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Adaptive induction of NF-E2-related factor-2-driven antioxidant genes in endothelial cells in response to hyperglycemia

Zoltan Ungvari,1 Lora Bailey-Downs,1 Tripti Gautam,1 Rosario Jimenez,2 Gyorgy Losonczy,3 Cuihua Zhang,4 Praveen Ballabh,5 Fabio A. Recchia,5 Donald C. Wilkerson,6 William E. Sonntag,1 Kevin Pearson,6,7 Rafael de Cabo,7 and Anna Csiszar1

1Reynolds Oklahoma Center on Aging, Department of Geriatric Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma; 2Department of Pharmacology, School of Pharmacy, Universidad de Granada, Granada, Spain; 3Department of Pulmonology, Semmelweis University, Budapest, Hungary; 4Departments of Internal Medicine, Medical Pharmacology and Physiology, and Nutritional Sciences, University of Missouri, Columbia, Missouri; 5Departments of Pediatrics, Cell Biology, and Physiology, New York Medical College-Westchester Medical Center, Valhalla, New York; 6Graduate Center for Nutritional Sciences, University of Kentucky, Lexington, Kentucky; and 7Laboratory of Experimental Gerontology, National Institute on Aging, Baltimore, Maryland

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Ungvari Z, Bailey-Downs L, Gautam T, Jimenez R, Losonczy G, Zhang C, Ballabh P, Recchia FA, Wilkerson DC, Sonntag WE, Pearson K, de Cabo R, Csiszar A. Adaptive induction of NF-E2-related factor-2-driven antioxidant genes in endothelial cells in response to hyperglycemia. Am J Physiol Heart Circ Physiol 300: H1133–H1140, 2011. First published July 1, 2011; doi:10.1152/ajpheart.00402.2010.—Hyperglycemia in diabetes mellitus promotes oxidative stress in endothelial cells, which contributes to development of cardiovascular diseases. Nuclear factor erythroid 2-related factor-2 (Nrf2) is a transcription factor activated by oxidative stress that regulates expression of numerous reactive oxygen species (ROS) detoxifying and antioxidant genes. This study was designed to elucidate the homeostatic role of adaptive induction of Nrf2-driven free radical detoxification mechanisms in endothelial protection under diabetic conditions. Using a Nrf2/antioxidant response element (ARE)-driven luciferase reporter gene assay we found that in a cultured coronary arterial endothelial cell model hyperglycemia (10–30 mmol/l glucose) significantly increases transcriptional activity of Nrf2 and upregulates the expression of the Nrf2 target genes NQO1, GCLC, and HMOX1. These effects of high glucose were significantly attenuated by small interfering RNA (siRNA) downregulation of Nrf2 or overexpression of Keap-1, which inactivates Nrf2. High-glucose-induced upregulation of NQO1, GCLC, and HMOX1 was also prevented by pretreatment with polyethylene glycol (PEG)-catalase or N-acetylcysteine, whereas administration of H2O2 mimicked the effect of high glucose. To test the effects of metabolic stress in vivo, Nrf2+/+ and Nrf2−/− mice were fed a high-fat diet (HFD). HFD elicited significant increases in mRNA expression of Gclc and Hmox1 in aortas of Nrf2+/+ mice, but not Nrf2−/− mice, compared with respective standard diet-fed control mice. Additionally, HFD-induced increases in vascular ROS levels were significantly greater in Nrf2−/− than Nrf2+/+ mice. HFD-induced endothelial dysfunction was more severe in Nrf2−/− mice, as shown by the significantly diminished acetylated linoleic-induced relaxation of aorta of these animals compared with HFD-fed Nrf2+/+ mice. Our results suggest that adaptive activation of the Nrf2/ARE pathway confers endothelial protection under diabetic conditions.

IN DIABETIC PATIENTS poorly controlled hyperglycemia leads to multiple vascular complications, including diabetic microvas-

Address for reprint requests and other correspondence: A. Csiszar, Reynolds Oklahoma Center on Aging, Dept. of Geriatric Medicine, Univ. of Oklahoma HSC, 975 N.E. 10th St.-BRC 1303, Oklahoma City, OK 73104 (e-mail: anna-csiszar@ouhs.edu, zoltan-ungvari@ouhs.edu).

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Cell density at transfection was 30%. Experiments were performed on day 2 after the transfection, when gene silencing was optimal. Keap-1 overexpression was achieved in CAECs by transfection with a Keap-1 full-length cDNA encoding plasmid (Origen) as described previously (10). In separate experiments, CAECs were pretreated with N-acetylcyesteine (25 mmol/l) or polyethylene glycol (PEG)-catalase (1,000 U/ml) and then exposed to high glucose (30 mmol/l). In other studies, CAECs were treated with H2O2 (10−5 mol/l) or the prototypical Nrf2 activator sulforaphane (2.5 μmol/l) to induce Nrf2-mediated gene transcription.

Quantitative real-time RT-PCR. A quantitative real-time RT-PCR technique was used to analyze mRNA expression of the Nrf2/ARE target genes NAD(P)H:quinone oxidoreductase 1 (NQO1), heme oxygenase-1 (Hmox1), and γ-glutamylcysteine synthetase (Gclc) and mRNA expression of the NF-κB target gene ICAM1 and the proinflammatory cytokine TNF in high-glucose-treated CAECs and in aortic segments, as previously reported (11, 15, 40, 42). In brief, total RNA was isolated with a Mini RNA Isolation Kit (Zymo Research, Orange, CA) and was reverse transcribed with SuperScript III reverse transcriptase (Invitrogen) as described previously (13, 15). A real-time RT-PCR technique was used to analyze mRNA expression with the Stratagene MX3000, as reported previously (13). Amplification efficiencies were determined with a dilution series of a standard vascular sample. Quantification was performed with the efficiency-corrected ΔΔCt method (where Ct is quantification cycle). The relative quantities of the human reference genes GAPDH, HPRT, and ACTB (β-actin) and the mouse reference genes Hprt, Ywhaz, and Actb were determined, and a normalization factor was calculated based on their geometric mean for internal normalization. Oligonucleotides used for quantitative real-time RT-PCR are listed in Table 1. Fidelity of the PCR reaction was determined by melting temperature analysis and visualization of product on a 2% agarose gel.

Table 1. Oligonucleotides for real-time RT-PCR

<table>
<thead>
<tr>
<th>mRNAs Targets</th>
<th>Descriptions</th>
<th>Species</th>
<th>Sense</th>
<th>Antisense</th>
</tr>
</thead>
<tbody>
<tr>
<td>NQO1</td>
<td>NAD(P)H:quinone oxidoreductase 1 (NAD(P)H dehydrogenase, quinine 1)</td>
<td>Homo sapiens</td>
<td>AGACCTTGTTGATATTTGGAGCT</td>
<td>GGAGGTTGTAAGTGGAG</td>
</tr>
<tr>
<td>Gclc</td>
<td>γ-Glutamylcysteine synthetase (glutamate-cysteine ligase, catalytic subunit)</td>
<td>H. sapiens</td>
<td>CAGTGCGATGTTGCTT</td>
<td>ATTTGATGATGTTGCTTAC</td>
</tr>
<tr>
<td>HMOX1</td>
<td>Heme oxygenase-1</td>
<td>H. sapiens</td>
<td>AAAATGACCTTGTTGACAGCAG</td>
<td>TGGGACAGAAGAAGAG</td>
</tr>
<tr>
<td>ICAM1</td>
<td>Intercellular adhesion molecule 1</td>
<td>H. sapiens</td>
<td>CACTATGCTCGTCAGCACC</td>
<td>GAAGATGCGTGGGACAC</td>
</tr>
<tr>
<td>HPRT</td>
<td>Hypoxanthine phosphoribosyltransferase 1</td>
<td>H. sapiens</td>
<td>CCCTGTGTCGAGGAGGAC</td>
<td>AACCTGCTGAAAGAGGAC</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>H. sapiens</td>
<td>AAGCAGAATTGGCTACAG</td>
<td>AGGTCATCTGTATTGATGAC</td>
</tr>
<tr>
<td>ACTB</td>
<td>β-Actin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nqo1</td>
<td>NAD(P)H:quinone oxidoreductase 1</td>
<td>Mus musculus</td>
<td>ATGAGGAGGCTTGGTACAG</td>
<td>AGATGCGTGGGAGGACAC</td>
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<tr>
<td>Gclc</td>
<td>γ-Glutamylcysteine synthetase</td>
<td>M. musculus</td>
<td>AGATATGACTGGATGAC</td>
<td>CAGAATGACTGAGCACA</td>
</tr>
<tr>
<td>Hmox1</td>
<td>Heme oxygenase-1</td>
<td>M. musculus</td>
<td>CTTGGACATCTGGTACAG</td>
<td>AACTGTGTGAGTGATGAC</td>
</tr>
<tr>
<td>Icam1</td>
<td>Intercellular adhesion molecule 1</td>
<td>M. musculus</td>
<td>TTCTGAGCTGGATAGAAACT</td>
<td>CTTGACAGACCTTCTTA</td>
</tr>
<tr>
<td>Tnf</td>
<td>Tumor necrosis factor (TNF superfamily, member 2)</td>
<td>M. musculus</td>
<td>GGAGGCTCAGAGGAGGCT</td>
<td>AGATGCGTGGGAGGACAC</td>
</tr>
<tr>
<td>Hprt</td>
<td>Hypoxanthine phosphoribosyltransferase 1</td>
<td>M. musculus</td>
<td>TGCTGGCTGCGGAGGACT</td>
<td>ATCGTTCGAGGAGGAGG</td>
</tr>
<tr>
<td>Ywhaz</td>
<td>Tyrosine 3-monooxygenase/trypophan 5-monooxygenase activation protein, ξ polypeptide</td>
<td>M. musculus</td>
<td>ACTGTGTTGACACGAAAGGAGG</td>
<td>GGGTCGTAAGAGGATGAC</td>
</tr>
<tr>
<td>Actb</td>
<td>β-Actin</td>
<td>M. musculus</td>
<td>AATGAGGAGGCTTGGGAG</td>
<td>AGATGCGTGGGAGGACAC</td>
</tr>
</tbody>
</table>
with high glucose. A significant, concentration-dependent increase in luciferase activity compared with the vector control was noted upon stimulation with glucose (Fig. 1A).

**High glucose upregulates Nrf2-ARE-driven genes in CAECs: role for increased ROS production.** Glucose, in a concentration-dependent manner, significantly increased mRNA expression of the known Nrf2 targets *NQO1, GCLC*, and *HMOX1* (Fig. 1B). Overexpression of Keap-1 or siRNA knockdown of Nrf2 prevented high-glucose-induced upregulation of *NQO1, GCLC*, and *HMOX1* (Fig. 1C). Western blotting showed that protein expression of NQO1 was upregulated by high-glucose treatment, and this effect was prevented by siRNA downregulation of NRF2 (Fig. 1D).

**RESULTS**

**High glucose increases transcriptional activity of Nrf2 in CAECs.** To determine the effect of hyperglycemia on Nrf2 activation, we transiently transfected CAECs with a Nrf2 ARE-driven reporter gene construct and then treated the cells with high glucose. A significant, concentration-dependent increase in luciferase activity compared with the vector control was noted upon stimulation with glucose (Fig. 1A).
Nrf2 significantly augmented the proapoptotic effect of high-rate of apoptosis in CAECs (Fig. 4). siRNA knockdown of Nrf2 significantly increased the production of TUNEL-positive apoptotic cells was low in untreated samples. Treatment with high glucose significantly increased the ROS production induced mitochondrial oxidative stress, as shown by the significant increases in the mean fluorescence intensity of oxidized MitoSOX (flow cytometry data). Overexpression of Keap-1 significantly increases HG-induced mitochondrial $O_2^\cdot$ production. Data are means ± SE (n = 6 in each group). *P < 0.05 vs. baseline; #P < 0.05 vs. HG only.

Hyperglycemia significantly increases mitochondrial ROS production, as shown by the intense MitoSOX staining in high-glucose-treated CAECs (Fig. 2). Overexpression of Keap-1 significantly increased MitoSOX staining in high-glucose-treated CAECs (Fig. 2B). To determine the role of increased ROS in high-glucose-mediated induction of Nrf2 targets, we treated the CAECs with scavengers of $H_2O_2$. We found that pretreatment with both PEG-catalase and N-acetylcysteine prevented high-glucose-mediated upregulation of NQO1, GCLC, and HMOX1 (Fig. 3A). Treatment of CAECs with $H_2O_2$ also resulted in upregulation of NQO1, GCLC, and HMOX1, and these effects were prevented by knockdown of Nrf2 (Fig. 3B). The prototypical Nrf2 activator sulforaphane also induced NQO1, GCLC, and HMOX1, and these effects were prevented by both siRNA knockdown of Nrf2 and overexpression of Keap-1 (Fig. 3C).

Nrf2-dependent attenuation of high-glucose-induced endothelial apoptosis. To assess the role of adaptive Nrf2 activation in regulation of endothelial apoptosis, CAECs were treated with high glucose and TUNEL assay was performed. The level of TUNEL-positive apoptotic cells was low in untreated samples. Treatment with high glucose significantly increased the rate of apoptosis in CAECs (Fig. 4). siRNA knockdown of Nrf2 significantly augmented the proapoptotic effect of high-glucose treatment (Fig. 4).

Nrf2-dependent attenuation of high-glucose-induced NF-κB activation and proinflammatory gene expression in CAECs. To assess the role of adaptive Nrf2 activation in regulation of proinflammatory gene expression, CAECs were treated with high glucose and NF-κB activity and NF-κB-driven gene expression were assayed. Treatment with high glucose significantly increased transcriptional activity of NF-κB (Fig. 5A) and upregulated ICAM-1 (Fig. 5B). siRNA knockdown of Nrf2 significantly augmented both high-glucose-induced NF-κB activity (Fig. 5A) and high-glucose-induced ICAM-1 induction (Fig. 5B).

Effect of high-fat diet on various biomarkers in mouse sera. We compared male Nrf2+/+ and Nrf2−/− mice after 16 wk of HFD. Fasting blood glucose levels differed significantly between the two strains on the control diet, yet both strains developed comparable relative hyperglycemia on HFD (Nrf2+/+: control diet 5.5 ± 0.6 mmol/l, HFD 8.6 ± 0.4 mmol/l; Nrf2−/−: control diet 2.8 ± 0.2 mmol/l, HFD 5.7 ± 0.8 mmol/l). HFD tended to increase serum levels of free fatty acids both in wild type [Nrf2+/+: 0.88 ± 0.06 meq/l, Nrf2+/+ (HFD): 1.15 ± 0.08 meq/l; P < 0.05] and Nrf2−/− mice [Nrf2−/−: 0.88 ± 0.12 meq/l, Nrf2−/− (HFD): 0.92 ± 0.12 meq/l].
wild-type mice [Nrf2 shown). Serum adiponectin levels were decreased significantly by HFD feeding in either strain (data not shown; Fig. 5A). HFD-induced endothelial dysfunction was more severe in Nrf2−/− mice compared with responses obtained in vessels from HFD-fed Nrf2+/− mice (Fig. 5B).

Increased oxidative stress in aortas of HFD-fed Nrf2−/− mice. The HFD elicited significant increases in DHE and H2DCFDA fluorescent signals (measures of vascular O2·− and H2O2 production, respectively) in aortas of Nrf2−/− mice (Fig. 6A). Western blot experiments confirmed that genetic depletion of Nrf2 prevents HFD-induced upregulation of protein expression of Nrf2 targets (NQO1, GPX, and GCLC) in mouse aortas (Fig. 6B).

Diminished endothelial function in aortas of HFD-fed Nrf2−/− mice. The HFD elicited significant endothelial dysfunction in aortas of Nrf2−/− mice, as shown by the impaired relaxation responses to ACh (Fig. 7B). HFD-induced endothelial dysfunction was more severe in Nrf2−/− mice, as shown by the significantly diminished ACh-induced relaxations of aortas of these animals, compared with responses obtained in vessels from HFD-fed Nrf2+/− mice (Fig. 7B).

**Fig. 5.** A: reporter gene assay showing that in primary human CAECs high glucose (HG) significantly increased NfκB activity. Cells were transiently cotransfected with NF-κB-driven firefly luciferase and CMV-driven Renilla luciferase constructs followed by HG treatment. Cells were then lysed and subjected to luciferase activity assay. After normalization relative luciferase activity was assessed from 4–6 independent transfections. siRNA knockdown of Nrf2 (siNrf2) significantly augmented HG-induced endothelial NfκB activation. Data are means ± SE (n = 5 in each group). *P < 0.05 vs. untreated; #P < 0.05 vs. HG only.

**Fig. 6.** A: expression of Gclc and Hmox1 mRNA in aortas isolated from Nrf2+/+ mice and Nrf2−/− mice fed a standard diet (SD) or a high-fat diet (HFD). Data are means ± SE (n = 5 or 6 in each group). *P < 0.05 vs. SD; #P < 0.05 vs. Nrf2+/+. B: analysis of protein expression of NQO1, GPX, and GCLC in aortic segments from Nrf2+/+ mice and Nrf2−/− mice fed SD or HFD. Shown are spliced bands from Western blot experiments, organized according to the experimental groups. Numbers are normalized densitometric values for the respective bands (see METHODS). The molecular masses of the bands recognized by the antibodies directed against NQO1, GPX, and GCLC are approximately 31, 22, and 73 kDa, respectively.

0.07 meq/l]. Serum cholesterol levels were also elevated by feeding HFD in both strains [Nrf2+/+: 245 ± 5 mg/dl, Nrf2+/+ (HFD): 337 ± 20 mg/dl (P < 0.05); Nrf2−/−: 123 ± 8 mg/dl, Nrf2−/− (HFD): 267 ± 9 mg/dl (P < 0.05)]. Serum triglyceride and insulin levels (assessed in fed animals) were not significantly affected by HFD feeding in either strain (data not shown). Serum adiponectin levels were decreased in HFD-fed wild-type mice [Nrf2+/+: 23.2 ± 2.6 μg/ml, Nrf2+/+ (HFD): 13.6 ± 0.4 μg/ml; P < 0.05], whereas they were unchanged in HFD-fed Nrf2−/− mice [Nrf2−/−: 15.6 ± 0.8 μg/ml, Nrf2−/− (HFD): 15.3 ± 0.4 μg/ml; not significant].
mRNA expression of acetylcholine in aortic segments isolated from Nrf2 knockout mice is significantly greater than in vessels of HFD-fed wild-type mice (Fig. 1A) and upregulates several ARE-regulated genes involved in detoxification mechanisms in vessels of HFD-fed Nrf2 knockout mice (Fig. 6). We found that the HFD elicits significant oxidative stress in blood vessels of wild-type mice, which is further supported by the findings that exogenous administration of H2O2 significantly increased expression of Nrf2-driven genes, an effect that was abolished by knockdown of Nrf2 (Fig. 3B). The induction of GCLC, HMox1, and NQO1 was attenuated by treatment with the thiol antioxidant N-acetylcysteine, suggesting that thiol oxidation is largely mediating the effects of H2O2 on Nrf2-responsive genes (Fig. 3A).

Hyperglycemia-induced endothelial oxidative stress has been implicated in the development of diabetic complications, in part by inducing endothelial apoptosis and by promoting endothelial activation and vascular inflammation. Our present findings (Fig. 4) and results from previous investigations (28) suggest that induction of Nrf2-driven free radical detoxification pathways confers significant antiapoptotic effects in endothelial cells exposed to hyperglycemia. Furthermore, our results (Fig. 5) suggest that adaptive Nrf2 activation also effectively attenuates hyperglycemia-induced NF-κB activation and NF-κB-driven proinflammatory gene expression in endothelial cells, extending previous findings (3).

Increased proinflammatory gene expression in aortas of HFD-fed Nrf2 knockout mice. The HFD elicited significant increases in mRNA expression of Icam1 and Tnfα in aortas of Nrf2 knockout mice (Fig. 8, A and B, respectively). In aortas of HFD-fed Nrf2 knockout mice mRNA expression of Icam1 and Tnfα was significantly greater than in vessels of HFD-fed wild-type mice (Fig. 8, A and B, respectively).

**DISCUSSION**

Here we show for the first time that adaptive activation of the Nrf2/ARE pathway has a critical role in endothelial protection in response to diabetic conditions both in vitro and in vivo. We base this conclusion on the following lines of evidence. We demonstrate that in human CAECs hyperglycemia significantly increases the transcriptional activity of Nrf2 (Fig. 1A) and upregulates several ARE-regulated genes involved in free radical metabolism (Fig. 1, B–D), including NQO1 (a key component of the plasma membrane redox system), heme oxygenase-1, and γ-glutamylcysteine synthetase (the rate-limiting enzyme for glutathione synthesis). The aforementioned effects of high glucose are mediated predominantly by Nrf2, as siRNA knockdown of Nrf2 inhibits induction of antioxidant genes by high glucose (Fig. 1, C and D). Furthermore, overexpression of Keap-1 also abolished the adaptive antioxidant response in response to hyperglycemia (Fig. 1C). Keap-1 is a cytosolic repressor protein that interacts with Nrf2, preventing its nuclear translocation.

Our findings suggest that increased mitochondrial ROS generation is a major mechanism by which hyperglycemia promotes oxidative stress in human CAECs (Fig. 2). Similar conclusions have been reached previously in studies from other laboratories as well (27, 31, 32, 38). Disruption of Nrf2 signaling significantly increases hyperglycemia-induced mitochondrial oxidative stress, indicating that adaptive upregulation of Nrf2-driven antioxidant systems effectively attenuates cellular oxidative stress under diabetic conditions (Fig. 2). Because administration of catalase prevented high-glucose-induced upregulation of Nrf2 targets (Fig. 3A), it is likely that increased H2O2 levels have a central role in activation of Nrf2 in metabolically stressed endothelial cells. This concept is further supported by the findings that exogenous administration of H2O2 significantly increased expression of Nrf2-driven genes, an effect that was abolished by knockdown of Nrf2 (Fig. 3B). The induction of GCLC, HMOX1, and NQO1 was attenuated by treatment with the thiol antioxidant N-acetylcysteine, suggesting that thiol oxidation is largely mediating the effects of H2O2 on Nrf2-responsive genes (Fig. 3A).

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Furthermore, results from the present study show that in wild-type mice metabolic stress associated with HFD can upregulate Nrf2 target genes in the vasculature, whereas genetic deletion of Nrf2 prevents induction of free radical detoxification mechanisms in vessels of HFD-fed Nrf2 knockout mice (Fig. 6). We found that the HFD elicits significant oxidative stress in blood vessels of wild-type mice, which is associated with endothelial dysfunction (Fig. 7), extending earlier findings (29, 35). Genetic lack of a functional Nrf2/ARE pathway results in significant increases in vascular ROS levels...
and a more severe endothelial functional impairment in aortas of HFD-fed Nrf2−/− mice compared with vessels of HFD-fed wild-type control mice (Fig. 7). These findings provide evidence that Nrf2-driven free radical detoxification pathways are physiologically important endogenous homeostatic mechanisms that play an important role in vasoprotection in metabolic diseases. The HFD-fed mouse is an accepted model of early type 2 diabetes, which recapitulates many aspects of diabetes mellitus, including hyperglycemia, oxidative stress, and endothelial dysfunction. Because in this model hyperglycemia is not the only factor contributing to vascular impairment, we propose that activation of Nrf2 represents a common pathway by which oxidative stress induced by diverse stimuli associated with diabetes mellitus and consumption of a HFD (including hyperglycemia, hyperlipidemia, increased levels of oxidized lipids, and advanced glycation end products) activate homeostatic mechanisms by which the deleterious effects of metabolic stress are attenuated in the vasculature. This concept is supported by the observation that hyperglycemia, oxidized lipoproteins (1), and advanced glycation end products (19) can separately elicit an Nrf2-driven adaptive response in endothelial cells. It is likely that adaptive Nrf2 activation also protects other organs, including the liver (34), the brain (26), and the heart (20), from the deleterious effects of oxidative stress associated with a HFD. Further studies are warranted to test the vasoprotective role of adaptive Nrf2 activation in other models of hyperglycemia/type 2 diabetes.

We posit that pathological conditions that impair the ability of cells to mount an effective Nrf2/ARE-mediated antioxidant response render the vascular system vulnerable to the deleterious effects of metabolic diseases. Importantly, there is a significant age-related dysregulation of Nrf2-dependent pathways in vascular cells. As a result, the same level of oxidative stress that elicits significant induction of Nrf2-dependent genes in arteries of young rats fails to upregulate Nrf2-dependent free radical detoxification pathways in vessels of aged rats (Csiszar and Ungvari, unpublished observations). Similarly, aortic expression of Nrf2-driven antioxidant enzymes markedly increases in young mice fed a HFD but tends to decrease or only modestly increase in middle-aged mice fed a HFD, despite the fact that vascular oxidative stress is greater in HFD-fed middle-aged mice than in young mice (5). The intimate link between aging and vascular Nrf2 activation is also underscored by the observations that arteries of extremely long-lived muroid rodents (Peromyscus leucopus, maximal life span: ~8 yr) exhibit an increased expression of Nrf2-driven antioxidant enzymes, decreased cellular and mitochondrial levels of ROS, and increased resistance to the proinflammatory and proapoptotic effects of hyperglycemia compared with vessels of shorter-lived Mus musculus (12, 24, 36, 37). Moreover, many of the effects of caloric restriction, which decreases cellular and mitochondrial levels of ROS and exerts anti-inflammatory and antiapoptotic vascular effects in aging (8), also depend on the presence of functional Nrf2 (30).

Because the metabolic stress-induced Nrf2-dependent adaptive response is relatively weak and cannot compensate completely for the increased cellular oxidative stress in diabetes (especially in aging), there is a clear opportunity for pharmacological intervention to facilitate the efficiency of Nrf2-driven homeostatic mechanisms. In that regard, it is significant that in endothelial cells and other cell types Nrf2 can be activated pharmacologically by the polyphenol resveratrol (2, 21, 25, 33, 35) or by sulforaphane (43), which results in significant induction of cellular antioxidant systems (23, 33, 39) (Fig. 3C), increases in GSH levels (23, 38), and consequential reduction of oxidative stress (23, 33, 39, 43). Importantly, both resveratrol and sulforaphane can effectively attenuate vascular ROS production and improve endothelial function in animal models of diabetes mellitus (29, 45) and/or attenuate hyperglycemia-induced endothelial oxidative stress (38, 43). Thus we posit that facilitation of the induction of Nrf2-driven homeostatic pathways by pharmacological treatments can contribute importantly to an intervention strategy for the prevention of vascular diseases in diabetic patients.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

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