitochondrial membranes may be essential to modulate the redox state in favor of antioxidants and to enhance mitochondrial transport of substrates and metabolites. Di Noia *et al.* showed restoration of six mitochondrial carriers, i.e., carnitine, citrate, phosphate, ATP and dicarboxylate, as a result of an increase in HO-1 protein and HO activity in diabetic rats [63,74]. Specific human HO-1 gene transfer to diabetic rats has also resulted in the restoration of mitochondrial carriers, including ADP/ATP and dicarboxylate [63]. Peterson *et al.* [66] and Li *et al.* [75,76] have shown that the increase in HO-1 in diabetic rats is associated with increased eNOS and phosphorylated protein kinase activator (pAKT). An increase in AKT phosphorylation is critical to cell survival in diabetes [77,78]. Increases in AKT phosphorylation and Bcl-XL levels have been shown to prevent the loss of beta cells in diabetes [79,80]. It is interesting to note that the alteration in mitochondrial function *in vitro* and *in vivo* has been shown to correlate with the levels of activation of AKT and the Bcl-2 family of proteins [81-84]. A decrease in Bcl-2 family members has been sug-

gested to contribute to apoptosis and the translocation of cytochrome C from the mitochondria to cytosol [82,83,85]. Activation of AKT has been shown to augment ATP synthesis [86] and promote the association of hexokinase with the voltage-dependent anion channel (VDAC) and, in so doing, promote VDAC closure, thus blocking release of cytochrome C [87].

The role of the heme degradation products CO and biliverdin/bilirubin in preventing the deleterious effects of hyperglycemia in diabetes has been examined in detail. Li *et al.* [75] reported the interdiction of the diabetic state in NOD mice by sustained induction of HO-1 and suggested a possible role of CO and bilirubin. This was associated with a decrease in infiltrated CD11c+ dendritic cells (Fig. 3).

HO-1 upregulation has proven to be capable of providing cytoprotection to vascular function [24,59] and to pancreatic beta cells *in vivo* [11]. Li *et al.* [76] have shown that an increase in HO-1 levels, i.e., CO and bilirubin, have a salutary effect, modulating the pancreas phenotype, as reflected by the increases in the antiapoptotic proteins, AKT and Bcl-XL, thus rendering beta cells resistant to oxidant stress and, hence, preventing the development of Type I diabetes (Fig. 4). These novel findings provide a link between the increase in HO-1 and a decrease in infiltrated type 1 transmembrane protein (CD11c+ dendritic cells), and suggest that the induction of HO activity can be used to enhance cell survival and moderate the diabetic state [76]. Similarly, others have shown that increased levels of HO-1 protein slows the progression to overt diabetes in prediabetic NOD mice by downregulating the phenotypic maturity of dendritic cells and T helper (Th1) effector function. CO appears to mediate, at least partly, the beneficial effect of HO-1 in this disease setting [88]. Hyperglycemia is also known to increase the levels of cellular heme and O_2^- as a result of a decrease in HO activity (for review, see Abraham and Kappas [1], and Abraham and Drummond [89]).

A question arises as to whether vascular endothelial cell damage in diabetes can be prevented or salvaged by altering the balance between the pro- and anti-apoptotic pathways regulated by the mito-

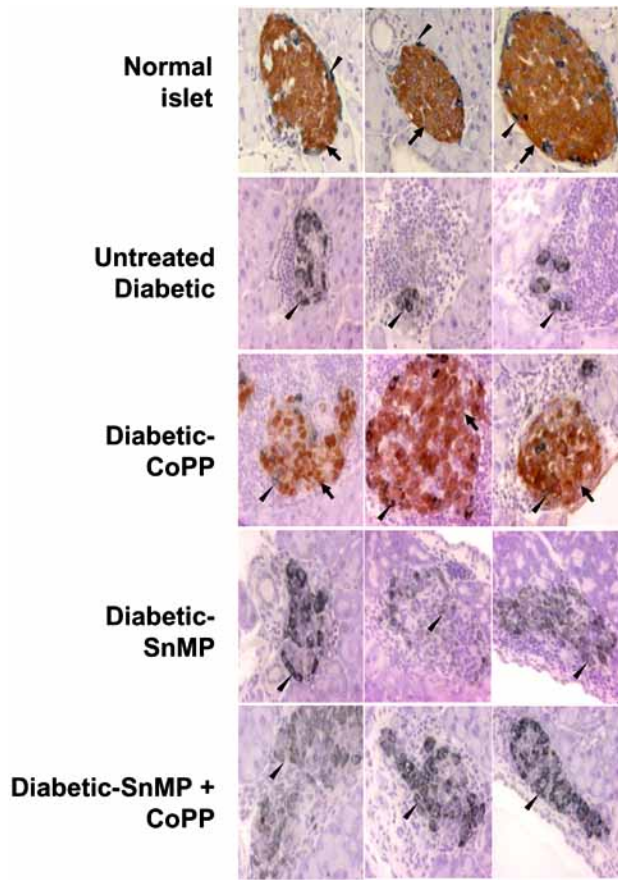


Fig. (3). Insulin and glucagon expression. Insulin-producing cells (brown color) were observed in normal islets (arrow) in pre-diabetic (arrows) and in CoPP-treated NOD mice administered SnMP. No beta cells were present in untreated, SnMP-treated or CoPP-treated NOD mice administered SnMP. Glucagon-producing cells (black color) were observed in the islets of each group (arrowheads) (original magnification X 400). Representative slides are shown [75,76].

chondria. It has been reported that the overexpression of HO-1 enhances cell proliferation through activation of AKT [90]. Similarly, a lack of HO-1 or HO-2 in either transgenic mice or in humans significantly increases apoptotic cell death [54,91-94]. The report that HO-1 regulates mitochondrial transport carriers and function [63] suggests that HO-1 by activating Bcl-2 and Bcl-XL, prevents cytochrome C release and the activation of caspases [63]. While these results suggest that it may be possible to favorably modulate the balance between pro- and anti-apoptotic mechanisms, neither HO-1 nor the effects of bilirubin and CO have been tested for significant clinical applicability as therapeutic measures in diabetes.

The beneficial effect of cytosolic HO-1 in vascular protection may be considered a result of the activation of EC-SOD (scavenging O₂⁻), enhancing NO bioavailability and preserving endothelial function. In addition, several reports suggest that beta cell destruction caused by elevated intracellular levels of ROS, including superoxide radicals, hydrogen peroxide and NO, is a process that occurs through both apoptotic and necrotic mechanisms [12-16,95]. T-cell-mediated infiltration of the pancreas leads to the generation of ROS and pro-inflammatory cytokines. The HO-1 system has been shown to regulate T-cell proliferation and immune response [96,97]. Studies have shown that CD4⁺ dendritic cells and T cells express HO-1 in response to CoPP, and that the lack of HO-1 modulates T-cell proliferation and maturation [98,99].

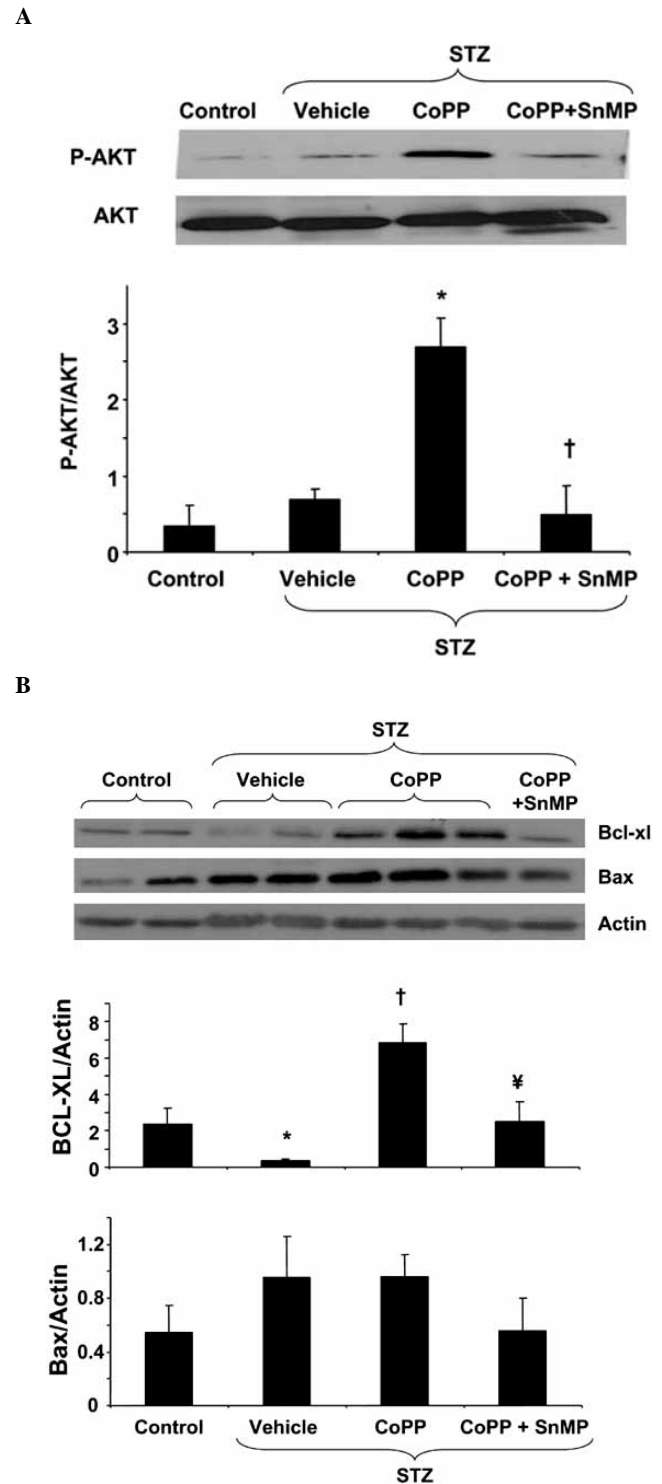


Fig. (4,A). Effect of diabetes and HO-1 expression on phospho (p) p-AKT and total AKT in renal tissue. Quantitative densitometry evaluation of p-AKT and total AKT proteins ratio was determined. *p<0.02 vs. diabetic rats; †p<0.02 vs STZ-CoPP; and (B) Effect of diabetes and HO-1 expression on Bcl-XL and Bax, in renal tissue. Quantitative densitometry evaluation of Bcl-XL and β-actin protein ratio were determined. *p<0.05 vs. control ; †p<0.001 vs. diabetic rats and ‡p<0.01 vs STZ-CoPP. Representative immunoblots (n= 6) are shown [63].

Antihypertensive drugs can help prevent renal complications by blockade of the renin-angiotensin system. Renal HO-1 is induced by several transcriptional factors, including nuclear factor E2-related factor 2 (Nrf2), to protect the kidney from injury in a model

of hepatic ischemia / reperfusion [100]. A protective effect is also seen in diabetes where insulin induces HO-1 in renal cells through the phosphoinositide 3-kinase (PI3k)/AKT pathway and Nrf2 transcription factor [101]. Thus HO-1 appears to be clinically important as a focal point in the development of strategies to reverse the detrimental side effects of diabetes.

EFFECT OF HO-1 INDUCTION ON SERUM ADIPONECTIN LEVELS AND INFLAMMATORY CYTOKINES, IL-1, IL-6 AND TNF- α

Adiponectin is an abundant, adipocyte-derived plasma protein that modulates vascular function. Adiponectin downregulates the generation of pro-inflammatory risk factors that mediate vascular dysfunction. A reduction in adiponectin levels has been implicated in the development of obesity linked diseases including diabetes, vascular inflammation and cardiovascular diseases [35]. Increased HO-1 expression is known to improve vascular function by decreasing superoxide and increasing antioxidant levels. This prompted us to examine the relationship between increased HO-1 expression and serum adiponectin levels.

To determine the effect of HO induction on adiponectin, we measured the adiponectin level in the Zucker diabetic fat rat (ZDF) and Zucker lean rat (ZL) before and the following treatment with CoPP for 6 weeks. As seen in Fig. 1, adiponectin was notably lower in 22-week-old ZDF compared to ZL ($P < 0.05$). After the treatment with CoPP, serum adiponectin in ZDF was increased by 165% compared to control ZDF ($P < 0.01$). Moreover, the increase in adiponectin in ZDF treated with CoPP attained a level significantly above that in ZL controls. When CoPP and SnMP were co-administered to ZDF, adiponectin levels did not increase because HO activity was inhibited (Kim and Abraham, manuscript in preparations). Pro-inflammatory cytokines, including IL-1, IL-6, and TNF- α , positively correlate with obesity, impaired glucose tolerance, insulin resistance and vascular inflammation [27,102]. To investigate the connection between HO induction and classical risk factors of type 2 diabetes, we assessed IL-6 and TNF- α in ZDF and ZL in response to treatment with CoPP for 4 weeks. All three cytokines were significantly increased in ZDF compared to ZL. The levels of IL-6 and TNF- α were lower than IL-6 in ZL control. Following treatment with CoPP both IL-6, TNF- α levels were significantly decreased in treated ZDF compared to control ZDF.

Weight and fat content were measured in ZDF following HO-1 induction to examine the role of adiponectin. HO-1 induction prevented the development of obesity. CoPP treatment (0.5mg/100g BW, once a week) maintained ZDF body wt. In contrast ZDF animals treated with either vehicle or SnMP continued to gain weight. Doubling the CoPP dose (1mg/100g, once a week for 6 weeks) resulted in ZDF animals losing weight and body fat. CoPP (1mg/100g) treated ZL exhibited no weight gain while ZL-vehicle treated gained ~90 grams ($p < 0.01$). Body fat mass was examined and was lower in CoPP-treated ZDF compared to SnMP-treated and vehicle treated (Fig. 5A). Similarly visceral fat content was reduced in ZDF CoPP treated animals (Fig. 5B). In summary, these results demonstrate a temporal link between HO-1 and adiponectin and suggests that HO-1 may be regarded as a target gene in the treatment of obesity which is considered a world wide problem of increasing magnitude.

Acute and Chronic Effects of HO-1

The degradation of heme is now considered critical in cellular defense for two contrasting reasons. First, the pro-oxidant heme is removed. Second, the increased production of bilirubin (antioxidant) and CO (anti-apoptotic) is now regarded as beneficial and critical to cellular defense mechanisms. Iron, which can stimulate free radical formation, is immediately bound by ferritin. Thus, CO and bilirubin are seminal to the protection that occurs from elevated levels of HO-1 expression and HO activity.

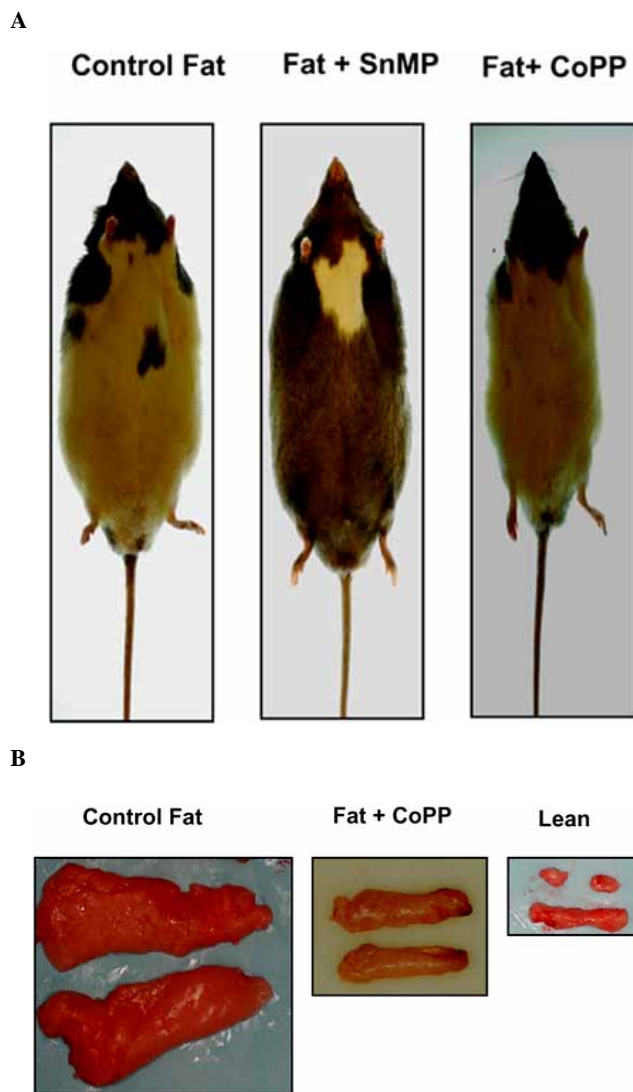


Fig. (5). Effect of upregulation of body weight and visceral fat content in lean and obese rats after 6 weeks of CoPP treatment (A) representative photograph for obese mice show mouse from each group after 6 weeks of treatment, and (B) the appearance of visceral fat deposit (abdominal fat) at the end of the treatment (6 weeks).

Early studies examined the acute increase in HO activity resulting from the administration of a broad spectrum of inducers. Chronic induction of HO-1 may have both beneficial and detrimental effects. The role of HO in the acquisition of resistance to H₂O₂ and hemin toxicity was evaluated in renal epithelial cells. Adapted by long-term exposure to H₂O₂, renal epithelial cells had a twofold increase in basal HO activity and HO-1 protein expression which was beneficial. Acute treatment with H₂O₂ and hemin produced an increase in HO-1 that was associated with a decrease in the viability of renal epithelial cells. Long-term exposure to both stressors resulted, however, in the acquisition of some resistance to further acute challenges of oxidant stress in these cells [103]. Thus it appears that resistance built up, suggesting that, to be effective, increased HO-1 protein expression should occur before the onset of chronic disease. The acute induction, by several metals, of HO-1 has been shown to have a beneficial effect due to the rapid decrease in undesired heme and cytochrome P450. Sacerdoti *et al.* [104] first reported the benefits of an acute effect, describing that treatment with SnCl₂ prevented the development of high blood pressure. Subsequently, others reported that acute and chronic expression of HO-

1 decreased vasoconstrictors, such as 20-hydroxyeicosatetraenoic acids [105,106], thromboxane synthase activity [107] and cyclooxygenase 2 activity [108]. Heme arginate, or heme, which is used clinically for the treatment of porphyria [109], has been shown to have a beneficial effect on acute induction of HO-1 and lowers blood pressure in hypertensive rats [110-113]. Hemin, a critical component of hemoglobin, is an active ingredient of a biologic therapeutic agent approved by the USA-FDA for the treatment of acute porphyrias.

The results described above leads to the question, can chronic induction of HO lead to adverse effects *in vivo*? In cell cultures, the chronic induction of HO activity by SnCl₂ resulted in a time- and dose-dependent decrease in heme and cGMP levels, due to the limitations of heme and culture media and the limitation in the heme synthesis [114]. However, chronic induction of HO-1, using iron as an inducer, did not affect cellular heme or cytochrome P450 content due to the rapid increase in heme turnover. Wang *et al.* elegantly showed that heme used for chronic treatment led to the continuous expression of HO-1, lowered blood pressure, and modestly decreased cellular heme [113]. Similarly, Levere *et al.* [115], showed that acute induction of HO-1 by heme arginate has beneficial effects including lowered blood pressure. Heme administration has been shown to reduce NADPH oxidase and administration of the HO inhibitor SnMP reversed that effect and increased NADPH [116]. These authors showed that inhibition of NADPH oxidase was minimized by bilirubin [116].

In contrast, inducers such as CoPP or CoCl₂ will, at high concentrations, inhibit heme synthesis and decrease cytochrome P450 levels [117,118]. Investigators have administered high concentrations of CoPP or CoCl₂ which inhibit ferrochelatase and heme synthesis and may be toxic due to the severe decrease in heme P450 [119,120]. However, low concentrations of CoPP, administered once a week in experimental diabetes, moderately decreased cellular heme, and P450 (unpublished results), and increased eNOS, pAKT and cell survival [55,56]. Small doses of CoPP, which produce long lasting and chronic induction of HO-1, attenuated the coronary constrictor response to ischemia-reperfusion [48]. This modest and chronic expression of HO-1 resulted in a significant increase in serum adiponectin [121]. The HO-1 mediated increase in adiponectin provides the heart and vascular system with tolerance and resistance to oxidative stress generated not only in diabetes, but also to other types of vascular stress [121]. One can speculate that, in anemias, or when a genetic or environmental decrease in heme synthesis occurs, chronic induction of HO activity would exacerbate the disease state. Similarly, in porphyrias, where the synthesis of heme is impaired, chronic increases in HO activity would only serve to worsen the disease through the depletion of heme. Thus, careful review of the individual circumstances is necessary before embarking on chronic manipulation of HO activity.

There is now a considerable body of evidence, both in animal models and in humans, on the use of metalloporphyrins to inhibit/increase HO activity and expression. Kappas [122] summarized some of these studies. In one study, tin protoporphyrin IX dichloride (SnPP) was administered to mice with hemolytic anemia over a several month period without deleterious effects on cytochrome P450 content and cytochrome P450 drug-dependent enzyme activity. In another study, two Crigler-Najjar type 1 patients were treated for extended periods of time without adverse events, other than mild erythema. Chronic induction or long-term expression of HO-1 by CoPP has been shown to attenuate vascular dysfunction and restore the acetylcholine-dilatory effect in Type I diabetes [55-57]. More recently, long-term induction by CoPP prevented beta-cell destruction in NOD mice, interdicting the diabetic state [75,76], as a result of the suppression of infiltrating dendritic cells and decreased superoxide formation in pancreatic tissues. Thus, by the judicious use of metals and metalloporphyrins, with

respect to dose and frequency of administration, beneficial effects can be achieved without deleterious side effects when used in chronic treatment.

HO-1 Gene: A Target for Drug Development in Cardiovascular Disease

HO-1 can be delivered directly by gene therapy or can be induced pharmacologically. The latter is fraught with difficulties due to dose and time of HO-1 expression considerations. Thus adverse effects associated with long term use on HO-1 expression and heme synthesis should be elucidated prior to use in the clinic. Nevertheless several commonly used drugs have been reported to modulate HO-1 expression in tissue. The PPAR δ agonist, pioglitazone, has been reported to improve insulin secretory capacity and prevent a reduction in the mass of pancreatic beta cells in obese diabetic db/db mice [123]. HO-1 expression is transcriptionally regulated by both PPAR α and PPAR δ agonists implying that the mechanism by which PPAR provides anti-inflammatory effects is due to an increase in HO-1 derived CO and/or bilirubin [44]. The recent report of the parallel increase in HO-1 expression and HO activity and serum adiponectin, a protein hormone that can modulate a number of metabolic processes including glucose metabolism, provides an insight into possible mechanism(s) involving PPAR δ . The identification of HO-1 as a target gene for PPAR widens the options for the development of drugs for the management of cardiovascular disease.

Aspirin has been reported to increase HO-1 expression and HO activity in a dose dependent manner in human umbilical vein endothelial cells [124] and to increase ferritin synthesis in endothelial cell (EC) through HO-1 induction [125,126]. The dose of aspirin used in these studies was substantially higher than used clinically. Various statins have shown to increase HO-1, mRNA levels in EC and vascular smooth muscle cell [127-130] and when administered orally to mice [131]. These results may explain the beneficial actions of statins as by the maintenance of low levels of free radicals through the continuous elevation of HO-1 expression. Several cardiovascular drugs such as probucol [132,133], losartan [134], paclitaxel [132] and rapamycin [135] increase HO-1 expression. Curcumin has been reported to induce HO-1 expression [136]. Increased HO activity is an important component in curcumin-mediated cytoprotection against oxidative stress [137]. In addition, resveratrol, a component in red wine grapes, has been reported to precondition the heart through an HO-1 dependent NO mediated mechanism [138] and to reduce infarct size [139] through a HO-1 mediated mechanism [140].

The common thread that links the disparate compounds described above is the increased expression of HO-1. This serves to focus on the critical role that HO-1 plays in the protection against metabolic insults and presents an unique opportunity as an obvious target in the synthesis of pharmacological compounds that would have clinical application in preventing the deleterious side effects associated with a number of disease states.

SUMMARY

The findings discussed above highlight the central role that HO-1 plays in modulating the deleterious complications that arise in diabetes as a result of oxidative stress. Thus, pharmacological or genetic manipulations that result in enhanced HO-1 expression and HO activity provide the basis for the vascular system to commence a host defense response to resist the deleterious effects of diabetes and obesity-mediated complications as a result of oxidative stress via an increase in adiponectin and decreases inflammatory molecules. Thus targeting the HO-1 gene to increase HO-1 protein levels and HO activity may be considered the primary defense against these complications and, as such, offers a portal for clinical intervention in these costly, in both monetary and human terms, diseases, including diabetes, metabolic syndrome and obesity.

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ABBREVIATIONS

AKT	=	Protein kinase (activator)
CO	=	Carbon monoxide
CoPP	=	Cobalt Protoporphyrin IX dichloride
CO-RM	=	Carbon monoxide releasing molecule
D-4F	=	Apolipoprotein mimetic peptide
EC	=	Endothelial cell
EC-SOD	=	Extracellular superoxide dismutase
eNOS	=	Endothelial nitric oxide synthase
HDL	=	High-density lipoprotein
HMW	=	High-molecular weight
HO	=	Heme oxygenase
HO-1	=	Heme oxygenase isozyme 1, inducible form
HO-2	=	Heme oxygenase isozyme 2, constitutive form
IL	=	Interleukin
iNOS	=	Inducible nitric oxide synthase
LDL	=	Low-density lipoprotein
LMW	=	Low-molecular weight
NF- κ B	=	Nuclear factor κ B
NO	=	Nitric oxide
NOD	=	Nonobese diabetic
Nrf ₂	=	Nuclear factor E2-related factor 2
PI3k	=	Phosphoinositide 3-kinase
p450	=	Cytochrome p-540
pAKT	=	Phosphorylated protein kinase (activator)
PPAR	=	Peroxisome proliferator-activated receptor
ROS	=	Reactive oxygen species
RPE	=	Retinal pigment epithelium
SnMP	=	Tin mesoporphyrin IX dichloride
SnPP	=	Tin protoporphyrin IX dichloride
STZ	=	Streptozotocin
Th1	=	T helper
TNF- α	=	Tumor necrosis factor-alpha
TZD	=	Thiazolidinedione
VDAC	=	Voltage-dependent anion channel
ZDF	=	Zucker diabetic fat rat
ZL	=	Zucker lean rat

REFERENCES

References 141-143 are related articles recently published in *Current Pharmaceutical Design*.

- Abraham NG, Kappas A. Heme oxygenase and the cardiovascular-renal system. *Free Radic Biol Med* 2005; 39: 1-25.
- Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. *Diabetes Care* 1996; 19: 257-67.
- Rabinovitch A, Suarez-Pinzon WL, Sorensen O, Bleackley RC. Inducible nitric oxide synthase (iNOS) in pancreatic islets of nonobese diabetic mice: identification of iNOS-expressing cells and relationships to cytokines expressed in the islets. *Endocrinology* 1996; 137: 2093-9.
- The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Eng J Med* 1993; 320: 977-86.
- Wolff SP. Diabetes mellitus and free radicals. Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. *Br Med Bull* 1993; 49: 642-52.
- Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes* 1991; 40: 405-12.
- Tesfamariam B. Free radicals in diabetic endothelial cell dysfunction. *Free Radic Biol Med* 1994; 16: 383-91.
- Bottino R, Balamurugan AN, Bertera S, Pietropaolo M, Trucco M, Piganelli JD. Preservation of human islet cell functional mass by anti-oxidative action of a novel SOD mimic compound. *Diabetes* 2002; 51: 2561-7.
- Ihara Y, Toyokuni S, Uchida K, Odaka H, Tanaka T, Ikeda H, *et al.* Hyperglycemia causes oxidative stress in pancreatic beta-cells of GK rats, a model of type 2 diabetes. *Diabetes* 1999; 48: 927-32.
- Eizirik DL, Flodstrom M, Karlens AE, Welsh N. The harmony of the spheres: inducible nitric oxide synthase and related genes in pancreatic beta cells. *Diabetologia* 1996; 39: 875-90.
- Pileggi A, Molano RD, Berney T, Cattani P, Vizzardelli C, Oliver R, *et al.* Heme oxygenase-1 induction in islet cells results in protection from apoptosis and improved *in vivo* function after transplantation. *Diabetes* 2001; 50: 1983-91.
- Kaneto H, Fujii J, Seo HG, Suzuki K, Matsuoka T, Nakamura M, *et al.* Apoptotic cell death triggered by nitric oxide in pancreatic beta-cells. *Diabetes* 1995; 44: 733-8.
- Kurrer MO, Pakala SV, Hanson HL, Katz JD. Beta cell apoptosis in T cell-mediated autoimmune diabetes. *Proc Natl Acad Sci USA* 1997; 94: 213-8.
- O'Brien BA, Harmon BV, Cameron DP, Allan DJ. Apoptosis is the mode of beta-cell death responsible for the development of IDDM in the nonobese diabetic (NOD) mouse. *Diabetes* 1997; 46: 750-7.
- Chervonsky AV, Wang Y, Wong FS, Visintin I, Flavell RA, Janeway CA, Jr., *et al.* The role of Fas in autoimmune diabetes. *Cell* 1997; 89: 17-24.
- Itoh N, Imagawa A, Hanafusa T, Waguri M, Yamamoto K, Iwahashi H, *et al.* Requirement of Fas for the development of autoimmune diabetes in nonobese diabetic mice. *J Exp Med* 1997; 186: 613-8.
- National diabetes fact sheet: General information and national estimates on diabetes in the United States. CDC 2005.
- Sullivan PW, Morrato EH, Ghushchyan V, Wyatt HR, Hill JO. Obesity, inactivity, and the prevalence of diabetes and diabetes-related cardiovascular comorbidities in the U.S., 2000-2002. *Diabetes Care* 2005; 28: 1599-603.
- DeFronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 1991; 14: 173-94.
- Fonseca VA. Rationale for the use of insulin sensitizers to prevent cardiovascular events in type 2 diabetes mellitus. *Am J Med* 2007; 120: S18-S25.
- Robertson RP. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *J Biol Chem* 2004; 279: 42351-4.
- Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest* 2005; 115: 1111-9.
- Bahia L, Aguiar LG, Villela N, Bottino D, Godoy-Matos AF, Geloneze B, *et al.* Relationship between adipokines, inflammation, and vascular reactivity in lean controls and obese subjects with metabolic syndrome. *Clinics* 2006; 61: 433-40.
- Kruger AL, Peterson S, Turkseven S, Kaminski PM, Zhang FF, Quan S, *et al.* D-4F induces heme oxygenase-1 and extracellular superoxide dismutase, decreases endothelial cell sloughing, and improves vascular reactivity in rat model of diabetes. *Circulation* 2005; 111: 3126-34.
- Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. *Circ Res* 2005; 96: 939-49.
- Koh KK, Han SH, Quon MJ. Inflammatory markers and the metabolic syndrome. *J Am Coll Cardiol* 2005; 46: 1978-85.
- Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 2004; 89: 2548-56.
- Kubota N, Terauchi Y, Kubota T, Kumagai H, Itoh S, Satoh H, *et al.* Pioglitazone ameliorates insulin resistance and diabetes by both

- adiponectin-dependent and -independent pathways. *J Biol Chem* 2006; 281: 8748-55.
- [29] Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. *Endocr Rev* 2005; 26: 439-51.
- [30] Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. *Endocrinology* 2004; 145: 2273-82.
- [31] Kondo H, Shimomura I, Matsukawa Y, Kumada M, Takahashi M, Matsuda M, *et al.* Association of adiponectin mutation with type 2 diabetes: a candidate gene for the insulin resistance syndrome. *Diabetes* 2002; 51: 2325-8.
- [32] Lara-Castro C, Luo N, Wallace P, Klein RL, Garvey WT. Adiponectin multimeric complexes and the metabolic syndrome trait cluster. *Diabetes* 2006; 55: 249-59.
- [33] Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, *et al.* Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999; 257: 79-83.
- [34] Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, *et al.* Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 2001; 86: 1930-5.
- [35] Hopkins TA, Ouchi N, Shibata R, Walsh K. Adiponectin actions in the cardiovascular system. *Cardiovasc Res* 2007; 74: 11-8.
- [36] Bahia L, Aguiar LG, Vilella N, Bottino D, Godoy-Matos AF, Geloneze B, *et al.* Adiponectin is associated with improvement of endothelial function after rosiglitazone treatment in non-diabetic individuals with metabolic syndrome. *Atherosclerosis* 2006; [epub ahead of print].
- [37] Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. *J Biol Chem* 2003; 278: 45021-6.
- [38] Matsuda M, Shimomura I, Sata M, Arita Y, Nishida M, Maeda N, *et al.* Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. *J Biol Chem* 2002; 277: 37487-91.
- [39] Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y, Kumada M, *et al.* Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 2002; 106: 2767-70.
- [40] Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, *et al.* Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 1999; 100: 2473-6.
- [41] Yilmaz MI, Sonmez A, Caglar K, Celik T, Yenicesu M, Eyleten T, *et al.* Effect of antihypertensive agents on plasma adiponectin levels in hypertensive patients with metabolic syndrome. *Nephrology (Carlton)* 2007; 12: 147-53.
- [42] Ouedraogo R, Gong Y, Berzins B, Wu X, Mahadev K, Hough K, *et al.* Adiponectin deficiency increases leukocyte-endothelium interactions *via* upregulation of endothelial cell adhesion molecules *in vivo*. *J Clin Invest* 2007; 117: 1718-26.
- [43] Iwaki M, Matsuda M, Maeda N, Funahashi T, Matsuzawa Y, Makishima M, *et al.* Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes* 2003; 52: 1655-63.
- [44] Kronke G, Kadl A, Ikonomu E, Bluml S, Furnkranz A, Sarembock IJ, *et al.* Expression of heme oxygenase-1 in human vascular cells is regulated by peroxisome proliferator-activated receptors. *Arterioscler Thromb Vasc Biol* 2007; 27: 1276-82.
- [45] Abraham NG, Kappas A. Pharmacological and Physiological Role of Heme Oxygenase In Normal and Pathological Circumstances. *Pharmacol Rev* 2007; (submitted).
- [46] Abraham NG, Kushida T, McClung J, Weiss M, Quan S, Lafaro R, *et al.* Heme Oxygenase-1 Attenuates Glucose-Mediated Cell Growth Arrest and Apoptosis in Human Microvessel Endothelial Cells. *Circ Res* 2003; 93: 507-14.
- [47] Abraham NG, Rezzani R, Rodella L, Kruger A, Taller D, Li VG, *et al.* Overexpression of human heme oxygenase-1 attenuates endothelial cell sloughing in experimental diabetes. *Am J Physiol Heart Circ Physiol* 2004; 287: H2468-77.
- [48] L'Abbate A, Neglia D, Vecoli C, Novelli M, Ottaviano V, Baldi S, *et al.* Beneficial effect of heme oxygenase-1 expression in myocardial ischemia-reperfusion increases adiponectin in mildly diabetic rats. *Am J Physiol Heart Circ Physiol* 2007; 293: H3532-41.
- [49] Jung TW, Lee JY, Shim WS, Kang ES, Kim JS, Ahn CW, *et al.* Adiponectin protects human neuroblastoma SH-SY5Y cells against acetaldehyde-induced cytotoxicity. *Biochem Pharmacol* 2006; 72: 616-23.
- [50] da Silva JL, Stoltz RA, Dunn MW, Abraham NG, Shibahara S. Diminished heme oxygenase-1 mRNA expression in RPE cells from diabetic donors as quantitated by competitive RT/PCR. *Curr Eye Res* 1997; 16: 380-6.
- [51] Bruce CR, Carey AL, Hawley JA, Febbraio MA. Intramuscular heat shock protein 72 and heme oxygenase-1 mRNA are reduced in patients with type 2 diabetes: evidence that insulin resistance is associated with a disturbed antioxidant defense mechanism. *Diabetes* 2003; 52: 2338-45.
- [52] Adaikalakeswari A, Balasubramanyam M, Rema M, Mohan V. Differential gene expression of NADPH oxidase (p22phox) and hemoxygenase-1 in patients with Type 2 diabetes and microangiopathy. *Diabet Med* 2006; 23: 666-74.
- [53] Murphy RC, Falck JR, Lumin S, Yadagiri P, Zirrolli JA, Balazy M, *et al.* 12[R]-hydroxyeicosatrienoic acid: a vasodilator cytochrome P-450-dependent arachidonate metabolite from the bovine corneal epithelium. *J Biol Chem* 1988; 263: 17197-202.
- [54] Quan S, Kaminski PM, Yang L, Morita T, Inaba M, Ikehara S, *et al.* Heme oxygenase-1 prevents superoxide anion-associated endothelial cell sloughing in diabetic rats. *Biochem Biophys Res Commun* 2004; 315: 509-16.
- [55] Turkseven S, Kruger A, Mingone CJ, Kaminski P, Inaba M, Rodella LF, *et al.* Antioxidant mechanism of heme oxygenase-1 involves an increase in superoxide dismutase and catalase in experimental diabetes. *Am J Physiol Heart Circ Physiol* 2005; 289: H701-H707.
- [56] Ahmad M, Turkseven S, Mingone CJ, Gupte SA, Wolin MS, Abraham NG. Heme oxygenase-1 gene expression increases vascular relaxation and decreases inducible nitric oxide synthase in diabetic rats. *Cell Mol Biol (Noisy -le-grand)* 2005; 51: 371-6.
- [57] Di Pascoli M, Rodella L, Sacerdoti D, Bolognesi M, Turkseven S, Abraham NG. Chronic CO levels have [corrected] a beneficial effect on vascular relaxation in diabetes. *Biochem Biophys Res Commun* 2006; 340: 935-43.
- [58] Robertson RP, Zhang HJ, Pyzdrowski KL, Walseth TF. Preservation of insulin mRNA levels and insulin secretion in HIT cells by avoidance of chronic exposure to high glucose concentrations. *J Clin Invest* 1992; 90: 320-5.
- [59] Abraham NG, Kushida T, McClung J, Weiss M, Quan S, Lafaro R, *et al.* Heme oxygenase-1 attenuates glucose-mediated cell growth arrest and apoptosis in human microvessel endothelial cells. *Circ Res* 2003; 93: 507-14.
- [60] Zou MH, Shi C, Cohen RA. High glucose *via* peroxynitrite causes tyrosine nitration and inactivation of prostacyclin synthase that is associated with thromboxane/prostaglandin H2 receptor-mediated apoptosis and adhesion molecular expression in cultured human aortic endothelial cells. *Diabetes* 2002; 51: 198-203.
- [61] Ceriello A, dello RP, Amstad P, Cerutti P. High glucose induces antioxidant enzymes in human endothelial cells in culture. Evidence linking hyperglycemia and oxidative stress. *Diabetes* 1996; 45: 471-7.
- [62] Sacerdoti D, Olszanecki R, Li VG, Colombrita C, Scapagnini G, Abraham NG. Heme oxygenase overexpression attenuates glucose-mediated oxidative stress in quiescent cell phase: linking heme to hyperglycemia complications. *Curr Neurovasc Res* 2005; 2: 103-11.
- [63] Di Noia MA, Van DS, Palmieri F, Yang LM, Quan S, Goodman AI, *et al.* Heme oxygenase-1 enhances renal mitochondrial transport carriers and cytochrome C oxidase activity in experimental diabetes. *J Biol Chem* 2006; 281: 15687-93.
- [64] Kappas A. Development of heme oxygenase inhibitors for the prevention of severe jaundice in infants: studies from laboratory bench to newborn nursery. In: Abraham NG, Alam J, Nath KA, editors. *Heme Oxygenase in Biology and Medicine*. New York, NY: Kluwer Academic/Plenum Publishers 2002; pp. 3-17.
- [65] Kruger AL, Peterson SJ, Schwartzman ML, Fusco H, McClung JA, Weiss M, *et al.* Up-regulation of heme oxygenase provides vascular protection in an animal model of diabetes through its antioxidant and antiapoptotic effects. *J Pharmacol Exp Ther* 2006; 319: 1144-52.

- [66] Peterson SJ, Husney D, Kruger AL, Olszanecki R, Ricci F, Rodella LF, *et al.* Long-term treatment with the apolipoprotein A1 mimetic Peptide increases antioxidants and vascular repair in type I diabetic rats. *J Pharmacol Exp Ther* 2007; 322: 514-20.
- [67] Goodman AI, Chander PN, Rezzani R, Schwartzman ML, Regan RF, Rodella L, *et al.* Heme oxygenase-2 deficiency contributes to diabetes-mediated increase in superoxide anion and renal dysfunction. *J Am Soc Nephrol* 2006; 17: 1073-81.
- [68] Shen X, Zheng S, Thongboonkerd V, Xu M, Pierce WM, Jr., Klein JB, *et al.* Cardiac mitochondrial damage and biogenesis in a chronic model of type I diabetes. *Am J Physiol Endocrinol Metab* 2004; 287: E896-E905.
- [69] Rosenberg MJ, Agarwala R, Bouffard G, Davis J, Fiermonte G, Hilliard MS, *et al.* Mutant deoxynucleotide carrier is associated with congenital microcephaly. *Nat Genet* 2002; 32: 175-9.
- [70] Stanley CA, Hale DE, Berry GT, Deleew S, Boxer J, Bonnefont JP. Brief report: a deficiency of carnitine-acylcarnitine translocase in the inner mitochondrial membrane. *N Engl J Med* 1992; 327: 19-23.
- [71] Sparks LM, Xie H, Koza RA, Mynatt R, Hulver MW, Bray GA, *et al.* A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. *Diabetes* 2005; 54: 1926-33.
- [72] Hsieh RH, Lien LM, Lin SH, Chen CW, Cheng HJ, Cheng HH. Alleviation of oxidative damage in multiple tissues in rats with streptozotocin-induced diabetes by rice bran oil supplementation. *Ann N Y Acad Sci* 2005; 1042: 365-71.
- [73] Detaille D, Guigas B, Chauvin C, Batandier C, Fontaine E, Wiernsperger N, *et al.* Metformin prevents high-glucose-induced endothelial cell death through a mitochondrial permeability transition-dependent process. *Diabetes* 2005; 54: 2179-87.
- [74] Turkseven S, Drummond G, Rezzani R, Rodella L, Quan S, Ikehara S, *et al.* Impact of silencing HO-2 on EC-SOD and the mitochondrial signaling pathway. *J Cell Biochem* 2007; 100: 815-23.
- [75] Li M, Peterson S, Husney D, Inaba M, Guo K, Terada E, *et al.* Interdiction of the diabetic state in NOD mice by sustained induction of heme oxygenase: possible role of carbon monoxide and bilirubin. *Antioxid Redox Signal* 2007; 9: 855-63.
- [76] Li M, Peterson S, Husney D, Inaba M, Guo K, Kappas A, *et al.* Long-lasting expression of HO-1 delays progression of type I diabetes in NOD mice. *Cell Cycle* 2007; 6: 567-71.
- [77] Tsang A, Hausenloy DJ, Mocanu MM, Carr RD, Yellon DM. Preconditioning the diabetic heart: the importance of akt phosphorylation. *Diabetes* 2005; 54: 2360-4.
- [78] Varma S, Lal BK, Zheng R, Breslin JW, Saito S, Pappas PJ, *et al.* Hyperglycemia Alters PI3k and Akt Signaling and Leads to Endothelial Cell Proliferative Dysfunction. *Am J Physiol Heart Circ Physiol* 2005; 289: H1744-51.
- [79] Kowluru A. Novel regulatory roles for protein phosphatase-2A in the islet beta cell. *Biochem Pharmacol* 2005; 69: 1681-91.
- [80] Dai C, Li Y, Yang J, Liu Y. Hepatocyte growth factor preserves beta cell mass and mitigates hyperglycemia in streptozotocin-induced diabetic mice. *J Biol Chem* 2003; 278: 27080-7.
- [81] Huang TJ, Sayers NM, Verkhatsky A, Fernyhough P. Neurotrophin-3 prevents mitochondrial dysfunction in sensory neurons of streptozotocin-diabetic rats. *Exp Neurol* 2005; 194: 279-83.
- [82] Bojunga J, Nowak D, Mitrou PS, Hoelzer D, Zeuzem S, Chow KU. Antioxidative treatment prevents activation of death-receptor- and mitochondrion-dependent apoptosis in the hearts of diabetic rats. *Diabetologia* 2004; 47: 2072-80.
- [83] Sun F, Kawasaki E, Akazawa S, Hishikawa Y, Sugahara K, Kamihira S, *et al.* Apoptosis and its pathway in early post-implantation embryos of diabetic rats. *Diabetes Res Clin Pract* 2005; 67: 110-8.
- [84] Chen W, Salojin KV, Mi QS, Grattan M, Meagher TC, Zucker P, *et al.* Insulin-like growth factor (IGF)-I/IGF-binding protein-3 complex: therapeutic efficacy and mechanism of protection against type I diabetes. *Endocrinology* 2004; 145: 627-38.
- [85] Srinivasan S, Stevens M, Wiley JW. Diabetic peripheral neuropathy: evidence for apoptosis and associated mitochondrial dysfunction. *Diabetes* 2000; 49: 1932-8.
- [86] Huang TJ, Verkhatsky A, Fernyhough P. Insulin enhances mitochondrial inner membrane potential and increases ATP levels through phosphoinositide 3-kinase in adult sensory neurons. *Mol Cell Neurosci* 2005; 28: 42-54.
- [87] Gottlob K, Majewski N, Kennedy S, Kandel E, Robey RB, Hay N. Inhibition of early apoptotic events by Akt/PKB is dependent on the first committed step of glycolysis and mitochondrial hexokinase. *Genes Dev* 2001; 15: 1406-18.
- [88] Hu CM, Lin HH, Chiang MT, Chang PF, Chau LY. Systemic expression of heme-oxygenase-1 ameliorates type I diabetes in NOD mice. *Diabetes* 2007; 56: 1240-7.
- [89] Abraham NG, Drummond G. CD163-Mediated hemoglobin-heme uptake activates macrophage HO-1, providing an antiinflammatory function. *Circ Res* 2006; 99: 911-4.
- [90] Salinas M, Diaz R, Abraham NG, Ruiz De Galarreta CM, Cuadrado A. Nerve growth factor protects against 6-hydroxydopamine-induced oxidative stress by increasing expression of heme oxygenase-1 in a phosphatidylinositol 3-kinase-dependent manner. *J Biol Chem* 2003; 278: 13898-904.
- [91] Nascimento AL, Luscher P, Tyrrell RM. Ultraviolet A (320-380 nm) radiation causes an alteration in the binding of a specific protein/protein complex to a short region of the promoter of the human heme oxygenase 1 gene. *Nucleic Acids Res* 1993; 21: 1103-9.
- [92] Poss KD, Tonegawa S. Reduced stress defense in heme oxygenase 1-deficient cells. *Proc Natl Acad Sci USA* 1997; 94: 10925-30.
- [93] Dennerly PA, Spitz DR, Yang G, Tatarov A, Lee CS, Shegog ML, *et al.* Oxygen toxicity and iron accumulation in the lungs of mice lacking heme oxygenase-2. *J Clin Invest* 1998; 101: 1001-11.
- [94] Quan S, Yang L, Abraham NG, Kappas A. Regulation of human heme oxygenase in endothelial cells by using sense and antisense retroviral constructs. *Proc Natl Acad Sci USA* 2001; 98: 12203-8.
- [95] Rabinovitch A, Suarez WL, Thomas PD, Strynadka K, Simpson I. Cytotoxic effects of cytokines on rat islets: evidence for involvement of free radicals and lipid peroxidation. *Diabetologia* 1992; 35: 409-13.
- [96] Choi BM, Pae HO, Jeong YR, Kim YM, Chung HT. Critical role of heme oxygenase-1 in Foxp3-mediated immune suppression. *Biochem Biophys Res Commun* 2005; 327: 1066-71.
- [97] Pae HO, Oh GS, Choi BM, Chae SC, Kim YM, Chung KR, *et al.* Carbon monoxide produced by heme oxygenase-1 suppresses T cell proliferation *via* inhibition of IL-2 production. *J Immunol* 2004; 172: 4744-51.
- [98] Chen S, Kapturczak MH, Wasserfall C, Glushakova OY, Campbell-Thompson M, Deshane JS, *et al.* Interleukin 10 attenuates neointimal proliferation and inflammation in aortic allografts by a heme oxygenase-dependent pathway. *Proc Natl Acad Sci USA* 2005; 102: 7251-6.
- [99] Chauveau C, Remy S, Royer PJ, Hill M, Tanguy-Royer S, Hubert FX, *et al.* Heme oxygenase-1 expression inhibits dendritic cell maturation and proinflammatory function but conserves IL-10 expression. *Blood* 2005; 106: 1694-702.
- [100] Tanaka Y, Maher JM, Chen C, Klaassen CD. Hepatic ischemia-reperfusion induces renal heme oxygenase-1 *via* NF-E2-related factor 2 in rats and mice. *Mol Pharmacol* 2007; 71: 817-25.
- [101] Harrison EM, McNally SJ, Devey L, Garden OJ, Ross JA, Wigmore SJ. Insulin induces heme oxygenase-1 through the phosphatidylinositol 3-kinase/Akt pathway and the Nrf2 transcription factor in renal cells. *FEBS J* 2006; 273: 2345-56.
- [102] Koh KK, Han SH, Quon MJ. Inflammatory markers and the metabolic syndrome: insights from therapeutic interventions. *J Am Coll Cardiol* 2005; 46: 1978-85.
- [103] da Silva JL, Morishita T, Escalante B, Staudinger R, Drummond G, Goligorsky MS, *et al.* Dual role of heme oxygenase in epithelial cell injury: contrasting effects of short-term and long-term exposure to oxidant stress. *J Lab Clin Med* 1996; 128: 290-6.
- [104] Sacerdoti D, Escalante B, Abraham NG, McGiff JC, Levere RD, Schwartzman ML. Treatment with tin prevents the development of hypertension in spontaneously hypertensive rats. *Science* 1989; 243: 388-90.
- [105] Laniado-Schwartzman M, Abraham NG, Sacerdoti D, Escalante B, McGiff JC. Effect of acute and chronic treatment of tin on blood pressure in spontaneously hypertensive rats. *Tohoku J Exp Med* 1992; 166: 85-91.
- [106] da Silva JL, Tiefenthaler M, Park E, Escalante B, Schwartzman ML, Levere RD, *et al.* Tin-mediated heme oxygenase gene activa-

- tion and cytochrome P450 arachidonate hydroxylase inhibition in spontaneously hypertensive rats. *Am J Med Sci* 1994; 307: 173-81.
- [107] Sessa WC, Abraham NG, Escalante B, Schwartzman ML. Manipulation of cytochrome P-450 dependent renal thromboxane synthase activity in spontaneously hypertensive rats. *J Hypertens* 1989; 7: 37-42.
- [108] Abraham NG. Therapeutic applications of human heme oxygenase gene transfer and gene therapy. *Curr Pharm Des* 2003; 9: 2513-24.
- [109] Kordac V, Kozakova M, Martasek P. Changes of myocardial functions in acute hepatic porphyrias. Role of heme arginate administration. *Ann Med* 1989; 21: 273-6.
- [110] Martasek P, Schwartzman ML, Goodman AI, Solangi KB, Levere RD, Abraham NG. Hemin and L-arginine regulation of blood pressure in spontaneous hypertensive rats. *J Am Soc Nephrol* 1991; 2: 1078-84.
- [111] Schwartzman ML, Martasek P, Rios AR, Levere RD, Solangi K, Goodman AI, *et al.* Cytochrome P450-dependent arachidonic acid metabolism in human kidney. *Kidney Int* 1990; 37: 94-9.
- [112] Ndisang JF, Wu L, Zhao W, Wang R. Induction of heme oxygenase-1 and stimulation of cGMP production by hemin in aortic tissues from hypertensive rats. *Blood* 2003; 101: 3893-900.
- [113] Wang R, Shamloul R, Wang X, Meng Q, Wu L. Sustained normalization of high blood pressure in spontaneously hypertensive rats by implanted hemin pump. *Hypertension* 2006; 48: 685-92.
- [114] Abraham NG, Mieyal PA, Quan S, Yang L, Burke-Wolin T, Mingone CJ, *et al.* Modulation of cyclic GMP by retrovirus-mediated human heme oxygenase-1 gene transfer in microvessel endothelial cells. *Am J Physiol* 2002; 283: L1117-L1124.
- [115] Levere RD, Martasek P, Escalante B, Schwartzman ML, Abraham NG. Effect of heme arginate administration on blood pressure in spontaneously hypertensive rats. *J Clin Invest* 1990; 86: 213-9.
- [116] Datla SR, Dusting GJ, Mori TA, Taylor CJ, Croft KD, Jiang F. Induction of heme oxygenase-1 *in vivo* suppresses NADPH oxidase derived oxidative stress. *Hypertension* 2007; 50: 636-42.
- [117] Drummond GS, Kappas A. The cytochrome P-450-depleted animal: an experimental model for *in vivo* studies in chemical biology. *Proc Natl Acad Sci USA* 1982; 79: 2384-8.
- [118] Lin JH, Villalon P, Martasek P, Abraham NG. Regulation of heme oxygenase gene expression by cobalt in rat liver and kidney. *Eur J Biochem* 1990; 192: 577-82.
- [119] Abraham NG, Lin JH, Schwartzman ML, Levere RD, Shibahara S. The physiological significance of heme oxygenase. *Int J Biochem* 1988; 20: 543-58.
- [120] Abraham NG, Friedland ML, Levere RD. Heme metabolism in hepatic and erythroid cells. In: Brown E, editor. *Progress in Hematology*. Vol. XIII ed. New York: Grune and Stratton 1983; pp. 75-130.
- [121] Abraham N, Li M, Kim D, Kawakami T, Tsenovoy P, Rodella L, *et al.* Adiponectin is a downstream signal for heme oxygenase-1-mediated vascular protection. *Diabetes Res Clin Pract* 2008; (submitted).
- [122] Kappas A. A method for interdicting the development of severe jaundice in newborns by inhibiting the production of bilirubin. *Pediatrics* 2004; 113: 119-23.
- [123] Ishida H, Takizawa M, Ozawa S, Nakamichi Y, Yamaguchi S, Katsuta H, *et al.* Pioglitazone improves insulin secretory capacity and prevents the loss of beta-cell mass in obese diabetic db/db mice: Possible protection of beta cells from oxidative stress. *Metabolism* 2004; 53: 488-94.
- [124] Grosser N, Abate A, Oberle S, Vreman HJ, Dennery PA, Becker JC, *et al.* Heme oxygenase-1 induction may explain the antioxidant profile of aspirin. *Biochem Biophys Res Commun* 2003; 308: 956-60.
- [125] Nascimento-Silva V, Arruda MA, Barja-Fidalgo C, Villela CG, Fierro IM. Novel lipid mediator aspirin-triggered lipoxin A4 induces heme oxygenase-1 in endothelial cells. *Am J Physiol Cell Physiol* 2005; 289: C557-C563.
- [126] Becker JC, Grosser N, Boknik P, Schroder H, Domschke W, Pohle T. Gastroprotection by vitamin C--a heme oxygenase-1-dependent mechanism? *Biochem Biophys Res Commun* 2003; 312: 507-12.
- [127] Grosser N, Hemmerle A, Berndt G, Erdmann K, Hinkelmann U, Schurgerc S, *et al.* The antioxidant defense protein heme oxygenase 1 is a novel target for statins in endothelial cells. *Free Radic Biol Med* 2004; 37: 2064-71.
- [128] Grosser N, Erdmann K, Hemmerle A, Berndt G, Hinkelmann U, Smith G, *et al.* Rosuvastatin upregulates the antioxidant defense protein heme oxygenase-1. *Biochem Biophys Res Commun* 2004; 325: 871-6.
- [129] Lee TS, Chang CC, Zhu Y, Shyy JY. Simvastatin induces heme oxygenase-1: a novel mechanism of vessel protection. *Circulation* 2004; 110: 1296-302.
- [130] Oberle S, Abate A, Grosser N, Hemmerle A, Vreman HJ, Dennery PA, *et al.* Endothelial protection by pentaerythritol trinitrate: bilirubin and carbon monoxide as possible mediators. *Exp Biol Med (Maywood)* 2003; 228: 529-34.
- [131] Hsu M, Muchova L, Morioka I, Wong RJ, Schroder H, Stevenson DK. Tissue-specific effects of statins on the expression of heme oxygenase-1 *in vivo*. *Biochem Biophys Res Commun* 2006; 343: 738-44.
- [132] Choi BM, Kim YM, Jeong YR, Pae HO, Song CE, Park JE, *et al.* Induction of heme oxygenase-1 is involved in anti-proliferative effects of paclitaxel on rat vascular smooth muscle cells. *Biochem Biophys Res Commun* 2004; 321: 132-7.
- [133] Wu BJ, Kathir K, Witting PK, Beck K, Choy K, Li C, *et al.* Antioxidants protect from atherosclerosis by a heme oxygenase-1 pathway that is independent of free radical scavenging. *J Exp Med* 2006; 203: 1117-27.
- [134] Ishizaka N, De Leon H, Laursen JB, Fukui T, Wilcox JN, De Keulenaer G, *et al.* Angiotensin II-induced hypertension increases heme oxygenase-1 expression in rat aorta. *Circulation* 1997; 96: 1923-9.
- [135] Morice MC, Serruys PW, Sousa JE, Fajadet J, Ban HE, Perin M, *et al.* A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. *N Engl J Med* 2002; 346: 1773-80.
- [136] Scapagnini G, Foresti R, Calabrese V, Giuffrida Stella AM, Green CJ, Motterlini R. Caffeic acid phenethyl ester and curcumin: a novel class of heme oxygenase-1 inducers. *Mol Pharmacol* 2002; 61: 554-61.
- [137] Motterlini R, Foresti R, Bassi R, Green CJ. Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. *Free Radic Biol Med* 2000; 28: 1303-12.
- [138] Das S, Fraga CG, Das DK. Cardioprotective effect of resveratrol *via* HO-1 expression involves p38 map kinase and PI-3-kinase signaling, but does not involve NFkappaB. *Free Radic Res* 2006; 40: 1066-75.
- [139] Kaga S, Zhan L, Matsumoto M, Maulik N. Resveratrol enhances neovascularization in the infarcted rat myocardium through the induction of thioredoxin-1, heme oxygenase-1 and vascular endothelial growth factor. *J Mol Cell Cardiol* 2005; 39: 813-22.
- [140] Huang HM, Liang YC, Cheng TH, Chen CH, Juan SH. Potential mechanism of blood vessel protection by resveratrol, a component of red wine. *Ann N Y Acad Sci* 2005; 1042: 349-56.
- [141] Barbaro G. Visceral fat as target of highly active antiretroviral therapy-associated metabolic syndrome. *Curr Pharm Des* 2007; 13(21): 2208-13.
- [142] Chaldakov GN, Fiore M, Tonchev AB, Dimitrov D, Pancheva R, Rancic G, *et al.* Homo obesus: a metabotrophin-deficient species. *Pharmacology and nutrition insight. Curr Pharm Des* 2007; 13(21): 2176-9.
- [143] Kulkarni RG, Achaiah G, Sastry G N. Novel targets for antiinflammatory and antiarthritic agents. *Curr Pharm Des* 2006; 12(19): 2437-54.