Chapter 19
Selenoproteins in Cardiovascular Redox Pathology

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Abstract Redox-active selenoproteins, such as the mammalian glutathione peroxidases (GPxs), are essential components of the antioxidant defense systems that serve to limit the damaging accumulation of intracellular and extracellular oxidants. Accumulating evidence from epidemiological and experimental studies indicates that deficiencies in these key antioxidant proteins promote cardiovascular disease and that their excess is often protective against injury and stress. In this chapter, we will examine the role of GPxs in cardiovascular diseases, highlighting their role in modulating vascular function, thrombosis, and atherogenesis.

19.1 Introduction

A role for selenium in heart disease has been known for some time. In human populations, a cardiomyopathy, Keshan disease, is endemic in provinces of China with low selenium in the soil [1]. Decreased expression of selenoproteins is characteristic of this disease, and replacement of selenium in the diet increases selenoprotein expression and is a successful preventive treatment. Experimental evidence confirms a role for individual selenoproteins in complex cardiovascular diseases, such as atherosclerosis and stroke, primarily through modulating the damaging effects of reactive oxygen species (ROS). Here, we focus on the role of the glutathione peroxidases (GPxs) in modulating vascular function and cardiovascular disease risk.

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19.2 Mammalian Glutathione Peroxidase 1 (GPx-1)

GPx-1 is a ubiquitously expressed member of the GPx-family that contains a selenocysteine (Sec) at its catalytic site [2] and exists as a tetramer. It was the first Sec-containing protein to be identified [3]. GPx-1 is found intracellularly, primarily in cytosolic and mitochondrial compartments [4], where it reduces intracellular hydrogen and lipid hydroperoxides using glutathione as an obligate cofactor. Although knockout of GPx-1 in mouse models is not fatal, GPx-1 is necessary for optimal protection following direct exposure to oxidants [5, 6], or to stress-induced oxidants, such as those produced during ischemia-reperfusion or inflammation [7–11]. Thus, decreased expression of GPx-1 leads to enhanced susceptibility to injury in many disease models. Similarly, in individuals with coronary artery disease red blood cell GPx-1 activity was found to be a strong predictor of future cardiovascular events with individuals in the lowest tertile having over a threefold increase in cardiovascular disease risk compared with those in the highest tertile of activity [12]. Consistent with these findings in human populations, excess GPx-1 has been found to protect the cardiovascular system from oxidative stress-induced injury in transgenic mice overexpressing this selenoprotein.

19.2.1 Ischemia-Reperfusion Injury and GPx-1

GPx-1 has been found to modulate ischemia-reperfusion injury in both cerebral (stroke) and cardiac models of injury. In brain, GPx-1 deficient neurons were found to be more susceptible to apoptosis following middle cerebral artery (MCA) occlusion [7]. In addition, in this model, lack of GPx-1 results in increased production of oxidative stress markers and enhanced activation of NFκB, a process dependent, in part, on oxidant production [7, 13]. Furthermore, in GPx-1 deficient mice, cerebral injury was exacerbated by vascular dysfunction that limited microvascular blood flow following ischemia [14]. Injury following MCA occlusion could be limited by treatment with ebselen, a GPx-mimetic, which attenuated infarct size and improved vascular function in GPx-1 deficient mice [14]. (As discussed further in Sect. 19.2.3, endothelial dysfunction is a hallmark of endothelial redox imbalance that is caused by GPx-1 deficiency.) Although ebselen has a broader substrate specificity than GPx-1 and may have additional antioxidant effects on cells, similar protective effects of excess GPx-1 were found in transgenic GPx-1 overexpressing mice, which had significantly less cerebral injury following MCA ischemia-reperfusion injury than mice with normal levels of GPx-1 [15]. Overall these findings suggest a critical role for redox-balance in modulating neuronal protection in response to cerebral ischemia-reperfusion. Interestingly, it has been reported that ebselen may also improve neurological outcomes following stroke in human subjects [16]; however, these early studies have not been replicated.

In mouse cardiac ischemia-reperfusion injury models, GPx-1 has also been found to preserve cardiac function, as hearts from transgenic mice overexpressing GPx-1

are more resistant to myocardial ischemia-reperfusion injury than those from non-transgenic controls. Consistent with this finding, we found that aged GPx-1 deficient mice with 50% of the normal GPx-1 (heterozygous knockout mice) have structural abnormalities in the myocardial vasculature and diastolic dysfunction following myocardial ischemia-reperfusion [8]. Subsequent studies found increased susceptibility to myocardial injury following ischemia-reperfusion in hearts from male, but not female, GPx-1-deficient mice [17]. These findings may be attributed, in part, to other compensatory antioxidant mechanisms in female GPx-1 deficient mice that preserve pools of reduced ascorbate and augment the conversion of nitrate to nitrite, a possible cardio-protective species. Although the underlying basis for additional redox-protection in female mice is not known for certain, additional evidence suggests that estrogen may offer some protection in females. In addition to enhancing GPx-1 expression [18], estrogen may also control the expression of other redox-active selenoproteins, including GPx-3, that are upregulated in females compared to males [19, 20]. Most studies examining the role of GPx-1 in mice have been limited to male mice, where compensatory mechanisms are not sufficient to offer protection against oxidant stress in the context of GPx-1 deficiency. In a separate study, excess reactive oxygen generation in male GPx-1-deficient hearts subjected to ischemia-reperfusion injury correlated with diminished mitochondrial function, characterized by increased damage to mitochondrial DNA, decreased levels of mitochondrial protein expression, and reduced NADH and ATP generation [21]. GPx-1 is one of many antioxidant enzymes found in mitochondria, possibly to limit damage due to ROS normally generated in this organelle during respiration and following stress, such as during reperfusion following ischemia. Thus, ischemia-reperfusion injury generates excess ROS, in part, via increased mitochondrial output of oxidants and the damaging effects of mitochondria oxidants may be augmented by lack of GPx-1. In support of a crucial role of GPx-1 in mitochondrial ROS-flux, absence of GPx-1 has been shown to be accompanied by increased mitochondrial production of hydrogen peroxide [21, 22].

19.2.2 Cardiac Hypertrophy

Angiotensin II (AII) is a vasoactive peptide that promotes hypertension, vascular remodeling, and cardiac hypertrophy, in part, via AII-receptor 1 mediated activation of NADPH oxidases to increase superoxide generation. In studies by Ardanaz et al. [23], GPx-1 deficiency was found to augment specifically AII-induced left ventricular hypertrophy, increase myocyte cross-sectional area and intraventricular septal thickness, and lower cardiac shortening fraction after only 7 days of administration. Under this short-term AII treatment, there was a similar increase in blood pressure between the control and GPx-1-deficient mice, with no structural changes in aorta and no differences in cardiac fibrosis. Although the mechanistic basis for the increased cardiac hypertrophy and dysfunction is not fully known, it is likely that it is related to excess ROS caused by GPx-1 deficiency.

19.2.3 Endothelial Dysfunction and Vascular Tone

Endothelial dysfunction is in part characterized by a decrease in bioavailable nitric oxide (NO) and a subsequent loss in normal endothelium-dependent vasorelaxation responses to flow or to NO-dependent agonists, such as acetylcholine or bradykinin [24]. Excess ROS promotes endothelial dysfunction by reducing bioavailable NO directly (for instance by the reaction of NO with superoxide to form peroxynitrite) or by activating the production of superoxide from NADPH oxidase. In addition, ROS can decrease the availability of cofactors necessary for the activation of endothelial NO synthase (eNOS), which produces NO. Importantly, loss of bioavailable NO contributes to platelet activation, proliferation of vascular smooth muscle cells, and pro-inflammatory activation of the endothelium. We have found that GPx-1 plays an essential role in modulating endothelial function by preserving bioavailable NO. In mouse knockout models, both heterozygous and homozygous GPx-1 deficient mice [8, 25, 26] have endothelial dysfunction, consistent with a crucial role of normal levels of GPx-1 for vascular homeostasis. In fact, we found that GPx-1 deficiency resulted in a (paradoxical) vasoconstrictor response to the vasoactive agonists that cause vasodilation in normal vessels. Furthermore, endothelium-independent responses to NO generators, such as sodium nitroprusside, are preserved in GPx-1-deficient mice, indicating that the smooth muscle responses to NO are intact in these mice. Consistent with a role for GPx-1 in reducing oxidants, we found that plasma and aortic levels of the isoprostane, $iPF_{2\alpha}$-III, were increased by GPx-1 deficiency [8, 25]. Compensatory treatment with L-2-oxothiazolidine-4-carboxylic acid to increase intracellular thiol pools restored vasorelaxation responses in GPx-1-deficient mice [25], and lowered $iPF_{2\alpha}$-III levels.

Other studies indicate that excess GPx-1 can compensate for the negative effects of the vasoactive peptide AII [26]. Thus, carotid arteries from heterozygous GPx-1 knockout mice showed diminished vasodilatory responses to acetylcholine at low doses of AII that had no effect on endothelium-dependent vasodilation in wild type vessels [26], whereas carotid arteries from GPx-1 overexpressing mice were resistant to dysfunction caused by higher doses of AII that compromised vascular function in wild type vessels. Suppression of GPx-1 can also lead to endothelial dysfunction. Thus, in a mouse model of hyperhomocysteinemia caused by partial deficiency of the cystathionine-beta-synthase gene, we found that GPx-1 expression is suppressed [27], in part, by mechanisms that reduce the translation of GPx-1 [28]. As in the genetic knockout models, diminished GPx-1 expression in hyperhomocysteinemia also resulted in endothelial dysfunction [29] characterized by a reduction in bioavailable NO. Other studies have reported diminished endothelial function caused by a combination of GPx-1 deficiency and hyperhomocysteinemia [30]. Overexpression of GPx-1 in the context of hyperhomocysteinemia prevented the loss of bioavailable NO and restored normal endothelial vasodilatory responses [27]. Although studies in human populations clearly show an effect of homocysteine on cardiovascular risk [31], there is a growing controversy regarding the importance of homocysteine in human cardiovascular disease as simple B-vitamin therapies

that lower homocysteine levels fail to reduce disease risk [32, 33]. Nonetheless, in coronary artery disease patients, homocysteine and GPx-1 activity are predictors of cardiovascular disease, with the combination of lowest GPx-1 activity and highest plasma homocysteine conferring the greatest risk [31]. Furthermore, in human hypertensive patients, recent studies suggest that GPx-1 activity is inversely correlated with endothelium-dependent vasodilation responses, illustrating the importance of GPx-1 in modulating vascular function in humans as well as in animal models [34] and indicating that modest alterations in GPx-1 levels may significantly diminish endothelial function. Paradoxically, in some vascular beds, hydrogen peroxide may modulate arachidonic acid-mediated vasodilation [35], suggesting excess GPx-1 may limit these responses by reducing hydrogen peroxide essential for vessel relaxation. In support of this concept, excess GPx-1 has been shown to decrease vasodilatory responses to low micromolar concentrations of hydrogen peroxide in isolated cerebral vessels [36]. The specific consequence of decreased GPx-1 on these pathways is unclear; however, these and other studies suggest that there are complex effects of ROS on vascular function that may depend on many factors, including the amount of ROS, the type of ROS, and the time course of its production.

19.2.4 Inflammation and Atherogenesis

Endothelial dysfunction and oxidative stress are thought to promote atherogenesis; yet in the context of a high fat diet, GPx-1 deficiency on a C57Bl/6 background did not promote atherogenesis. Rather, GPx-1 deficient mice had decreased severity of aortic sinus lesions [37] possibly due, in part, to compensatory upregulation of glutaredoxin-2, a redox-active enzyme that can preserve protein thiol redox state [38]. In the context of ApoE deficiency, however, lack of GPx-1 was found to increase atherogenesis in response to a Western diet [39] and in combination with streptozotocin-induced diabetes mellitus [40]. In each of these models, GPx-1 deficiency was found to augment inflammatory changes associated with the development of atherosclerotic lesions. Notably, compared to ApoE-deficient mice, ApoE/GPx-1 double knockout mice showed excess aortic ROS production, enhanced NADPH-stimulated ROS production, and enhanced mitochondrial ROS generation, indicating increased vascular oxidant stress caused by lack of GPx-1 [39]. Other studies have found that ebselen, a GPx-mimic, decreases aortic lesion formation in ApoE-deficient diabetic mice, illustrating a role of oxidant stress in atherogenesis in ApoE-deficient mice. As mentioned above, ebselen has a broader substrate specificity than GPx-1 and can effectively reduce membrane phospholipids that are normally reduced intracellularly by GPx-4. As discussed further below, GPx-4 overexpression was also found to slow atherosclerotic lesion development in ApoE-deficient mice [41].

Other studies in endothelial cells suggest that GPx-1 modulates pro-atherogenic gene expression in response to intracellular oxidants generated during cyclic stress [42] or following endotoxin exposure [11]. In fact, GPx-1 deficiency alone promotes upregulation of adhesion molecules in human microvascular endothelial cells [11],

resulting in a pro-inflammatory state. In human subjects, the greatest risk of cardiovascular [43] events was found in individuals with a combination of the lowest levels of GPx-1 activity and most extensive atherosclerosis [44], suggesting that deficiencies in GPx-1 can potentiate human atherogenesis. These findings are consistent with the initial Athero*Gene* studies of coronary heart disease patients that reported a significant protective effect of increased levels of red blood cell GPx-1 activity against future cardiovascular events [12]. Furthermore, in humans, there is a genetic polymorphism of GPx-1 involving a T for a C substitution that results in an amino acid difference (Leu substitution for Pro) at position 198 (Pro198Leu) in the GPx-1 protein. It has been suggested that the Leu variant may be associated with decreased expression of GPx-1 under conditions where selenium is limited [45, 46]; studies indicate that the Leu variant may contribute to risk in Keshan disease [46], which is caused, in part, by selenium insufficiency. Similarly, in a case-control study of coronary artery disease patients in China, the presence of the Leu allele was associated with increased disease risk [47]. Other studies from Japan found an association of the Leu allele with increased risk of restonosis following stenting [48], and in other studies, enhanced vascular disease in Japanese type 2 diabetic subjects [43, 49]. Further analysis, however, is necessary to understand the significance of these GPx-1 polymorphisms and to determine if these variant proteins alter in vivo GPx-1 activity to modulate cardiovascular risk.

19.3 Glutathione Peroxidase-3 (GPx-3)

GPx-3 is a secreted glycoprotein, often referred to as plasma GPx. The major source of human plasma GPx-3 is renal proximal tubules [50], although recent findings suggest that adipose tissue may also contribute to circulating levels of GPx-3, at least in the mouse [51]. In both mice and humans, GPx-3 has been found in many other tissues including lung, heart, liver, brain, breast, placenta, skeletal muscle, and spleen. Similar to GPx-1, GPx-3 contains Sec at the active site, exists as a tetramer, and reduces hydrogen and lipid hydroperoxides. Unlike GPx-1, GPx-3 may utilize thioredoxin and glutaredoxin, as well as glutathione, as reducing cofactors [52]. Recent findings suggest that lack of GPx-3 in knockout mice is not fatal [53], although, functionally, lack of GPx-3 has been shown to cause NO insufficiency and promote thrombosis [54, 55], as discussed in the following section.

19.3.1 GPx-3, Stroke, and Thrombosis

In 1996, we found a causal relationship between a deficiency of GPx-3 and thrombotic stroke in two brothers [54] with childhood cerebrovascular thrombotic disease. Mechanistically, excess peroxides caused by the deficiency of GPx-3 promoted platelet activation by inactivating NO, a known inhibitor of platelet activation. Subsequent studies by our group found evidence for GPx-3 insufficiency in other

families predisposed to childhood stroke [55]. Although the underlying genetic defects in these families are unknown, the deficiency appears to be due to a dominantly inherited defect that reduces plasma GPx-3 activity approximately 50% in affected patients. Concurrent with a decrease in plasma GPx-3, NO fails to block platelet P-selectin expression and platelet aggregation in studies with normal gel-filtered platelets mixed with GPx-3 deficient plasma. These findings suggest that modest alterations in circulating GPx-3 can alter platelet homeostasis, thereby contributing to platelet-dependent thrombosis and stroke. Additional studies in our laboratory found that GPx-3 expression was transcriptionally upregulated by hypoxia [56], suggesting that increased expression of GPx-3 in response to lower oxygen tension, as in ischemic stroke, may guard against ROS-induced damage during reoxygenation. In our subsequent analysis of human thrombotic disorders, we have identified a variant haplotype (H_2) in the GPx-3 gene promoter that correlated with reduced transcriptional activity under normoxic and hypoxic conditions [57]. Furthermore, we found that this haplotype is a strong, independent risk factor for cerebral venous thrombosis [58] and that it is associated with increased risk of arterial ischemic stoke in young individuals [57]. Independent studies by Nowak-Gottl et al. [59] in a German cohort confirmed that the H_2-haplotype was a risk factor in arterial ischemic stroke in children. This latter study found no association between GPx-3 genotypes and thromboembolic or cerebral sinovenous thrombosis in children.

To study further the role of GPx-3 in maintaining the balance between hemostasis and thrombosis, we developed a GPx-3 knockout model. Consistent with altered platelet function in GPx-3 deficient patients, we found attenuated bleeding times, elevated soluble P-selectin (a marker of platelet and endothelial activation), and increased platelet aggregation in response to ADP infusion in an in vivo model of platelet activation as well as increased ADP-activation of platelets in in vitro platelet assays [60]. Several observations suggest the presence of NO insufficiency in these mice: circulating levels of cGMP are decreased, and vascular beds have endothelial dysfunction. To determine whether alterations in platelet function would result in stroke injury, we used the cerebral MCA ischemia-reperfusion model. We found that GPx-3-deficient mice were more sensitive to cerebral injury following MCA ischemia-reperfusion, with increased infarct size and greater neurological impairment. Clopidogrel, a platelet inhibitor, significantly reduced stroke volume and improved neurological function, suggesting that platelet activation contributed to the extensive injury caused by GPx-3 deficiency in this model. Furthermore, use of MnTBAP, an antioxidant, was similarly able to reduce brain injury following MCA ischemia-reperfusion, indicating the importance of oxidative mechanisms in the underlying dysfunction caused by GPx-3 deficiency.

19.4 Glutathione Peroxidase-4 (GPx-4)

GPx-4 is a widely expressed, intracellular selenoprotein that exists as a monomer rather than a tetramer. This enzyme is often referred to as the phospholipid GPx, as it can effectively reduce oxidized membrane phospholipids. GPx-4 exists in several

forms in the cell, including a long form with a mitochondrial targeting sequence that is found in mitochondria, and a short form that is found outside of the mitochondria. Although enzymatically, GPx-4 can reduce hydrogen and lipid hydroperoxides, intracellularly, it primarily reduces oxidized membrane phospholipids and has little effect on hydrogen peroxide levels. In mice, knockout of GPx-4 was found to be lethal, and its deficiency in cells grown in culture has been shown to promote apoptosis [61]. Thus, in order to study its in vivo protective function, studies have examined the consequences of overexpression of this essential selenoprotein, rather than its deficiency. Overexpression of GPx-4 has been found to increase survival to oxidants in cells grown in culture as well as in a transgenic mouse model [62, 63].

19.4.1 GPx-4 and Cardiac Ischemia-Reperfusion

To study the role of mitochondrial oxidants on ischemia-reperfusion injury, a transgenic mouse was engineered to overexpress specifically a rat mitochondrial form of GPx-4 [64] and used in the Langendorff model of global no-flow ischemia-reperfusion injury. In these studies, overexpression of GPx-4 resulted in improved contractile function characterized by improved rates of contraction, developed pressure, and peak-systolic pressure compared to non-transgenic hearts. These functional improvements may be the result of decreased mitochondrial damage, as there was less overall lipid peroxidation in mitochondria and electron transport complexes had preserved function in hearts from GPx-4 transgenic mice. Overall, these findings suggest that excess GPx-4 in mitochondria effectively removes harmful oxidants during ischemia-reperfusion to lessen cardiac contractile dysfunction.

19.4.2 GPx-4 and Atherogenesis

Excess GPx-4 was also found to lessen atherogenesis in ApoE-deficient mice [41]. This protective effect correlated with a reduction in lipid peroxidation in aorta without any change in overall plasma lipid levels. In isolated mouse aortic endothelial cells, overexpression of GPx-4 reduced endothelial production of hydroperoxides and decreased adhesion molecule expression in response to oxidized phospholipids, suggesting that GPx-4 overexpression reduces lipid oxidation and inflammatory responses to lessen atherogenesis. These studies also found that treatment of isolated cells with exogenous catalase could reduce hydroperoxide release more effectively, suggesting that in these cultured mouse endothelial cells, hydrogen peroxide is the major hydroperoxide generated in response to exposure to oxidized lipids.

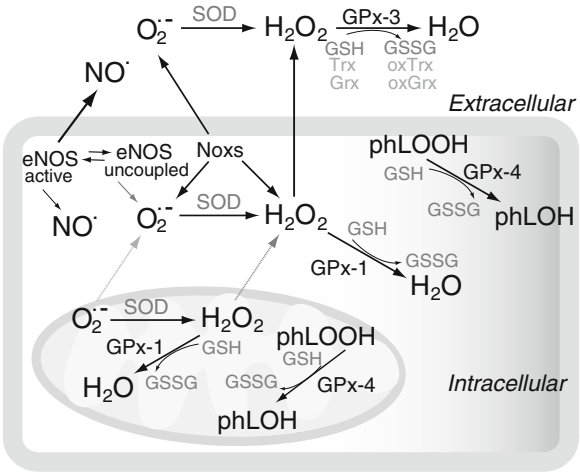

Fig. 19.1 Role of GPxs in modulating ROS-flux in endothelial cells. Superoxide is generated intracellularly from enzymatic sources as well as from mitochondrial respiration. Various NADPH oxidases (Noxs) may contribute to extracellular or intracellular superoxide pools and may also directly produce hydrogen peroxide. Other enzymatic sources, not represented in the figure, also contribute to superoxide and/or hydrogen peroxide production. Endothelial nitric oxide synthase (eNOS) is an essential source of nitric oxide (NO); however, in the absence of other reductive cofactors, this enzyme can become uncoupled leading to the production of superoxide. Superoxide is converted to hydrogen peroxide spontaneously or by a family of superoxide dismutases (SOD), one of which is in the mitochondria, one is cytoplasmic, and one is extracellular. Superoxide and other reactive oxygen species (ROS) contribute to phospholipid oxidation (phLOOH). GPx-1 and GPx-4 are both found in the cytoplasm and mitochondria where they reduce hydrogen and phospholipid hydroperoxides, respectively, using glutathione (GSH) as a reducing cofactor. GPx-3 is an extracellular glycoprotein that may utilize GSH, thioredoxin (Trx), or glutaredoxin (Grx) as cofactors in the enzymatic reduction of hydrogen peroxide. Reduction of cellular ROS maintains bioavailable nitric oxide. NO can readily combine with superoxide to produce peroxynitrite. In addition, excess ROS can diminish eNOS activity, leading to its uncoupling and further production of superoxide

19.5 Concluding Remarks

Clinical and experimental models suggest a crucial role for redox-active selenoproteins in modulating endothelial function to preserve bioavailable nitric oxide, regulate platelet homeostasis, and lessen atherogenesis. In addition, through their regulation of cellular oxidant accumulation, these enzymes also serve to moderate damage during ischemia/reperfusion, as is found following stroke or myocardial infarction. This chapter has focused on the role of the intracellular GPxs, GPx-1 and GPx-4, and the extracellular GPx-3, that together function to maintain optimal protection against soluble and membrane hydroperoxides (Fig. 19.1). Genetic and epidemiological studies indicate that GPx-1 and GPx-3 may both modulate cardiovascular disease

risk in human subjects, suggesting the importance of understanding the mechanisms by which these selenoproteins regulate the underlying disease processes.

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References

1. Cheng WH, Ho YS, Valentine BA et al (1998) J Nutr 128:1070
2. Papp LV, Lu J, Holmgren A et al (2007) Antioxid Redox Signal 9:775
3. Flohe L, Gunzler WA, Schock HH (1973) FEBS Lett 32:132
4. Esworthy RS, Ho YS, Chu FF (1997) Arch Biochem Biophys 340:59
5. de Haan JB, Bladier C, Griffiths P et al (1998) J Biol Chem 273:22528
6. Fu Y, Cheng WH, Ross DA et al (1999) Proc Soc Exp Biol Med 222:164
7. Crack PJ, Taylor JM, Flentjar NJ et al (2001) J Neurochem 78:1389
8. Forgione MA, Cap A, Liao R et al (2002) Circulation 106:1154
9. Lim CC, Bryan NS, Jain M et al (2009) Am J Physiol Heart Circ Physiol 297:H2144
10. Li C, Liu J, Waalkes MP et al (2003) Toxicol Lett 144:397
11. Lubos E, Mahoney CE, Leopold JA et al (2010) FASEB J 24:2525
12. Blankenberg S, Rupprecht HJ, Bickel C et al (2003) N Engl J Med 349:1605
13. Crack PJ, Taylor JM, Ali U et al (2006) Stroke 37:1533
14. Wong CH, Bozinovski S, Hertzog PJ et al (2008) J Neurochem 107:241
15. Weisbrot-Lefkowitz M, Reuhl K, Perry B et al (1998) Brain Res Mol Brain Res 53:333
16. Yamaguchi T, Sano K, Takakura K, Saito I et al (1998) Stroke 29:12
17. Chew P, Yuen DY, Koh P et al (2009) Arterioscler Thromb Vasc Biol 29:823
18. Borras C, Gambini J, Gomez-Cabrera MC et al (2005) Aging Cell 4:113
19. Lundholm L, Putnik M, Otsuki M et al (2008) J Endocrinol 196:547
20. Riese C, Michaelis M, Mentrup B, Gotz F et al (2006) Endocrinology 147:5883
21. Thu VT, Kim HK, Ha SH et al (2010) Pflugers Arch 460:55
22. Esposito LA, Kokoszka JE, Waymire KG et al (2000) Free Radic Biol Med 28:754
23. Ardanaz N, Yang XP, Cifuentes ME et al (2010) Hypertension 55:116
24. Harrison DG (1997) J Clin Invest 100:2153
25. Forgione MA, Weiss N, Heydrick S, Cap A et al (2002) Am J Physiol Heart Circ Physiol 282:H1255
26. Chrissobolis S, Didion SP, Kinzenbaw DA et al (2008) Hypertension 51:872
27. Weiss N, Zhang YY, Heydrick S et al (2001) Proc Natl Acad Sci USA 98:12503
28. Handy DE, Zhang Y, Loscalzo J (2005) J Biol Chem 280:15518
29. Eberhardt RT, Forgione MA, Cap A et al (2000) J Clin Invest 106:483
30. Dayal S, Brown KL, Weydert CJ et al (2002) Arterioscler Thromb Vasc Biol 22:1996
31. Schnabel R, Lackner KJ, Rupprecht HJ et al (2005) J Am Coll Cardiol 45:1631
32. Antoniades C, Antonopoulos AS, Tousoulis D et al (2009) Eur Heart J 30:6
33. Joseph J, Handy DE, Loscalzo J (2009) Cardiovasc Toxicol 9:53
34. de la Sierra A, Larrousse M (2010) J Hum Hypertens 24:373
35. Oltman CL, Kane NL, Miller FJ et al (2003) Am J Physiol Heart Circ Physiol 285:H2309
36. Modrick ML, Didion SP, Lynch CM et al (2009) J Cereb Blood Flow Metab 29:1130
37. de Haan JB, Witting PK, Stefanovic N et al (2006) J Lipid Res 47:1157
38. Beer SM, Taylor ER, Brown SE et al (2004) J Biol Chem 279:47939
39. Torzewski M, Ochsenhirt V, Kleschyov AL et al (2007) Arterioscler Thromb Vasc Biol 27:850
40. Lewis P, Stefanovic N, Pete J et al (2007) Circulation 115:2178

41. Guo Z, Ran Q, Roberts LJ II, Zhou L, Richardson A, Sharan C, Wu D, Yang H (2008) Free Radic Biol Med 44:343
42. Wagner AH, Kautz O, Fricke K et al (2009) Arterioscler Thromb Vasc Biol 29:1894
43. Nemoto M, Nishimura R, Sasaki T et al (2007) Cardiovasc Diabetol 6:23
44. Espinola-Klein C, Rupprecht HJ, Bickel C et al (2007) Am J Cardiol 99:808
45. Hu YJ, Diamond AM (2003) Cancer Res 63:3347
46. Lei C, Niu X, Wei J et al (2009) Clin Chim Acta 399:102
47. Tang NP, Wang LS, Yang L et al (2008) Clin Chim Acta 395:89
48. Oguri M, Kato K, Hibino T et al (2007) Atherosclerosis 194:e172
49. Hamanishi T, Furuta H, Kato H et al (2004) Diabetes 53:2455
50. Avissar N, Ornt DB, Yagil Y et al (1994) Am J Physiol 266:C367
51. Lee YS, Kim AY, Choi JW et al (2008) Mol Endocrinol 22:2176
52. Bjornstedt M, Xue J, Huang W et al (1994) J Biol Chem 269:29382
53. Olson GE, Whitin JC, Hill KE et al (2010) Am J Physiol Renal Physiol 298:F1244
54. Freedman JE, Loscalzo J, Benoit SE et al (1996) J Clin Invest 97:979
55. Kenet G, Freedman J, Shenkman B et al (1999) Arterioscler Thromb Vasc Biol 19:2017
56. Bierl C, Voetsch B, Jin RC et al (2004) J Biol Chem 279:26839
57. Voetsch B, Jin RC, Bierl C et al (2007) Stroke 38:41
58. Voetsch B, Jin RC, Bierl C et al (2008) Stroke 39:303
59. Nowak-Gottl U, Fiedler B, Huge A et al (2011) J Thromb Haemost 9:33
60. Jin RC, Mahoney CE, Anderson LC, et al (2011) Circulation, 123:1963
61. Seiler A, Schneider M, Forster H et al (2008) Cell Metab 8:237
62. Yagi K, Komura S, Kojima H et al (1996) Biochem Biophys Res Commun 219:486
63. Ran Q, Liang H, Gu M et al (2004) J Biol Chem 279:55137
64. Dabkowski ER, Williamson CL, Hollander JM (2008) Free Radic Biol Med 45:855