Endothelial Nitric Oxide Synthase

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Endothelial function is largely based on endothelial nitric oxide synthase (eNOS) function and activity. Likewise, oxidative stress can lead to the loss of eNOS activity or even “uncoupling” of the enzyme by adverse regulation of well-defined “redox switches” in eNOS itself or up-/down-stream signaling molecules.

Keywords: eNOS ; Cardiovascular Disease ; Redox

1. Introduction

There are classical regulatory mechanisms of eNOS activity, such as calcium/calmodulin, caveolin, HSP90, palmitoylation, and myristoylation, which also control the activating phosphorylation by protein kinase B (Akt) or AMP-activated protein kinase (AMPK) at Ser1177 (Ser1179) as well as the localization of eNOS. The non-classical regulation of eNOS activity is based on the formation of redox-active species that trigger adverse phosphorylation by redox-active kinases at Thr495/Tyr657 (e.g., kinases PKC and PYK-2), S-glutathionylation, oxidative tetrahydrobiopterin depletion, dysregulation of asymmetric dimethylarginine (ADMA) formation/degradation, and disruption of the zinc-sulfur-complex stabilizing the eNOS dimer (reviewed in [1][2]). These “redox switches“ in eNOS confer alterations in enzymatic eNOS activity and they may contribute to the uncoupling of eNOS. eNOS uncoupling is characterized by leakage of electrons from the transport chain in the reductase domain (from NADPH over flavins FMN and FAD) and transfer to molecular oxygen yielding superoxide instead of NO, thereby switching the enzyme from a nitric oxide to a superoxide source [3][4]. NO can be antagonized by superoxide anion radicals, leading to the formation of the cytotoxic oxidant peroxynitrite, as shown by Gryglewski, Palmer and Moncada [5]. Also, down-stream of eNOS, the NO/cGMP signaling pathway contains a number of redox switches. The soluble guanylyl cyclase (sGC) can be inactivated via thiol oxidation, S-nitros(yl)ation, or ROS-triggered loss of the heme-group [6][2]. Likewise, cGMP-degrading phosphodiesterases are activated by ROS [4][7]. Other regulatory pathways of vascular function, such as endothelin-1 and prostaglandins, are under redox control. The endothelin-1 mRNA promoter is stabilized by ROS and vice versa endothelin-1 can stimulate NADPH oxidase activity and expression levels [2]. Finally, prostaglandin formation is redox-regulated by the “peroxide tone” needed for the activity of cyclooxygenase-1/2, inactivation of prostacyclin synthase by tyrosine nitration, and as a consequence of these two oxidative processes a switch of the prostanoid synthesis to thromboxane inducing a vasoconstrictory and aggregatory phenotype [2].

2. Oxidative Depletion of Tetrahydrobiopterin

Oxidative loss of tetrahydrobiopterin (BH_4) as a trigger for eNOS uncoupling is the best characterized “redox switch” in eNOS and meanwhile well documented in hypertension, diabetes, and atherosclerosis [8][9][10][11][12][13]. Reviews on the role of BH_4 deficiency in almost all cardiovascular diseases provide detailed insights on the mechanisms [14][15][16][17][18]. The enzymatic source for BH_4 synthesis, GTP-cyclohydrolase-1 (GCH-1), was identified as an important regulator of eNOS and endothelial function [19]. The stoichiometry between eNOS and GCH-1 expression controls endothelial function and eNOS overexpression without matched increase in BH_4 levels will ultimately result in eNOS uncoupling [12]. In accordance with the concept of direct oxidative depletion of BH_4 by peroxynitrite, an oxidant-driven proteasomal degradation of the GCH-1 has been demonstrated via peroxynitrite- or superoxide-mediated activation of the proteasome 26S [20][21][22]. Recently, Chuaiphichai et al. have demonstrated that genetic endothelial-specific GCH-1 deficiency in $Gch1^{fl/fl}$Tie2cre mice causes eNOS uncoupling (evident by increased endothelial superoxide formation) and an impaired endothelium-dependent relaxation in arterial resistance arteries [23][24]. These data indicate that endothelial GCH-1 is the essential regulator of eNOS functionality and it can prevent uncoupling of eNOS. In a just published work, the same group show that BH_4 deficiency in endothelial and macrophages is required to trigger endothelial dysfunction and the enhancement of atherosclerosis by using endothelial/myeloid-specific GCH-1 and ApoE global knockout ($Gch1^{fl/fl}$Tie2CreApoE$^{-/-}$) mice and bone marrow chimeras thereof [25][26]. These data indicate that NOS isoforms in the vasculature (eNOS) and immune cells (iNOS) need tight regulation by BH_4 in order to prevent the progression of

atherosclerosis, as already suggested [27], thereby providing another strong link between vascular function, cardiovascular health, and inflammation. Besides the GCH-1 dependent de novo synthesis of BH_4, the so called “salvage pathway”, is of high physiological importance, consisting of the recycling of oxidized BH_2 back to BH_4 by dihydrofolate reductase (DHFR) [28][29]. Also, DHFR is subject to 26S proteasome-dependent degradation, a process that was prevented by S-nitros(yl)ation of DHFR by eNOS-derived NO [30]. Therefore, the BH_4 regulatory system itself provides multiple pharmacological targets for therapeutic prevention of endothelial dysfunction and the progression of cardiovascular disease[31]. As suggested previously, a combination of antioxidant therapy and BH_4 supplementation may be required in order to successfully treat cardiovascular diseases [32].

3. Oxidative Disruption of the Zinc-Sulfur-Complex ($ZnCys_4$) in the Binding Region of the eNOS Dimer

Another direct redox-regulatory pathway for eNOS function is the oxidative disruption of the zinc-sulfur-complex ($ZnCys_4$) in the binding region of the eNOS dimer, resulting in a loss of sodium dodecyl sulfate (SDS)-resistant eNOS dimers, which has been first described by Zou and coworkers for peroxynitrite-mediated oxidation of eNOS [33]. The reports on this “redox switch” reflected by a decreased eNOS dimer/monomer ratio were previously summarized until the year 2010 [1][14]. More recent studies reported impaired endothelial function and a decrease in eNOS dimer/monomer ratio by CRP treatment of rats [34], in old rats with improvement by arginase inhibition [35], in mild hyperhomocysteinemia in mice with heterozygous gene deletion of methylenetetrahydrofolate reductase ($Mthfr^{+/-}$) [36], and in 8-nitro-cGMP treated mice [37]. Similar observations were made in caveolin-1 depleted and angiotensin-II stimulated endothelial cells [38], in diabetic rats with improvement by nicorandil [39], in diabetic rats with improvement by green tea extract [40], in hypoxic pigs with improvement by L-citrulline [41], and in tachycardia/sympathetic over-activation in mice by isoproterenol [42]. eNOS dimer/monomer ratio was also decreased in diabetic db/db mice and normalized by saxagliptin [43], in erectile tissue of type 2 diabetes mellitus (T2DM) men [44], in mice with doxorubicin-induced cardiomyopathy and improvement by folic acid [45], in spontaneously hypertensive rats [46], and the improvement of eNOS coupling by increased eNOS dimer/monomer ratio by exercise training [47][48]. The critical role of this zinc-sulfur-complex for proper eNOS dimer formation was demonstrated by significant monomerization in a knock-in mouse expressing a C101A-eNOS mutant with impaired zinc-sulfur-complex forming ability [49]. Of note, these C101A-eNOS-transgenic mice displayed normal systolic blood pressure despite higher levels of eNOS, whereas mice overexpressing wild type eNOS showed significant hypotension [50]. According to another hypothesis, the dimer formation is mainly regulated by BH_4 binding in a pocket of the dimer interface and prevention of the BH_4 binding by mutation of the critical tryptophan 447 caused monomerization of the eNOS enzyme [51].

4. S-glutathionylation of the eNOS Reductase Domain

S-glutathionylation represents another important “redox switch” in eNOS. Zweier and coworkers showed that eNOS is adversely regulated and uncoupled (leading to superoxide formation) by S-glutathionylation at cysteine residues Cys689 and Cys908 in the reductase domain [54][55]. The reports on this “redox switch” that were reflected by eNOS S-glutathionylation were previously summarized until the year 2011 [1][14][56]. More recent in vivo studies reported on an association of eNOS S-glutathionylation with eNOS uncoupling and/or endothelial dysfunction in isosorbide-5-mononitrate treated mice [57], in hypertensive mice with improvement by inhibition of mitochondrial permeability transition pore opening [58], in aged mice with potentiation by glutathione peroxidase-1 deficiency [59], in atherosclerotic mice treated with carbamylated low-density lipoprotein [60], in mice with lung injury after lipopolysaccharide challenges [61], in mice with cardiac pressure-overload and improvement by N-acetylcysteine [62], in rats with T2DM and erectile dysfunction [63], and in mice with doxorubicin-induced cardiomyopathy and improvement by folic acid [45]. Also, environmental risk factors, such as traffic noise exposure, were associated with eNOS S-glutathionylation in mice exposed to aircraft noise for several days [64][65]. eNOS S-glutathionylation in mice with pressure overload produced by transverse aortic constriction was normalized by physical exercise [66]. eNOS S-glutathionylation was also observed in a knock-in mouse expressing a C101A-eNOS mutant with impaired zinc-sulfur-complex forming ability, which was also associated with increased superoxide production and protein tyrosine nitration [50]. Also, ex vivo experiments yielded similar results and eNOS S-glutathionylation with eNOS uncoupling was observed in human aortic endothelial cells that were treated with ultrafine particles [67] and in endothelial cells upon hypoxia/reoxygenation (associated with BH_4 depletion) [68]. This redox-regulatory mechanism gained even more biological relevance when Zweier and colleagues showed that eNOS S-glutathionylation can be reversed by glutaredoxin-1 [69]. An essential role of glutaredoxin-1 for the control of eNOS S-glutathionylation was also demonstrated in mice with necrotizing enterocolitis [70][71]. eNOS S-glutathionylation in experimental fibrosis is directly connected to glutathione biosynthesis [72]. eNOS S-glutathionylation is obviously connected to BH_4 deficiency [73] and also a direct connection between nNOS function and eNOS S-glutathionylation was

shown in the heart [74]. It remains to be established whether eNOS S-glutathionylation represents a drug-targetable pathomechanism. However, it at least represents a surrogate marker to monitor the successful recoupling of eNOS by pharmacological or non-pharmacological therapy.

5. Phosphorylation at Thr495 and Tyr657

Regulation of eNOS is also mediated by phosphorylation via redox-sensitive kinases. Whereas, eNOS phosphorylation at Ser1177 via Akt pathway is of activating nature [75], phosphorylation at Tyr657 mediated by the protein tyrosine kinase-2 (PYK-2) is of inactivating nature [76]. Also, phosphorylation at Thr495 that is mediated by protein kinase C (PKC) leads to the inactivation and potential uncoupling of eNOS [77][78][79], and PKC is also a potent activator of NADPH oxidases NOX-1 and NOX-2. PKC has redox-sensitive thiols in its phorbol ester/diacylglycerol binding site and it can be activated by hydrogen peroxide [80][81]. PYK-2 was reported to be stimulated by authentic hydrogen peroxide as well as by angiotensin-II stimulated ROS formation in cell culture and in vivo [76]. The reports on this “redox switch” reflected by adverse phosphorylation by redox-sensitive kinases were previously reviewed in detail [1][14][56], but some old reports and new studies were not considered and they are highlighted here. Homocysteine obviously causes dysregulation of eNOS via PKC activation and adverse (de)phosphorylation of eNOS in human platelets [82]. Treatment of human aortic endothelial cells with CRP caused the dephosphorylation of eNOS at Ser1177 and phosphorylation at Thr495 [83]. In isolated, pre-contracted, endothelium-intact porcine coronary arteries, amlodipine increased eNOS phosphorylation at Ser1177 and it decreased the one at Thr495, which was associated with an improvement of bradykinin-dependent relaxation [84]. Likewise, the AT1-receptor blocker telmisartan improved the ratio of phosphoThr495/phosphoSer1177 of eNOS in cultured endothelial cells [85] and nitroglycerin-treated rats [86]. Simvastatin was reported to increase NO formation, eNOS phosphorylation at Ser1177, to decrease phosphorylation at Thr495, and improve cardiac function in patients undergoing cardiac surgery [87]. Other therapeutic strategies for improvement of the ratio of phosphoThr495/phosphoSer1177 in eNOS were reported for exercise of mice [88][89], inhibition of the ROCK and ERK kinase pathways by MEKK1/2 inhibitor U0126 and ROCK inhibitor Y27632, thereby preventing the oxidative activation of PKC in human umbilical vein endothelial cells (HUVECs) [90]. Conversely, there seems to be a feedback mechanism to prevent over-activation of the “NO pathway”, as demonstrated by a decrease in Ser1177 and an increase in Thr495 phosphorylation of eNOS by chronic administration of high dose inorganic nitrate, which also resulted in impaired endothelium-dependent relaxation [91], providing a possible explanation as to why chronic NO donor therapy is not suitable for targeting the NO/cGMP pathway.

6. ADMA Formation and Degradation by DDAH

Asymmetric dimethylarginine (ADMA) is a potent endogenous inhibitor of eNOS [92], which may trigger the uncoupling of eNOS [93]. ADMA serum/plasma levels have prognostic value for future cardiovascular events in patients at increased risk [94][95]. ADMA as an eNOS “redox switch” was previously reviewed in more detail and is based on increased ADMA generation (e.g., by the activation of S-adenosylmethionine-dependent protein arginine methyltransferase (PRMT, type I)) and on decreased ADMA degradation (e.g., by inhibition of hydrolyzing dimethylarginine dimethylaminohydrolase (DDAH) enzymes) under oxidative stress conditions [1][14][56]. The relationship between ADMA, eNOS activity, and oxidative stress was also highlighted by Sydow and Münzel [93]. According to an overview on circulating biomarkers in diabetic patients, ADMA may be used as an indicator of endothelial dysfunction in diabetes [96]. Rosuvastatin attenuated isoproterenol-induced hypertrophy, remodeling/dysfunction of the ventricle, and increased myocardial NO in rats, which was associated with normalized ADMA and PRMT1/DDAH2 expression levels [97]. Simvastatin increased DDAH1 expression levels and decreased ADMA concentrations in cultured endothelial cells [98]. Another study reported that coronary artery disease patients with too high ADMA serum concentrations are not protected anymore by simvastatin therapy against cardiovascular events, which however could be prevented by DDAH2 overexpression in a related experimental animal model [98]. Serum ADMA levels were significantly increased and endothelial function and DDAH activity were inhibited in hyperlipidemic rabbits, all of which were improved by therapy with the angiotensin converting enzyme (ACE) inhibitor captopril [99].

7. L-Arginine Deficiency

L-Arginine is the endogenous substrate of eNOS and “L-arginine deficiency” may contribute to the uncoupling of eNOS [100]. However, the K_m (the concentration of L-arginine that is necessary for half maximal saturation) of eNOS for L-arginine is approximately 2.9 µM, whereas intracellular L-arginine concentrations are usually in the mM range [1][14][56]. Therefore, L-arginine depletion as a regulator of eNOS was considered to be unlikely. Nevertheless, a number of pre-clinical and clinical studies reported on highly beneficial effects by oral L-arginine supplementation and also increased

superoxide formation by isolated NOS enzymes in the absence of L-arginine was observed. The direct antioxidant effects of the guanidino-group L-arginine may be one explanation for these observations [101]. Improved export of ADMA from endothelial cells by high dose L-arginine represents another explanation, which would become even more attractive when the y^+L amino acid transporters (y^+LAT-1 and -2) are impaired (e.g., by genetic deficiency) and the cationic amino acid transporter (CAT-1) takes over using cationic amino acids, such as L-arginine for the export of ADMA [102].

L-arginine deficiency can also occur as a result of arginase induction. Arginases are major L-arginine-consuming enzymes that metabolize L-arginine to urea and L-ornithine. The up-regulation of arginase limits L-arginine bioavailability for eNOS, which represents a mechanism of eNOS uncoupling [105]. In the PVAT of diet-induced obese mice, arginase expression was enhanced and L-arginine content was reduced. These changes were associated with an uncoupling of PVAT eNOS [106]. Importantly, the incubation of PVAT-containing aorta with a combination of L-arginine and an arginase inhibitor for 30 min ex vivo in organ chambers restored the vasodilator effects of PVAT-containing aortas from obese mice [106]. These results demonstrate that L-arginine deficiency is indeed a reason for PVAT eNOS dysfunction and that eNOS uncoupling is causally involved in obesity-induced vascular dysfunction [107].

References

1. Schulz, E.; Wenzel, P.; Munzel, T.; Daiber, A. Mitochondrial redox signaling: Interaction of mitochondrial reactive oxygen species with other sources of oxidative stress. Antioxid. Redox Signal. 2014, 20, 308–324.
2. Daiber, A.; Di Lisa, F.; Oelze, M.; Kroller-Schon, S.; Steven, S.; Schulz, E.; Munzel, T. Crosstalk of mitochondria with NADPH oxidase via reactive oxygen and nitrogen species signalling and its role for vascular function. Br. J. Pharmacol. 2017, 174, 1670–1689.
3. Forstermann, U.; Munzel, T. Endothelial nitric oxide synthase in vascular disease: From marvel to menace. Circulation 2006, 113, 1708–1714.
4. Munzel, T.; Daiber, A.; Ullrich, V.; Mulsch, A. Vascular consequences of endothelial nitric oxide synthase uncoupling for the activity and expression of the soluble guanylyl cyclase and the cGMP-dependent protein kinase. Arterioscler. Thromb. Vasc. Biol. 2005, 25, 1551–1557.
5. Gryglewski, R.J.; Palmer, R.M.; Moncada, S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. Nature 1986, 320, 454–456.
6. Daiber, A.; Steven, S.; Weber, A.; Shuvaev, V.V.; Muzykantov, V.R.; Laher, I.; Li, H.; Lamas, S.; Munzel, T. Targeting vascular (endothelial) dysfunction. Br. J. Pharmacol. 2017, 174, 1591–1619.
7. Munzel, T.; Daiber, A.; Gori, T. Nitrate therapy: New aspects concerning molecular action and tolerance. Circulation 2011, 123, 2132–2144.
8. Landmesser, U.; Dikalov, S.; Price, S.R.; McCann, L.; Fukai, T.; Holland, S.M.; Mitch, W.E.; Harrison, D.G. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J. Clin. Investig. 2003, 111, 1201–1209.
9. Vasquez-Vivar, J.; Kalyanaraman, B.; Martasek, P.; Hogg, N.; Masters, B.S.; Karoui, H.; Tordo, P.; Pritchard, K.A., Jr. Superoxide generation by endothelial nitric oxide synthase: The influence of cofactors. Proc. Natl. Acad. Sci. USA 1998, 95, 9220–9225.
10. Guzik, T.J.; Mussa, S.; Gastaldi, D.; Sadowski, J.; Ratnatunga, C.; Pillai, R.; Channon, K.M. Mechanisms of increased vascular superoxide production in human diabetes mellitus: Role of NAD(P)H oxidase and endothelial nitric oxide synthase. Circulation 2002, 105, 1656–1662.
11. Alp, N.J.; Mussa, S.; Khoo, J.; Cai, S.; Guzik, T.; Jefferson, A.; Goh, N.; Rockett, K.A.; Channon, K.M. Tetrahydrobiopterin-dependent preservation of nitric oxide-mediated endothelial function in diabetes by targeted transgenic GTP-cyclohydrolase I overexpression. J. Clin. Investig. 2003, 112, 725–735.
12. Bendall, J.K.; Alp, N.J.; Warrick, N.; Cai, S.; Adlam, D.; Rockett, K.; Yokoyama, M.; Kawashima, S.; Channon, K.M. Stoichiometric relationships between endothelial tetrahydrobiopterin, endothelial no synthase (eNOS) activity, and eNOS coupling in vivo: Insights from transgenic mice with endothelial-targeted GTP cyclohydrolase 1 and eNOS overexpression. Circ. Res. 2005, 97, 864–871.
13. Laursen, J.B.; Somers, M.; Kurz, S.; McCann, L.; Warnholtz, A.; Freeman, B.A.; Tarpey, M.; Fukai, T.; Harrison, D.G. Endothelial regulation of vasomotion in apoE-deficient mice: Implications for interactions between peroxynitrite and tetrahydrobiopterin. Circulation 2001, 103, 1282–1288.
14. Daiber, A.; Oelze, M.; Daub, S.; Steven, S.; Schuff, A.; Kroller-Schon, S.; Hausding, M.; Wenzel, P.; Schulz, E.; Gori, T.; et al. Vascular redox signaling, redox switches in endothelial nitric oxide synthase and endothelial dysfunction. In

Systems Biology of Free Radicals and Antioxidants; Laher, I., Ed.; Springer: Berlin/Heidelberg, Germany, 2014; pp. 1177–1211.

15. Schulz, E.; Jansen, T.; Wenzel, P.; Daiber, A.; Munzel, T. Nitric oxide, tetrahydrobiopterin, oxidative stress, and endothelial dysfunction in hypertension. Antioxid. Redox Signal. 2008, 10, 1115–1126.
16. Harrison, D.G.; Chen, W.; Dikalov, S.; Li, L. Regulation of endothelial cell tetrahydrobiopterin pathophysiological and therapeutic implications. Adv. Pharmacol. 2010, 60, 107–132.
17. Bendall, J.K.; Douglas, G.; McNeill, E.; Channon, K.M.; Crabtree, M.J. Tetrahydrobiopterin in cardiovascular health and disease. Antioxid. Redox Signal. 2014, 20, 3040–3077.
18. Vasquez-Vivar, J. Tetrahydrobiopterin, superoxide, and vascular dysfunction. Free Radic. Biol. Med. 2009, 47, 1108–1119.
19. Alp, N.J.; Channon, K.M. Regulation of endothelial nitric oxide synthase by tetrahydrobiopterin in vascular disease. Arterioscler. Thromb. Vasc. Biol. 2004, 24, 413–420.
20. Xu, J.; Wang, S.; Wu, Y.; Song, P.; Zou, M.H. Tyrosine nitration of PA700 activates the 26S proteasome to induce endothelial dysfunction in mice with angiotensin II-induced hypertension. Hypertension 2009, 54, 625–632.
21. Xu, J.; Wu, Y.; Song, P.; Zhang, M.; Wang, S.; Zou, M.H. Proteasome-dependent degradation of guanosine 5′-triphosphate cyclohydrolase I causes tetrahydrobiopterin deficiency in diabetes mellitus. Circulation 2007, 116, 944–953.
22. Whitsett, J.; Picklo, M.J., Sr.; Vasquez-Vivar, J. 4-hydroxy-2-nonenal increases superoxide anion radical in endothelial cells via stimulated GTP cyclohydrolase proteasomal degradation. Arterioscler. Thromb. Vasc. Biol. 2007, 27, 2340–2347.
23. Munzel, T.; Daiber, A. Does endothelial tetrahydrobiopterin control the endothelial no synthase coupling state in arterial resistance arteries? Br. J. Pharmacol. 2017, 174, 2422–2424.
24. Chuaiphichai, S.; Crabtree, M.J.; McNeill, E.; Hale, A.B.; Trelfa, L.; Channon, K.M.; Douglas, G. A key role for tetrahydrobiopterin-dependent endothelial NOS regulation in resistance arteries: Studies in endothelial cell tetrahydrobiopterin-deficient mice. Br. J. Pharmacol. 2017, 174, 657–671.
25. Douglas, G.; Hale, A.B.; Patel, J.; Chuaiphichai, S.; Al Haj Zen, A.; Rashbrook, V.S.; Trelfa, L.; Crabtree, M.J.; McNeill, E.; Channon, K.M. Roles for endothelial cell and macrophage Gch1 and tetrahydrobiopterin in atherosclerosis progression. Cardiovasc. Res. 2018, 114, 1385–1399.
26. Munzel, T.; Daiber, A. Role of endothelial and macrophage tetrahydrobiopterin in development and progression of atherosclerosis: BH4 puzzle solved? Cardiovasc. Res. 2018, 114, 1310–1312.
27. Kossmann, S.; Hu, H.; Steven, S.; Schonfelder, T.; Fraccarollo, D.; Mikhed, Y.; Brahler, M.; Knorr, M.; Brandt, M.; Karbach, S.H.; et al. Inflammatory monocytes determine endothelial nitric-oxide synthase uncoupling and nitro-oxidative stress induced by angiotensin II. J. Biol. Chem. 2014, 289, 27540–27550.
28. Chalupsky, K.; Cai, H. Endothelial dihydrofolate reductase: Critical for nitric oxide bioavailability and role in angiotensin II uncoupling of endothelial nitric oxide synthase. Proc. Natl. Acad. Sci. USA 2005, 102, 9056–9061.
29. Ionova, I.A.; Vasquez-Vivar, J.; Whitsett, J.; Herrnreiter, A.; Medhora, M.; Cooley, B.C.; Pieper, G.M. Deficient BH4 production via de novo and salvage pathways regulates no responses to cytokines in adult cardiac myocytes. Am. J. Physiol. Heart Circ. Physiol. 2008, 295, H2178–H2187.
30. Cai, Z.; Lu, Q.; Ding, Y.; Wang, Q.; Xiao, L.; Song, P.; Zou, M.H. Endothelial nitric oxide synthase-derived nitric oxide prevents dihydrofolate reductase degradation via promoting S-nitrosylation. Arterioscler. Thromb. Vasc. Biol. 2015, 35, 2366–2373.
31. Munzel, T.; Daiber, A. Redox regulation of dihydrofolate reductase: Friend or troublemaker? Arterioscler. Thromb. Vasc. Biol. 2015, 35, 2261–2262.
32. Munzel, T.; Gori, T.; Bruno, R.M.; Taddei, S. Is oxidative stress a therapeutic target in cardiovascular disease? Eur. Heart J. 2010, 31, 2741–2748.
33. Zou, M.H.; Shi, C.; Cohen, R.A. Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. J. Clin. Investig. 2002, 109, 817–826.
34. Hein, T.W.; Singh, U.; Vasquez-Vivar, J.; Devaraj, S.; Kuo, L.; Jialal, I. Human C-reactive protein induces endothelial dysfunction and uncoupling of eNOS in vivo. Atherosclerosis 2009, 206, 61–68.
35. Kim, J.H.; Bugaj, L.J.; Oh, Y.J.; Bivalacqua, T.J.; Ryoo, S.; Soucy, K.G.; Santhanam, L.; Webb, A.; Camara, A.; Sikka, G.; et al. Arginase inhibition restores NOS coupling and reverses endothelial dysfunction and vascular stiffness in old rats. J. Appl. Physiol. 2009, 107, 1249–1257.

36. Lemarie, C.A.; Shbat, L.; Marchesi, C.; Angulo, O.J.; Deschenes, M.E.; Blostein, M.D.; Paradis, P.; Schiffrin, E.L. Mthfr deficiency induces endothelial progenitor cell senescence via uncoupling of eNOS and downregulation of SIRT1. Am. J. Physiol. Heart Circ. Physiol. 2011, 300, H745–H753.

37. Tokutomi, Y.; Kataoka, K.; Yamamoto, E.; Nakamura, T.; Fukuda, M.; Nako, H.; Toyama, K.; Dong, Y.F.; Ahmed, K.A.; Sawa, T.; et al. Vascular responses to 8-nitro-cyclic GMP in non-diabetic and diabetic mice. Br. J. Pharmacol. 2011, 162, 1884–1893.

38. Lobysheva, I.; Rath, G.; Sekkali, B.; Bouzin, C.; Feron, O.; Gallez, B.; Dessy, C.; Balligand, J.L. Moderate caveolin-1 downregulation prevents NADPH oxidase-dependent endothelial nitric oxide synthase uncoupling by angiotensin II in endothelial cells. Arterioscler. Thromb. Vasc. Biol. 2011, 31, 2098–2105.

39. Serizawa, K.; Yogo, K.; Aizawa, K.; Tashiro, Y.; Ishizuka, N. Nicorandil prevents endothelial dysfunction due to antioxidative effects via normalisation of NADPH oxidase and nitric oxide synthase in streptozotocin diabetic rats. Cardiovasc. Diabetol. 2011, 10, 105.

40. Faria, A.M.; Papadimitriou, A.; Silva, K.C.; Lopes de Faria, J.M.; Lopes de Faria, J.B. Uncoupling endothelial nitric oxide synthase is ameliorated by green tea in experimental diabetes by re-establishing tetrahydrobiopterin levels. Diabetes 2012, 61, 1838–1847.

41. Fike, C.D.; Dikalova, A.; Kaplowitz, M.R.; Cunningham, G.; Summar, M.; Aschner, J.L. Rescue treatment with L-citrulline inhibits hypoxia-induced pulmonary hypertension in newborn pigs. Am. J. Respir. Cell Mol. Biol. 2015, 53, 255–264.

42. Davel, A.P.; Brum, P.C.; Rossoni, L.V. Isoproterenol induces vascular oxidative stress and endothelial dysfunction via a Giα-coupled β2-adrenoceptor signaling pathway. PLoS ONE 2014, 9, e91877.

43. Solini, A.; Rossi, C.; Duranti, E.; Taddei, S.; Natali, A.; Virdis, A. Saxagliptin prevents vascular remodeling and oxidative stress in db/db mice. Role of endothelial nitric oxide synthase uncoupling and cyclooxygenase. Vasc. Pharmacol. 2016, 76, 62–71.

44. Musicki, B.; Burnett, A.L. Constitutive NOS uncoupling and NADPH oxidase upregulation in the penis of type 2 diabetic men with erectile dysfunction. Andrology 2017, 5, 294–298.

45. Octavia, Y.; Kararigas, G.; de Boer, M.; Chrifi, I.; Kietadisorn, R.; Swinnen, M.; Duimel, H.; Verheyen, F.K.; Brandt, M.M.; Fliegner, D.; et al. Folic acid reduces doxorubicin-induced cardiomyopathy by modulating endothelial nitric oxide synthase. J. Cell. Mol. Med. 2017, 21, 3277–3287.

46. Liu, M.; Zhang, X.; Wang, B.; Wu, Q.; Li, B.; Li, A.; Zhang, H.; Xiu, R. Functional status of microvascular vasomotion is impaired in spontaneously hypertensive rat. Sci. Rep. 2017, 7, 17080.

47. Couto, G.K.; Paula, S.M.; Gomes-Santos, I.L.; Negrao, C.E.; Rossoni, L.V. Exercise training induces eNOS coupling and restores relaxation in coronary arteries of heart failure rats. Am. J. Physiol. Heart Circ. Physiol. 2018, 314, H878–H887.

48. Santana, M.N.S.; Souza, D.S.; Miguel-Dos-Santos, R.; Rabelo, T.K.; Vasconcelos, C.M.L.; Navia-Pelaez, J.M.; Jesus, I.C.G.; Silva-Neto, J.A.D.; Lauton-Santos, S.; Capettini, L.; et al. Resistance exercise mediates remote ischemic preconditioning by limiting cardiac eNOS uncoupling. J. Mol. Cell. Cardiol. 2018, 125, 61–72.

49. Suvorava, T.; Nagy, N.; Pick, S.; Lieven, O.; Ruther, U.; Dao, V.T.; Fischer, J.W.; Weber, M.; Kojda, G. Impact of eNOS-dependent oxidative stress on endothelial function and neointima formation. Antioxid. Redox Signal. 2015, 23, 711–723.

50. Suvorava, T.; Pick, S.; Kojda, G. Selective impairment of blood pressure reduction by endothelial nitric oxide synthase dimer destabilization in mice. J. Hypertens. 2017, 35, 76–88.

51. Benson, M.A.; Batchelor, H.; Chuaiphichai, S.; Bailey, J.; Zhu, H.; Stuehr, D.J.; Bhattacharya, S.; Channon, K.M.; Crabtree, M.J. A pivotal role for tryptophan 447 in enzymatic coupling of human endothelial nitric oxide synthase (eNOS): Effects on tetrahydrobiopterin-dependent catalysis and eNOS dimerization. J. Biol. Chem. 2013, 288, 29836–29845.

52. Daiber, A.; Frein, D.; Namgaladze, D.; Ullrich, V. Oxidation and nitrosation in the nitrogen monoxide/superoxide system. J. Biol. Chem. 2002, 277, 11882–11888.

53. Campos-Mota, G.P.; Navia-Pelaez, J.M.; Araujo-Souza, J.C.; Stergiopulos, N.; Capettini, L.S.A. Role of ERK1/2 activation and nNOS uncoupling on endothelial dysfunction induced by lysophosphatidylcholine. Atherosclerosis 2017, 258, 108–118.

54. Chen, C.A.; Wang, T.Y.; Varadharaj, S.; Reyes, L.A.; Hemann, C.; Talukder, M.A.; Chen, Y.R.; Druhan, L.J.; Zweier, J.L. S-glutathionylation uncouples eNOS and regulates its cellular and vascular function. Nature 2010, 468, 1115–1118.

55. Zweier, J.L.; Chen, C.A.; Druhan, L.J. S-glutathionylation reshapes our understanding of endothelial nitric oxide synthase uncoupling and nitric oxide/reactive oxygen species-mediated signaling. Antioxid. Redox Signal. 2011, 14, 1769–1775.

56. Karbach, S.; Wenzel, P.; Waisman, A.; Munzel, T.; Daiber, A. eNOS uncoupling in cardiovascular diseases—The role of oxidative stress and inflammation. Curr. Pharm. Des. 2014, 20, 3579–3594.

57. Oelze, M.; Knorr, M.; Kroller-Schon, S.; Kossmann, S.; Gottschlich, A.; Rummler, R.; Schuff, A.; Daub, S.; Doppler, C.; Kleinert, H.; et al. Chronic therapy with isosorbide-5-mononitrate causes endothelial dysfunction, oxidative stress, and a marked increase in vascular endothelin-1 expression. Eur. Heart J. 2013, 34, 3206–3216.

58. Kroller-Schon, S.; Steven, S.; Kossmann, S.; Scholz, A.; Daub, S.; Oelze, M.; Xia, N.; Hausding, M.; Mikhed, Y.; Zinssius, E.; et al. Molecular mechanisms of the crosstalk between mitochondria and NADPH oxidase through reactive oxygen species-studies in white blood cells and in animal models. Antioxid. Redox Signal. 2014, 20, 247–266.

59. Oelze, M.; Kroller-Schon, S.; Steven, S.; Lubos, E.; Doppler, C.; Hausding, M.; Tobias, S.; Brochhausen, C.; Li, H.; Torzewski, M.; et al. Glutathione peroxidase-1 deficiency potentiates dysregulatory modifications of endothelial nitric oxide synthase and vascular dysfunction in aging. Hypertension 2014, 63, 390–396.

60. Speer, T.; Owala, F.O.; Holy, E.W.; Zewinger, S.; Frenzel, F.L.; Stahli, B.E.; Razavi, M.; Triem, S.; Cvija, H.; Rohrer, L.; et al. Carbamylated low-density lipoprotein induces endothelial dysfunction. Eur. Heart J. 2014, 35, 3021–3032.

61. Wu, F.; Szczepaniak, W.S.; Shiva, S.; Liu, H.; Wang, Y.; Wang, L.; Wang, Y.; Kelley, E.E.; Chen, A.F.; Gladwin, M.T.; et al. Nox2-dependent glutathionylation of endothelial NOS leads to uncoupled superoxide production and endothelial barrier dysfunction in acute lung injury. Am. J. Physiol.-Lung Cell. Mol. Physiol. 2014, 307, L987–L997.

62. Van Deel, E.D.; Octavia, Y.; de Boer, M.; Juni, R.P.; Tempel, D.; van Haperen, R.; de Crom, R.; Moens, A.L.; Merkus, D.; Duncker, D.J. Normal and high eNOS levels are detrimental in both mild and severe cardiac pressure-overload. J. Mol. Cell. Cardiol. 2015, 88, 145–154.

63. Musicki, B.; Hannan, J.L.; Lagoda, G.; Bivalacqua, T.J.; Burnett, A.L. Mechanistic link between erectile dysfunction and systemic endothelial dysfunction in type 2 diabetic rats. Andrology 2016, 4, 977–983.

64. Kroller-Schon, S.; Daiber, A.; Steven, S.; Oelze, M.; Frenis, K.; Kalinovic, S.; Heimann, A.; Schmidt, F.P.; Pinto, A.; Kvandova, M.; et al. Crucial role for nox2 and sleep deprivation in aircraft noise-induced vascular and cerebral oxidative stress, inflammation, and gene regulation. Eur. Heart J. 2018, 39, 3528–3539.

65. Munzel, T.; Daiber, A.; Steven, S.; Tran, L.P.; Ullmann, E.; Kossmann, S.; Schmidt, F.P.; Oelze, M.; Xia, N.; Li, H.; et al. Effects of noise on vascular function, oxidative stress, and inflammation: Mechanistic insight from studies in mice. Eur. Heart J. 2017, 38, 2838–2849.

66. Van Deel, E.D.; Octavia, Y.; de Waard, M.C.; de Boer, M.; Duncker, D.J. Exercise training has contrasting effects in myocardial infarction and pressure overload due to divergent endothelial nitric oxide synthase regulation. Int. J. Mol. Sci. 2018, 19, 1968.

67. Du, Y.; Navab, M.; Shen, M.; Hill, J.; Pakbin, P.; Sioutas, C.; Hsiai, T.K.; Li, R. Ambient ultrafine particles reduce endothelial nitric oxide production via S-glutathionylation of eNOS. Biochem. Biophys. Res. Commun. 2013, 436, 462–466.

68. De Pascali, F.; Hemann, C.; Samons, K.; Chen, C.A.; Zweier, J.L. Hypoxia and reoxygenation induce endothelial nitric oxide synthase uncoupling in endothelial cells through tetrahydrobiopterin depletion and S-glutathionylation. Biochemistry 2014, 53, 3679–3688.

69. Chen, C.A.; De Pascali, F.; Basye, A.; Hemann, C.; Zweier, J.L. Redox modulation of endothelial nitric oxide synthase by glutaredoxin-1 through reversible oxidative post-translational modification. Biochemistry 2013, 52, 6712–6723.

70. Shang, Q.; Bao, L.; Guo, H.; Hao, F.; Luo, Q.; Chen, J.; Guo, C. Contribution of glutaredoxin-1 to S-glutathionylation of endothelial nitric oxide synthase for mesenteric nitric oxide generation in experimental necrotizing enterocolitis. Transl. Res. 2017, 188, 92–105.

71. Li, X.; Li, X.; Shang, Q.; Gao, Z.; Hao, F.; Guo, H.; Guo, C. Fecal microbiota transplantation (FMT) could reverse the severity of experimental necrotizing enterocolitis (NEC) via oxidative stress modulation. Free Radic. Biol. Med. 2017, 108, 32–43.

72. Espinosa-Diez, C.; Miguel, V.; Vallejo, S.; Sanchez, F.J.; Sandoval, E.; Blanco, E.; Cannata, P.; Peiro, C.; Sanchez-Ferrer, C.F.; Lamas, S. Role of glutathione biosynthesis in endothelial dysfunction and fibrosis. Redox Biol. 2018, 14, 88–99.

73. Crabtree, M.J.; Brixey, R.; Batchelor, H.; Hale, A.B.; Channon, K.M. Integrated redox sensor and effector functions for tetrahydrobiopterin- and glutathionylation-dependent endothelial nitric-oxide synthase uncoupling. J. Biol. Chem. 2013, 288, 561–569.

74. Idigo, W.O.; Reilly, S.; Zhang, M.H.; Zhang, Y.H.; Jayaram, R.; Carnicer, R.; Crabtree, M.J.; Balligand, J.L.; Casadei, B. Regulation of endothelial nitric-oxide synthase (NOS) S-glutathionylation by neuronal NOS: Evidence of a functional interaction between myocardial constitutive NOS isoforms. J. Biol. Chem. 2012, 287, 43665–43673.

75. Dimmeler, S.; Fleming, I.; Fisslthaler, B.; Hermann, C.; Busse, R.; Zeiher, A.M. Activation of nitric oxide synthase in endothelial cells by AKT-dependent phosphorylation. Nature 1999, 399, 601–605.

76. Loot, A.E.; Schreiber, J.G.; Fisslthaler, B.; Fleming, I. Angiotensin ii impairs endothelial function via tyrosine phosphorylation of the endothelial nitric oxide synthase. J. Exp. Med. 2009, 206, 2889–2896.

77. Fleming, I.; Fisslthaler, B.; Dimmeler, S.; Kemp, B.E.; Busse, R. Phosphorylation of Thr(495) regulates Ca2+/calmodulin-dependent endothelial nitric oxide synthase activity. Circ. Res. 2001, 88, e68–e75.

78. Lin, M.I.; Fulton, D.; Babbitt, R.; Fleming, I.; Busse, R.; Pritchard, K.A., Jr.; Sessa, W.C. Phosphorylation of threonine 497 in endothelial nitric-oxide synthase coordinates the coupling of L-arginine metabolism to efficient nitric oxide production. J. Biol. Chem. 2003, 278, 44719–44726.

79. Matsubara, M.; Hayashi, N.; Jing, T.; Titani, K. Regulation of endothelial nitric oxide synthase by protein kinase C. J. Biochem. 2003, 133, 773–781.

80. Rathore, R.; Zheng, Y.M.; Niu, C.F.; Liu, Q.H.; Korde, A.; Ho, Y.S.; Wang, Y.X. Hypoxia activates NADPH oxidase to increase i and i through the mitochondrial ROS-PKCε signaling axis in pulmonary artery smooth muscle cells. Free Radic. Biol. Med. 2008, 45, 1223–1231.

81. Lin, D.; Takemoto, D.J. Oxidative activation of protein kinase Cγ through the C1 domain. Effects on gap junctions. J. Biol. Chem. 2005, 280, 13682–13693.

82. Signorello, M.G.; Segantin, A.; Passalacqua, M.; Leoncini, G. Homocysteine decreases platelet NO level via protein kinase C activation. Nitric Oxide 2009, 20, 104–113.

83. Singh, U.; Devaraj, S.; Vasquez-Vivar, J.; Jialal, I. C-reactive protein decreases endothelial nitric oxide synthase activity via uncoupling. J. Mol. Cell. Cardiol. 2007, 43, 780–791.

84. Lenasi, H.; Kohlstedt, K.; Fichtlscherer, B.; Mulsch, A.; Busse, R.; Fleming, I. Amlodipine activates the endothelial nitric oxide synthase by altering phosphorylation on Ser1177 and Thr495. Cardiovasc. Res. 2003, 59, 844–853.

85. Barauna, V.G.; Mantuan, P.R.; Magalhaes, F.C.; Campos, L.C.; Krieger, J.E. AT1 receptor blocker potentiates shear-stress induced nitric oxide production via modulation of eNOS phosphorylation of residues Thr495 and Ser1177. Biochem. Biophys. Res. Commun. 2013, 441, 713–719.

86. Knorr, M.; Hausding, M.; Kroller-Schuhmacher, S.; Steven, S.; Oelze, M.; Heeren, T.; Scholz, A.; Gori, T.; Wenzel, P.; Schulz, E.; et al. Nitroglycerin-induced endothelial dysfunction and tolerance involve adverse phosphorylation and S-glutathionylation of endothelial nitric oxide synthase: Beneficial effects of therapy with the AT1 receptor blocker telmisartan. Arterioscler. Thromb. Vasc. Biol. 2011, 31, 2223–2231.

87. Almansob, M.A.; Xu, B.; Zhou, L.; Hu, X.X.; Chen, W.; Chang, F.J.; Ci, H.B.; Yao, J.P.; Xu, Y.Q.; Yao, F.J.; et al. Simvastatin reduces myocardial injury undergoing noncoronary artery cardiac surgery: A randomized controlled trial. Arterioscler. Thromb. Vasc. Biol. 2012, 32, 2304–2313.

88. Yang, L.; Jia, Z.; Yang, L.; Zhu, M.; Zhang, J.; Liu, J.; Wu, P.; Tian, W.; Li, J.; Qi, Z.; et al. Exercise protects against chronic beta-adrenergic remodeling of the heart by activation of endothelial nitric oxide synthase. PLoS ONE 2014, 9, e96892.

89. Calvert, J.W.; Condit, M.E.; Aragon, J.P.; Nicholson, C.K.; Moody, B.F.; Hood, R.L.; Sindler, A.L.; Gundewar, S.; Seals, D.R.; Barouch, L.A.; et al. Exercise protects against myocardial ischemia-reperfusion injury via stimulation of β3-adrenergic receptors and increased nitric oxide signaling: Role of nitrite and nitrosothiols. Circ. Res. 2011, 108, 1448–1458.

90. Guterbaum, T.J.; Braunstein, T.H.; Fossum, A.; Holstein-Rathlou, N.H.; Torp-Pedersen, C.T.; Dominguez, H. Endothelial nitric oxide synthase phosphorylation at threonine 495 and mitochondrial reactive oxygen species formation in response to a high H2O2 concentration. J. Vasc. Res. 2013, 50, 410–420.

91. Carlstrom, M.; Liu, M.; Yang, T.; Zollbrecht, C.; Huang, L.; Peleli, M.; Borniquel, S.; Kishikawa, H.; Hezel, M.; Persson, A.E.; et al. Cross-talk between nitrate-nitrite-NO and NO synthase pathways in control of vascular NO homeostasis. Antioxid. Redox Signal. 2015, 23, 295–306.

92. Boger, R.H. Association of asymmetric dimethylarginine and endothelial dysfunction. Clin. Chem. Lab. Med. 2003, 41, 1467–1472.

93. Sydow, K.; Munzel, T. ADMA and oxidative stress. Atheroscler. Suppl. 2003, 4, 41–51.

94. Schnabel, R.; Blankenberg, S.; Lubos, E.; Lackner, K.J.; Rupprecht, H.J.; Espinola-Klein, C.; Jachmann, N.; Post, F.; Peetz, D.; Bickel, C.; et al. Asymmetric dimethylarginine and the risk of cardiovascular events and death in patients with coronary artery disease: Results from The AtheroGene Study. Circ. Res. 2005, 97, e53–e59.
95. Boger, R.H. When the endothelium cannot say ‘NO’ anymore. ADMA, an endogenous inhibitor of NO synthase, promotes cardiovascular disease. Eur. Heart J. 2003, 24, 1901–1902.
96. Tousoulis, D.; Papageorgiou, N.; Androulakis, E.; Siasos, G.; Latsios, G.; Tentolouris, K.; Stefanadis, C. Diabetes mellitus-associated vascular impairment: Novel circulating biomarkers and therapeutic approaches. J. Am. Coll. Cardiol. 2013, 62, 667–676.
97. Zhou, R.; Ma, P.; Xiong, A.; Xu, Y.; Wang, Y.; Xu, Q. Protective effects of low-dose rosuvastatin on isoproterenol-induced chronic heart failure in rats by regulation of DDAH-ADMA-NO pathway. Cardiovasc. Ther. 2017, 35, e12241.
98. Hsu, C.P.; Zhao, J.F.; Lin, S.J.; Shyue, S.K.; Guo, B.C.; Lu, T.M.; Lee, T.S. Asymmetric dimethylarginine limits the efficacy of simvastatin activating endothelial nitric oxide synthase. J. Am. Heart Assoc. 2016, 5, e003327.
99. Lin, Y.; Feng, M.; Lu, C.W.; Lei, Y.P.; He, Z.M.; Xiong, Y. Preservation of vascular DDAH activity contributes to the protection of captopril against endothelial dysfunction in hyperlipidemic rabbits. Eur. J. Pharmacol. 2017, 798, 43–48.
100. Bode-Boger, S.M.; Scalera, F.; Ignarro, L.J. The L-arginine paradox: Importance of the L-arginine/asymmetrical dimethylarginine ratio. Pharmacol. Ther. 2007, 114, 295–306.
101. Lass, A.; Suessenbacher, A.; Wolkart, G.; Mayer, B.; Brunner, F. Functional and analytical evidence for scavenging of oxygen radicals by L-arginine. Mol. Pharmacol. 2002, 61, 1081–1088.
102. Closs, E.I.; Ostad, M.A.; Simon, A.; Warnholtz, A.; Jabs, A.; Habermeier, A.; Daiber, A.; Forstermann, U.; Munzel, T. Impairment of the extrusion transporter for asymmetric dimethyl-L-arginine: A novel mechanism underlying vasospastic angina. Biochem. Biophys. Res. Commun. 2012, 423, 218–223.
103. Watson, C.P.; Pazarentzos, E.; Fidanboylu, M.; Padilla, B.; Brown, R.; Thomas, S.A. The transporter and permeability interactions of asymmetric dimethylarginine (ADMA) and L-arginine with the human blood-brain barrier in vitro. Brain Res. 2016, 1648, 232–242.
104. Speranza, L.; Franceschelli, S.; D’Orazio, N.; Gaeta, R.; Bucciarelli, T.; Felaco, M.; Grilli, A.; Riccioni, G. The biological effect of pharmacological treatment on dimethylaminohydrolases (DDAH-1) and cationic amino acid transporter-1 (CAT-1) expression in patients with acute congestive heart failure. Microvasc. Res. 2011, 82, 391–396.
105. Yang, Z.; Ming, X.F. Arginase: The emerging therapeutic target for vascular oxidative stress and inflammation. Front. Immunol. 2013, 4, 149.
106. Xia, N.; Horke, S.; Habermeier, A.; Closs, E.I.; Reifenberg, G.; Gericke, A.; Mikhed, Y.; Munzel, T.; Daiber, A.; Forstermann, U.; et al. Uncoupling of endothelial nitric oxide synthase in perivascular adipose tissue of diet-induced obese mice. Arterioscler. Thromb. Vasc. Biol. 2016, 36, 78–85.
107. Xia, N.; Li, H. The role of perivascular adipose tissue in obesity-induced vascular dysfunction. Br. J. Pharmacol. 2017, 174, 3425–3442.