Glucose-6 Phosphate Dehydrogenase Deficiency Decreases the Vascular Response to Angiotensin II

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Background—Glucose-6-phosphate dehydrogenase (G6PD) regulates production of the reduced form of NADPH through the pentose phosphate pathway. G6PD may therefore affect superoxide anion production via vascular NADPH oxidase, which is key in mediating the vascular response to angiotensin II (Ang II). We determined the hypertensive and vascular hypertrophic response to Ang II in G6PD-deficient mice.

Methods and Results—Ang II (0.7 mg/kg per day) was infused via subcutaneous osmotic pumps for 6 days in male hemizygote G6PD mutant (G6PDmut) and wild-type (WT) C3H mice. (1) Compared with WT, G6PDmut mouse aorta had 10% to 20% of G6PD activity and 50% less NADPH. (2) Basal systolic blood pressure was not significantly different in G6PDmut mice (WT 88±4 mm Hg versus G6PDmut 95±4 mm Hg), but Ang II increased blood pressure to a lower level in G6PDmut mice (WT 139±4 mm Hg versus G6PDmut 123±5 mm Hg; P<0.05). (3) Ang II increased aortic medial thickness less in G6PDmut mice (WT 71±2 μm versus G6PDmut 62±1 μm; P<0.01). (4) 3-α-Nitrotyrosine staining and dihydroethidium oxidation in the aorta was increased by Ang II less in G6PDmut mice. (5) Smooth muscle cells isolated from G6PDmut mice showed less Ang II–induced phosphorylation of Akt and p42/44 ERK.

Conclusions—G6PD deficiency may reduce vascular superoxide anion production by limiting production of the substrate for NADPH oxidase, thereby inhibiting oxidant-mediated Ang II–induced signaling pathways that contribute to hypertension and smooth muscle hypertrophy. (Circulation. 2005;112:257-263.)

Key Words: angiotensin ■ aorta ■ free radicals ■ hypertension ■ hypertrophy

Glucose-6-phosphate dehydrogenase (G6PD) regulates production of the reduced form of nicotinamide-adenine-dinucleotide phosphate (NADPH) through the pentose phosphate pathway. Various NADPH-dependent enzymes regulate cellular redox balance, including glutathione reductase, thioredoxin reductase, nitric oxide synthase, and NADPH oxidase. G6PD has been suggested to be an “anti-oxidant” enzyme because it provides NADPH to maintain glutathione (GSH) in its reduced form. G6PD activity may restore cellular GSH levels after its depletion by oxidative stress.1,2 In addition, G6PD regulates production of nitric oxide in endothelium and other types of cells.3–6 On the other hand, superoxide production by the neutrophil NADPH oxidase is decreased in G6PD-deficient granulocytes,3,4,7 suggesting that vascular NADPH oxidase–dependent superoxide production may be regulated by the concentration of NADPH that is available from the pentose phosphate pathway. In fact, pharmacological inhibition of G6PD was shown to lower NADPH and decrease superoxide production in bovine coronary arteries.8

There is increasing evidence that NADPH oxidase is a major source of superoxide anion in vascular tissue9,10 and that excess generation of superoxide anion/reactive oxygen species contributes to pathological vascular conditions such as hypertension and atherosclerosis. Angiotensin II (Ang II) is a potent vasoconstrictor and upregulates expression and activity of NADPH oxidase and increases superoxide anion and hydrogen peroxide (H2O2) production in vascular smooth muscle cells.11–13 These reactive oxygen species mediate Ang II–induced signaling events leading to Akt phosphorylation14–16 and vascular smooth muscle cell hypertrophy.15 Ang II–induced hypertension and vascular superoxide anion production are markedly blunted in gp91phox-deficient20 and p47phox-deficient mice,20 and aortic medial hypertrophy is also dependent on NADPH oxidase–derived superoxide anion.20 Thus, the effects of Ang II in the vasculature are tightly linked to NADPH oxidase–dependent reactive oxygen species.

An early clinical report suggested that blood pressure is increased in G6PD-deficient people,22 but it is not well studied whether risk for cardiovascular disease is affected by G6PD deficiency. We therefore examined the role of G6PD as a source of NADPH in regulating the vascular response to
Ang II infusion by using G6PD-deficient mutant mice. G6PD-deficient mice demonstrated a less marked hypertensive and vascular hypertrophic response to Ang II. Furthermore, aortic smooth muscle cells isolated from G6PD-deficient mice showed decreased Ang II–induced Akt and ERK phosphorylation, consistent with the lesser medial hypertrophy observed in vivo. These findings implicate a unique role of G6PD as a regulator of vascular NADPH levels, NADPH oxidase, and vascular oxidant signaling when the activity of NADPH oxidase is upregulated by Ang II.

**Methods**

**Animal Model**

The G6PD-deficient mouse model in the C57BL/6J strain was bred at our institution from frozen embryos obtained from Medical Research Council (Harwell, UK). This G6PD-deficient mouse line was originally created by Pretsch et al. and showed decreased translation of the protein caused by a single mutation in the untranslated region of the X-linked G6PD gene. Mice were genotyped by polymerase chain reaction as previously described. Hemizygous mutant (X<sup>y</sup>, G6PD<sup>−/−</sup>) and male wild-type (WT) C57BL/6J mice, aged 16 to 20 weeks, were used for Ang II infusion. The mice were anesthetized with inhaled isoflurane, and an incision was made in the midscapular region. Osmotic minipumps were used for Ang II infusion. The mice were anesthetized with inhaled isoflurane, and an incision was made in the midscapular region.

**Immunohistochemistry for 3-nitrotyrosine**

Mice were anesthetized with isoflurane and euthanized by exsanguination. The aorta was cleaned of adherent fat, placed in 4% formalin, and processed as previously described. 3-nitrotyrosine was detected using the avidin-biotin-peroxidase complex technique. The aortic cross sections were stained with hematoxylin and eosin and photographed at a magnification of ×100. The microscopic images of these sections were displayed on a computer with the use of Adobe Photoshop software. Four measurements of aortic medial thickness oriented at 90° were made per section with the use of NIH Image software. The increase in medial thickness caused by Ang II is comparable to that in medial area reported previously.

**Detection of Superoxide Anion by Dihydroethidium**

The fluorescent dye dihydroethidium (hydroethidine) was used to evaluate superoxide anion generation by a previously described method. Mouse aorta was quickly embedded in OCT compound (Miles) to freeze on dry ice and was cut into 10-μm sections. Immediately, dihydroethidium (2×10<sup>−6</sup> mol/L; Molecular Probes) was applied to cryostat sections at 37°C for 30 minutes in the dark. Images were obtained by fluorescence microscopy (AXIVERT S100TV) with the use of an excitation wavelength of 488 nm and an emission wavelength of 610 nm.

**Mouse Aortic Smooth Muscle Cells**

Vascular smooth muscle cells were isolated from aortas of WT and G6PD<sup>−/−</sup> mice by enzymatic digestion. Untreated mice aged 4 to 5 months were used for cell isolation. The smooth muscle layer of aortas from 2 mice were denuded of endothelium and incubated in serum-free medium containing collagenase II (218 U/mg, 2 mg/mL), elastase (grade II, 0.75 mg/mL), and trypsin inhibitor (type II, 0.5 mg/mL) at 37°C for 20 minutes. Cells were rinsed and plated in Dulbecco’s modified Eagle’s medium with nutrient mixture F-12 (DMEM/F12) supplemented with 10% fetal bovine serum, 100 μg/mL penicillin G, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B. Cells between passages 6 and 15 were cultured in DMEM/F12 with the 10% fetal bovine serum and made quiescent by replacing the media with DMEM/F12 with 0.1% bovine serum albumin for 48 to 72 hours before the experiments.

**Western Blot**

Confluent quiescent vascular smooth muscle cells were stimulated with Ang II (100 nmol/L) as indicated. Cells were washed twice with cold PBS and lysed in modified RIPA buffer (1% NP-40, 0.25% deoxycholic acid, 50 mmol/L Tris, pH 7.4, 1 mmol/L EDTA, 150 mmol/L NaCl, 1 mmol/L NaF, 1 mmol/L sodium orthovanadate, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL leupeptin). Protein concentration was determined with protein assay reagent (Bio-Rad). Samples were separated by SDS-PAGE, transferred onto nitrocellulose membranes, and incubated with primary antibody overnight at 4°C. The proteins were detected with the use of horseradish peroxidase–conjugated secondary antibodies and ECL reagents. Some of the mouse aortas were homogenized in modified RIPA buffer, and protein expression for G6PD and other molecules was studied as well. G6PD antibody (polyclonal) was obtained from Bethyl Laboratories. a-Actin antibody (monoclonal) was from Sigma, and β-actin antibody (monoclonal) was from Abcam. Man-ganese superoxide dismutase (MnSOD) antibody was from Stressgen. All other antibodies were from Cell Signaling Technology.

**Determination of G6PD Activity**

Tissue or cells were homogenized in 20 mmol/L Tris buffer with 0.35 mol/L sucrose and centrifuged at 12 000g for 5 minutes. Supernatant was analyzed for protein concentration, and enzymatic activity of G6PD was assayed according to the method described elsewhere. Briefly, samples were incubated in buffer (50 mmol/L Tris, 1 mmol/L MgCl<sub>2</sub>, pH 8.1) containing substrates 6-phosphogluconic acid and NADP with or without d-glucose 6-phosphate (final concentration: 250 μmol/L each). The increase in absorbance at 340 nm (V<sub>max</sub>) was recorded on a plate reader. Spectra MAX 340, equipped with analytical software SoftMax Pro3.0. The difference between readings with and without d-glucose 6-phosphate was taken as G6PD activity.

**Determination of NADPH in Aortic Homogenate**

The content of NADPH in G6PD<sup>−/−</sup> mouse aorta was determined by measuring the absorbance at 340 nm with or without glutathione reductase as described elsewhere. Similarly, the level of NADPH was measured in mouse smooth muscle cells after extraction in buffer (0.1 mol/L Tris, pH 8.0, 0.01 mol/L EDTA, 0.05% [vol/vol] Triton X-100).
Effect of G6PD Deficiency on Blood Pressure

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>WT</th>
<th>G6PD&lt;sup&gt;mut&lt;/sup&gt; Hemizygous</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Ang II infusion</td>
<td>88.1±4.4 (n=13)</td>
<td>94.9±4.1 (n=19)</td>
<td>0.26</td>
</tr>
<tr>
<td>After Ang II infusion</td>
<td>139.7±4.7 (n=13)</td>
<td>123.5±5.2 (n=19)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Change in pressure with Ang II infusion</td>
<td>51.6±3.0 (n=13)</td>
<td>35.3±5.6 (n=19)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
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Ang II (0.7 mg/kg per day) was infused through subcutaneous osmotic pumps for 6 days. Blood pressure was measured by tail cuff. Data are expressed as mean±SE (mm Hg). The pressor response to Ang II was significantly less in G6PD<sup>mut</sup> mice.

Data Analysis
Data are expressed as mean±SE. Statistical comparisons were performed by ANOVA and Student t test. Significance was accepted when P was <0.05.

Results

Blood Pressure and Body Weight

Although baseline blood pressure tended to be higher in G6PD<sup>mut</sup> mice compared with age-matched C3H WT mice, it was not significantly different (Table). After 6 days of Ang II infusion, blood pressure was significantly increased in both mouse strains, but the pressure increase was significantly less in G6PD<sup>mut</sup> mice, and the final blood pressure was significantly lower in G6PD<sup>mut</sup> mice (Table). Body weight was not significantly different between WT and G6PD<sup>mut</sup> mice before or after surgery (data not shown).

G6PD Activity and NADPH Content in Mouse Aorta

The G6PD enzyme activity in G6PD<sup>mut</sup> mouse aorta was 10% to 20% of that measured in WT mouse aorta and was not altered after Ang II infusion (Figure 1a). Protein expression of G6PD was obviously lower in G6PD<sup>mut</sup> mouse aorta and was not altered after Ang II infusion (Figure 1b). The content of NADPH in G6PD<sup>mut</sup> mouse aorta was 50% of that in WT mice (WT 123±7 nmol/mg protein, G6PD<sup>mut</sup> 63±1 nmol/mg protein; n=6; P<0.01).

Aortic Medial Thickness

The cross-sectional medial thickness was not different between WT mice and G6PD<sup>mut</sup> mice after sham infusion (WT 55±2.6 μm, G6PD<sup>mut</sup> 54±2.7 μm). Ang II infusion significantly increased aortic medial thickness to 71±2.0 μm in WT mice and to 62±1.5 μm in G6PD<sup>mut</sup> mice. Compared with WT, the hypertrophic response to Ang II was significantly less in G6PD<sup>mut</sup> mouse aorta (P<0.01; Figure 2).

Immunohistochemistry for 3-o-Nitrotyrosine

Ang II infusion increased 3-o-nitrotyrosine in mouse aorta, and this increase was attenuated in mice deficient in gp91phox or in mice that overexpress human superoxide dismutase. Therefore, formation of 3-o-nitrotyrosine in proteins was examined as a marker of superoxide generation and its reaction product with nitric oxide, peroxynitrite. In Ang II–infused WT mice, staining for 3-nitrotyrosine on aortic cross sections showed increased staining in the endothelium and the adventitia and to a lesser extent in the media, as shown previously. In contrast, the staining in Ang II–infused G6PD<sup>mut</sup> mouse aorta was obviously less (Figure 3a). Semiquantitative analysis showed that Ang II infusion significantly increased nitrotyrosine staining compared with saline infusion in WT mice but not in G6PD<sup>mut</sup> mice (Figure 3b).

Detection of Superoxide Anion by Dihydroethidine in Mouse Aorta

To further assess whether a lower NADPH level results in lower superoxide generation by NADPH oxidase, oxidation...
of dihydroethidium was examined in mouse aorta. Ang II–infused mouse aorta showed a higher fluorescent signal localized primarily in medial smooth muscle compared with saline-infused mouse aorta (Figure 4a). In addition, Ang II–infused WT mouse aorta showed a visibly greater signal than Ang II–infused G6PDmut mouse aorta (Figure 4b).

Ang II Signaling in Aortic Smooth Muscle Cells

Smooth muscle cells isolated from G6PDmut mouse aorta had growth rates similar to those of cells from WT mice. α-Actin was confirmed by immunoblot as a marker of smooth muscle cells and was expressed similarly and consistently in cells from WT and G6PDmut mice during passage. After 10 passages, the G6PD activity of cells from G6PDmut mice was <10% of that in cells from WT mice (data not shown). The content of NADPH in cultured cells from G6PDmut mice was significantly decreased compared with cells from WT mice (WT 135±15 nmol/mg protein, G6PDmut 88±4 nmol/mg protein; n=3; P<0.05). When quiescent cells were stimulated with Ang II, phosphorylation of Akt was lower in cells from G6PDmut mice than in cells from WT mice. Additionally, phosphorylation of p42/44 ERK responded less to Ang II in cells from G6PDmut (Figure 5).

Discussion

Our results indicate that vascular responses to Ang II are blunted in G6PD-deficient mice, which is consistent with less NADPH being available for superoxide generation by NADPH oxidase. G6PDmut mice showed a smaller pressor response and less medial hypertrophy, superoxide anion, and nitrotyrosine in the aorta. Cultured smooth muscle cells isolated from G6PDmut mice also demonstrated attenuated responses to Ang II, confirming that decreased G6PD activity is associated with a decreased response to Ang II at a cellular level.

G6PD is the rate-limiting enzyme in the pentose phosphate pathway that generates 2 molecules of NADPH from NADP⁺ through the oxidation of glucose-6-phosphate. Because the pentose phosphate pathway is a main source of cytosolic NADPH, the activity of G6PD may influence various NADPH-dependent reactions. For instance, GSH reductase requires NADPH to maintain GSH in its reduced form. Acute oxidative stress associated with depletion of GSH was shown to increase G6PD expression and to restore GSH levels. In response to acute ischemia/reperfusion, the G6PD-deficient mice used in the present study demonstrated greater cardiac dysfunction, associated with lower GSH levels. Thus, an inadequate supply of NADPH may be a disadvantage in G6PD deficiency by preventing recovery of acutely lowered GSH levels. In addition, G6PD activity may regulate nitric oxide synthase, which also requires NADPH as a cofactor. NAPDH also is required for the activity of NADPH oxidase, and decreased production of superoxide anion and hydrogen peroxide has been reported in G6PD-deficient granulocytes and in coronary arteries after pharmacological inhibition of G6PD. The Michaelis constant (Km) for NADPH, 0.04 mmol/L, is 100-fold higher for NADPH oxidase than for nitric oxide synthase and 5-fold higher than for glutathione reductase. Therefore, NADPH oxidase requires higher concentrations of NADPH to be active and thus may be more susceptible to a decreased supply of the substrate compared with these other NAPDH-dependent enzymes. This may be particularly so when oxidative stress is mediated by NADPH oxidase, particularly in more chronic conditions, G6PD deficiency would be expected to decrease superoxide anion generation.

It is possible that lower NADPH levels produce less endothelium-derived nitric oxide in G6PDmut mice. Consistent with this assumption, basal blood pressure tended to be higher in G6PDmut mice. Lower nitric oxide levels, not only lower superoxide, might also explain the decreased nitrotyrosine found in the aorta of G6PDmut mice. In preliminary studies, we examined the effect of inhibiting nitric oxide synthase with N⁴-nitro-L-arginine methyl ester (L-NAME) on blood pressure in G6PDmut mice. L-NAME (1 mg/mL added to the drinking water for 3 weeks) increased blood pressure to...
similar levels in WT and G6PD<sup>mut</sup> mice, suggesting that baseline nitric oxide production was not very different. When Ang II was infused together with L-NAME, 3 of 4 WT mice died during the 6 days infusion, whereas 3 of 4 G6PD<sup>mut</sup> mice survived. This suggests that the response to Ang II was blunted in G6PD<sup>mut</sup> mice even after inhibition of nitric oxide synthesis.

Nitrotyrosine staining, a footprint of peroxynitrite derived from superoxide and nitric oxide, was enhanced in adventitia and endothelium after Ang II infusion in WT mouse aorta, consistent with previous observations<sup>20,27</sup> and attributable to superoxide generation from adventitial fibroblasts.<sup>37</sup> However, dihydroethidine indicated intense superoxide production in the smooth muscle layer after Ang II infusion, in agreement with others.<sup>21</sup> Dihydroethidine is accumulated from the reaction of superoxide during a brief in vitro incubation, whereas nitrotyrosine accumulates over time in vivo. We speculate that the reaction between superoxide and nitric oxide occurs in vivo preferentially in adventitia and endothelium. G6PD<sup>mut</sup> mice may demonstrate less nitrotyrosine because of lower superoxide and nitric oxide levels, yielding less peroxynitrite.

One controversial issue relates to substrate specificity of vascular NADPH oxidase. Studies of the oxidase in homogenates of cultured rat aortic smooth muscle cells indicate that both NADH and NADPH can serve as substrates for producing superoxide,<sup>11,38,39</sup> whereas the phagocyte oxidase exclusively consumes NADPH.<sup>10</sup> However, NADPH was by far the preferred substrate in membrane fractions of rabbit aorta.<sup>40</sup> Our results are consistent with NADPH being the more important substrate for vascular NAD(P)H oxidase in vivo and are consistent with a recent publication showing that G6PD-dependent NADPH regulates basal superoxide generation in isolated arteries.<sup>41</sup>

The level of NADPH in the aorta or smooth muscle cells in culture was decreased in G6PD<sup>mut</sup> mice to ∼50% of WT mice, even though G6PD activity was 10% that of WT mice. This is likely due to other NADPH-producing enzymes such as malate dehydrogenase and isocitrate dehydrogenase. We speculate that the decrease in NADPH levels manifests itself only when NADPH consumption is unusually increased, such as with induction of an enzyme like NADPH oxidase that has a relatively high $K_m$ for the substrate.

Earlier studies demonstrated that Ang II activated vascular NADPH oxidase in the plasma membrane to generate super-
lular protein was extracted, and expression of each protein stimulated with Ang II (100 nmol/L) for the indicated times. Cel- 
erk phosphorylation was relatively decreased in cells from aortic smooth muscle cells. Ang II–induced Akt and p42/44 
we examined Ang II–mediated signaling in cultured mouse 
similar experiments are shown. 

Figure 5. Ang II–induced Akt and ERK phosphorylation in cul-
tured mouse aortic smooth muscle cells. Quiescent aortic 
smooth muscle cells isolated from WT and G6PDmut mice were stimulated with Ang II (100 nmol/L) for the indicated times. Cel-

lar protein was extracted, and expression of each protein (phospho-Akt, Akt, phospho-p42/44 ERK, p42/44 ERK, α-actin) was evaluated by Western blot. Representative blots from 4 similar experiments are shown. 

oxide/reactive oxygen species.11,42 Superoxide anion and its 
metabolite, hydrogen peroxide, are essential regulators of 
signaling induced by Ang II, leading to phosphorylation of 
Akt, increased protein synthesis, and medial hypertrophy.15,16 Because medial hypertrophy was decreased in G6PDmut mice, we examined Ang II–mediated signaling in cultured mouse aortic smooth muscle cells. Ang II–induced Akt and p42/44 ERK phosphorylation was relatively decreased in cells from G6PDmut mice compared with WT cells. In rat aortic smooth muscle cells, Ang II–induced Akt phosphorylation and pro-
tein synthesis are redox sensitive,16,19 but ERK phosphoryla-
tion may not be.17,19 However, there are also some reports that 
phosphorylation of ERK in response to Ang II is redox 
sensitive,43,44 Compared with rat aortic smooth muscle cells in culture, we found that it is more difficult to consistently 
decrease basal phosphorylation of ERK in quiescent mouse aortic smooth muscle cells. This difference in cultured cells 
could explain the difference in the response of ERK. The decreased response in Akt of cells from G6PDmut mice 
provides a molecular mechanism whereby lower levels of 
NADPH and less oxidant generation can result in decreased 
medial hypertrophy. 

The response to Ang II is not only important in hypertension 
but also plays a role as a mediator in atherosclerosis45,46 and angiogenesis.57 Insufficient substrate for NADPH oxida-
dase might therefore influence cellular responses in various 
pathologies in vivo. In fact, we found that G6PD deficiency also 
results in decreased aortic superoxide and atherosclerotic 
lesions in apolipoprotein E–deficient mice.88 

G6PD deficiency is the most common enzymopathy in the 
world and is particularly prevalent in Mediterranean and 
African countries. G6PD deficiency is known to manifest 
itself in chemically induced hemolytic anemia but also is 
associated with higher resistance to and survival from malar-

ia.49 It is not well studied whether cardiovascular risk is 
affected in people with G6PD deficiency. Although an early 
report suggested a higher incidence of hypertension,22 a 
recent cohort study suggested lower mortality from cardio-
vascular disease in people with G6PD deficiency.50 Although 

human cardiovascular diseases stem from multiple genetic 
and environmental factors, a decreased role of NADPH 
oxidase may provide a beneficial effect among people with 
G6PD deficiency. In conclusion, our data suggest that G6PD 
deficiency may provide an advantage by protecting against 
Ang II–dependent hypertension by decreasing the contribu-
tion of NADPH oxidase–derived superoxide anion to the 
pressor and hypertrophic vascular response. 

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References 
Enhanced glutathione levels and oxidoreductase mediated by increased 
glucose-6-phosphate dehydrogenase expression. J Biol Chem. 1999;274: 
2750–2757. 
2. Leopold JA, Localdo J. Cyclic strain modulates resistance to oxidant 
stress by increasing G6PD expression in smooth muscle cells. 
3. Tsai KJ, Hung DJ, Chow CK, Stern A, Chao SS, Chiu DTY. Impaired 
production of nitric oxide, superoxide, and hydrogen peroxide in 
glucose-6-phosphate dehydrogenase–deficient granulocytes. FEBS Lett. 1998;436: 
411–414. 
4. Hothersall JS, Gorgde M, Noronha-Dutra AA. Inhibition of NADPH 
supply by 6-aminocinodiamine: effect on glutathione, nitric oxide, and 
5. Guo L, Zhang Z, Green K, Stanton RC. Suppression of interleukin-
1β-induced nitric oxide production in RINm5F cells by inhibition of 
6-phosphate dehydrogenase overexpression decreases endothelial cell 
oxidant stress and increases bioavailable nitric oxide. Arterioscler 
7. Pascale R, Garcea R, Ruggiu ME, Diano L, Frassetto S, Vanniini MG, 
Cozzolino P, Lenzieri L, Feo F, Schwartz AG. Decreased stimulation by 
12-0-tetradecanoylphorbol-13-acetate of superoxide radical production by 
neutrophil-type NAD(P)H oxidase in smooth muscle cells from human 
aortic smooth muscle cells. 
8. Gupte SA, Arshad M, Viola S, Kaminski PM, Ungvári Z, Rabbani G, 
Koller A, Wolin MS. Pentose phosphate pathway coordinates multiple 
redox-controlled relaxing mechanisms in bovine coronary arteries. 
9. Griendling KK, Ushio-Fukai M. Reactive oxygen species as mediators of 
10. Cifuentes ME, Rey FE, Carretero OA, Pagano PJ, Gupte SA, Arshad M, 
Lo Ban R, Quinn M, Pagano O, Schwartz AG. Decreased stimulation by 
12-0-tetradecanoylphorbol-13-acetate of superoxide radical production by 
neutrophil-type NAD(P)H oxidase in smooth muscle cells from human 
aortic smooth muscle cells. 
11. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angioten-
sin II stimulates NADH and NADPH oxidase activity in cultured vascular 
12. Cifuentes ME, Rey FE, Carretero OA, Pagano PJ. Upregulation of p67phox 
and gp91phox in aortas from angiotensin II–infused mice. Am J Physiol. 
RL. Expression of a functionally active gp91phox-containing 
neutrophil-type NAD(P)H oxidase in smooth muscle cells from human 
p22phox is a critical component of the superoxide-generating 
NADPH/NADPH oxidase system and regulates angiotensin II–induced 
hypertrophy in vascular smooth muscle cells. J Biol Chem. 1996;271: 
23317–23321.


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