Impact of glucose-6-phosphate dehydrogenase deficiency on the pathophysiology of cardiovascular disease

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Hecker PA, Leopold JA, Gupte SA, Recchia FA, Stanley WC. Impact of glucose-6-phosphate dehydrogenase deficiency on the pathophysiology of cardiovascular disease. Am J Physiol Heart Circ Physiol 304: H491–H500, 2013. First published December 15, 2012; doi:10.1152/ajpheart.00721.2012.—Glucose-6-phosphate dehydrogenase (G6PD) catalyzes the rate-determining step in the pentose phosphate pathway and produces NADPH to fuel glutathione recycling. G6PD deficiency is the most common enzyme deficiency in humans and affects over 400 million people worldwide; however, its impact on cardiovascular disease is poorly understood. The glutathione pathway is paramount to antioxidant defense, and G6PD-deficient cells do not cope well with oxidative damage. Limited clinical evidence indicates that G6PD deficiency may be associated with hypertension. However, there are also data to support a protective role of G6PD deficiency in decreasing the risk of heart disease and cardiovascular-associated deaths, perhaps through a decrease in cholesterol synthesis. Studies in G6PD-deficient (G6PDX) mice are mixed and provide evidence for both protective and deleterious effects. G6PD deficiency may provide a protective effect through decreasing cholesterol synthesis, superoxide production, and reductive stress. However, recent studies indicate that G6PDX mice are moderately more susceptible to ventricular dilation in response to myocardial infarction or pressure overload-induced heart failure. Furthermore, G6PDX hearts do not recover as well as nondeficient mice when faced with ischemia-reperfusion injury, and G6PDX mice are susceptible to the development of age-associated cardiac hypertrophy. Overall, the limited available data indicate a complex interplay in which adverse effects of G6PD deficiency may outweigh potential protective effects in the face of cardiac stress. Definitive clinical studies in large populations are needed to determine the effects of G6PD deficiency on the development of cardiovascular disease and subsequent outcomes.

G6PD; heart failure; NADPH; ROS; oxidative stress

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) catalyzes the conversion of G6P to 6-phosphogluconolactone and the formation of NADPH from NADP+. G6PD activity is a key determinant of the NADPH-to-NADP+ ratio in the cytoplasm and thus contributes to replenishment of the antioxidant glutathione system. In addition, by influencing the concentration of NADPH, G6PD activity can influence NADPH-dependent superoxide production (3, 83, 107, 110). G6PD deficiency is the most common enzyme deficiency in the world, with an estimated >400 million people with G6PD deficiency (12, 59, 109a). G6PD deficiency is common throughout sub-Saharan Africa, regions in the Mediterranean, and parts of Southeast Asia and is thought to be preserved in these populations because it results in few gross complications and it confers a selective advantage against malaria (59). Here we will review the biochemical and physiological effects of G6PD deficiency on the heart and discuss the potential impact on the development and progression of heart disease. Furthermore, we will address recent evidence suggesting adverse effects of G6PD deficiency on heart failure.

G6PD Deficiency

G6PD deficiency is caused by a diverse array of mutations in the g6pdx gene (82, 103, 109a), which is X-linked, and thus G6PD deficiency is most common in males. Common mutant

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G6PD Deficiency and Cardiovascular Disease

**Review**

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G6PD is a cytoplasmic enzyme that controls the entry of G6P into the pentose phosphate pathway (Fig. 1). The pentose phosphate pathway is characterized by the following reactions: 1) G6PD oxidizes G6P to 6-phosphogluconolactone; 2) lactonase hydrolyzes circular 6-phosphogluconolactone to form a linear product, 6-phosphoglucone; and 3) 6-phosphoglucone dehydrogenase (6PGD) converts 6-phosphogluconate to ribulose-5-phosphate. The reaction catalyzed by 6PGD cleaves the 1° carbon from 6-phosphogluconate to release CO₂. These three reactions are known as the oxidative phase of the pathway. G6PD and 6PGD both reduce NADP⁺ to NADPH, but the reaction catalyzed by G6PD is a rate-determining step of the pentose phosphate pathway, and decreasing the activity of G6PD lowers NADPH levels (27, 31, 32, 35, 44). In the nonoxidative phase, ribulose-5-phosphate may be used for nucleotide synthesis or aromatic amino acid synthesis or may be converted to fructose-6-phosphate and glyceraldehydes-3-phosphate through a series of aldolases and transketolases that reenter the pathway or are oxidized as fuel. It is important to note that there is also NADPH in the mitochondria which is maintained by isocitrate dehydrogenase, glutamate dehydrogenase, and malic enzyme and that isocitrate dehydrogenase has a cytoplasmic isofrom that produces NADPH; however, G6PD is the major producer of cytoplasmic NADPH (27, 44).

NADPH from G6PD is required by cellular antioxidant systems to reduce reactive oxygen species (ROS) (19, 21, 23, 24, 44, 45, 101). The glutathione system requires NADPH to remove excess hydrogen peroxide (H₂O₂) (Fig. 2). In this system, glutathione reductase uses NADPH to convert oxidized glutathione (GSSG) to its reduced form (GSH). GSH is then converted back to GSSG by glutathione peroxidase to reduce H₂O₂ to water. Thus NADPH fuels the removal of H₂O₂ by the glutathione system (63). This system is generally considered to be beneficial because excessive ROS would likely adversely affect cell function through damaging reactions with proteins, nucleotides, and lipids (99, 107). Because of the requirement for NADPH in the reduction of ROS, G6PD-deficient cells are sensitive to oxidizing stimuli and more easily succumb to oxidative stress than nondeficient cells (23, 24, 104), whereas the overexpression of G6PD protects cells against oxidative damage (91). This increased susceptibility for cell death in G6PD-deficient cells can lead to adverse physiological effects under conditions of increased oxidative stress in tissues where the specific cell type is indispensable (44, 45, 48, 75, 101, 105, 111, 115). In particular, cell types

Fig. 1. The pentose phosphate pathway. The oxidative phase of the pentose phosphate pathway produces NADPH from NADP⁺ and ribulose-5-phosphate. Ribulose-5-phosphate may be converted to glyceraldehyde-3-phosphate and fructose-6-phosphate through a series of nonoxidative reactions. 6PGD, 6-phosphogluconate dehydrogenase; G6PD, glucose-6-phosphate dehydrogenase.

**G6PD Biochemistry and Cell Biology**

G6PD is a cytoplasmic enzyme that controls the entry of G6P into the pentose phosphate pathway (Fig. 1). The pentose phosphate pathway is characterized by the following reactions: 1) G6PD oxidizes G6P to 6-phosphogluconolactone; 2) lactonase hydrolyzes circular 6-phosphogluconolactone to form a linear product, 6-phosphoglucone; and 3) 6-phosphogluconate dehydrogenase (6PGD) converts 6-phosphogluconate to ribulose-5-phosphate. The reaction catalyzed by 6PGD cleaves the 1° carbon from 6-phosphogluconate to release CO₂. These three reactions are known as the oxidative phase of the pathway. G6PD and 6PGD both reduce NADP⁺ to NADPH, but the reaction catalyzed by G6PD is a rate-determining step of the pentose phosphate pathway, and decreasing the activity of G6PD lowers NADPH levels (27, 31, 32, 35, 44). In the nonoxidative phase, ribulose-5-phosphate may be used for nucleotide synthesis or aromatic amino acid synthesis or may be converted to fructose-6-phosphate and glyceraldehydes-3-phosphate through a series of aldolases and transketolases that reenter the pathway or are oxidized as fuel. It is important to note that there is also NADPH in the mitochondria which is maintained by isocitrate dehydrogenase, glutamate dehydrogenase, and malic enzyme and that isocitrate dehydrogenase has a cytoplasmic isofrom that produces NADPH; however, G6PD is the major producer of cytoplasmic NADPH (27, 44).

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Fig. 2. Role of G6PD in both antioxidant and oxidant formation pathways. The pentose phosphate pathway produces NADPH through G6PD and 6PGD. NADPH supports the antioxidant glutathione pathway in which glutathione reductase (GR) uses NADPH to reduce oxidized glutathione (GSSG) to reduced glutathione (GSH) for use by glutathione peroxidase (GPx) to reduce H₂O₂ to H₂O. On the other hand, NADPH is also used to produce superoxide (O₂⁻) via NADPH oxidase, uncoupled nitric oxide synthase, and xanthine oxidase. O₂⁻ is converted to H₂O₂ by O₂⁻ dismutase (SOD), and the overproduction of these reactive oxygen species may adversely affect cell function.

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that normally have low G6PD expression in nondeficient individuals may be likely to succumb when faced with oxidant stress in deficient individuals where the G6PD expression is even lower than normal (39, 115) (Fig. 3).

The short-term effects of decreased G6PD activity have been examined in cardiomyocytes (44). In nondeficient cells, H₂O₂ stimulates G6PD activity and short-term pharmacological inhibition of G6PD in the absence of H₂O₂ decreased GSH/GSSG and increased ROS. The increase in ROS resulted in contractile dysfunction, as indicated by decreased cell shortening and prolonged relengthening. These functional impairments corresponded with impaired Ca²⁺ transport and were rescued by antioxidant treatment, but not by ribose supplementation, indicating that they were likely due to a decrease in the capacity of the antioxidant glutathione system.

It is important to note that the nonoxidative phase is critical for ribose synthesis and the subsequent production of nucleotides, and this could potentially impact the G6PD-deficient cell. This is particularly important under conditions of rapid growth, such as cancerous cells where extra ribose may be needed to produce DNA for replication (10); however, this topic is outside the focus of this review. The effects of ribose supplementation in the G6PD-deficient state have been investigated (44, 45, 105) and found that it did not impact the physiology or pathophysiology of G6PD deficiency in the heart (44, 45). On the other hand, the NADPH produced by G6PD is critical both for protecting the cell against oxidative damage and for cell growth (104, 105).

G6PD Deficiency in Human Cardiovascular Disease

Despite its known role in oxidant defense, there is a surprisingly limited amount of clinical information about G6PD deficiency in the cardiovascular system. G6PD deficiency may increase diabetes and renal failure. This is indicated by increased serum creatinine and an increased risk for diabetes in G6PD-deficient subjects (28, 74, 90, 108, 109) and albuminuria and metabolic dysfunction in deficient animals (40, 111, 115), which could have adverse cardiac effects. G6PD is upregulated in response to a number of stress-induced stimuli, including heart failure, (9, 32, 35, 38, 40, 44, 45, 101, 102), and deficient individuals may be unable to upregulate G6PD in response to stress (19–21, 68). An increase in the activity of G6PD is generally thought of as being protective because it should increase the capacity of antioxidant defense systems. G6PD activity was increased in postinfarct surviving myocardium and in failing myocardium in both human and animal models of disease (9, 32, 35, 38, 39, 45). This could be a compensatory mechanism necessary to oppose the increased generation of ROS in failing myocardium (9).

In 1967, cardiovascular parameters in association with G6PD deficiency were examined among 1,473 black American men (57). This study found a higher incidence of hypertension and idiopathic cardiomyopathy among those with G6PD deficiency. However, this study also found evidence of decreased coronary artery disease among G6PD-deficient patients compared with a normal population. Similar evidence has been observed more recently in two small studies that suggest G6PD deficiency may decrease the risk of coronary heart disease and cardiovascular associated death (15, 67). The first study reported on the mortality in 1,756 G6PD-deficient men in Sardinia (15) and found that during a 5-yr follow-up period, 29 men with G6PD deficiency died of cardiovascular-associated death, whereas 62.6 cardiovascular deaths were expected based on population data. This suggests that G6PD deficiency may decrease cardiovascular-associated death. The second study was a case-control study that reported that among 314 cases of Sardinian men with coronary artery disease, 11.8% were G6PD deficient, whereas among 424 controls, 18.6% were G6PD deficient (67). These findings suggest that G6PD deficiency protects against coronary heart disease. Thus, despite the role of G6PD in protecting against oxidative damage in cell and tissue based studies, limited population studies do not support an adverse role for G6PD deficiency in human cardiovascular disease, although findings from these studies are not definitive as they cannot exclude a survivor benefit.

Effects of Deficient G6PD Activity in Experimental Systems

Animal model of G6PD deficiency. Complete genetic deletion of G6PD produces embryonic lethality (58). However, the generation of G6PD-deficient (G6PDX) mice that recapitulate key aspects of clinical deficiency was reported in 1988 (84). This was done by treating male mice with a DNA ethylating agent that normally have low G6PD expression in nondeficient individuals may be likely to succumb when faced with oxidant stress in deficient individuals where the G6PD expression is even lower than normal (39, 115) (Fig. 3).
agent 1-ethyl-1-nitroso urea to induce DNA mutations, and G6PD deficiency was identified in subsequent offspring. G6PDX mice have an A:T mutation in the 5′-untranslated region at the splice site of the 3′-end of exon 1 (93). This mutation results in decreased translation of G6PD and leads to ∼20–40% residual G6PD activity (class III deficiency) in G6PDX mice compared with wild-type (WT) littermate control mice. G6PDX mice mature normally but have been reported to develop modest cardiac hypertrophy at 9 mo of age (44). Overall, G6PDX mice provide a clinically relevant model of G6PD deficiency that reflects the extent of limited G6PD activity commonly found in human patients with G6PD deficiency (6, 12).

Statin-like effect. A protective cardiovascular role for G6PD deficiency is conceivable in light of the effects of G6PD deficiency on cholesterol synthesis (71). The enzyme 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase is rate determining in the cholesterol synthesis pathway and is dependent on NADPH to catalyze its reaction. Decreased cholesterol synthesis was found in peripheral lymphomononuclear cells obtained from G6PD-deficient subjects (5), and evidence suggests that G6PD deficiency may decrease the overall cholesterol content in the blood of deficient subjects (71). We also found evidence for decreased cholesterol synthesis in G6PDX mice (88). Furthermore, in an apolipoprotein E-deficient mouse model of atherosclerosis, G6PD deficiency decreased cholesterol and the development of vascular lesions (63). Thus G6PD deficiency may provide a statin-like cholesterol-lowering effect and thereby protect against atherosclerosis (57, 70, 71, 94).

Reductive stress. In addition to decreasing cholesterol synthesis, G6PD deficiency may be protective under conditions of reductive stress (85). Reductive stress refers to an increase in NADPH and GSH and may result in adverse cellular events such as protein misfolding and aggregation, mitochondrial dysfunction, pathological gene expression, and increased susceptibility to apoptosis (11, 14, 60, 98, 106, 112, 114). Evidence for a role of reductive stress in heart failure was found in a mouse model of desmin-related cardiomyopathy induced by overexpression of mutant human αB-crystallin (hR120GCrAB) in mice (CryAB mice) (85–87). This cardiomyopathy model results in protein aggregation within the cell, hypertrophy, and failure of the ventricle (29, 64, 85). Support for the role of reductive stress in the cardiomyopathy observed in CryAB mice was provided by the observation that G6PD and glutathione reductase activities were increased with a concomitant increase in GSH and the GSH-to-GSSG ratio (GSH/GSSG) (85). An increase in GSH contrasts with the typically reported decrease in GSH/GSSG that is associated with oxidative stress (86). When CryAB mice were crossed with G6PDX mice, there was a decrease in protein aggregation and cardiac hypertrophy, thus supporting a protective role for G6PD deficiency in reducing reductive stress in protein aggregation-associated cardiomyopathy (85). Overall, the data suggest that decreasing G6PD activity may alleviate reductive stress (17).

G6PD fuels superoxide production. Although G6PD supplies the antioxidant glutathione system with NADPH, the NADPH produced by G6PD is also used to produce superoxide via NADPH oxidase (Nox), uncoupled nitric oxide synthase, and xanthine oxidase (Fig. 2) (3, 83, 107, 110). These reactions are all implicated in heart failure, and decreasing the production of ROS by these enzymes may exert beneficial effects on cardiac hypertrophy and dysfunction (18, 37, 41, 42, 49, 110). An upregulation of G6PD expression may fuel these superoxide-producing enzymes in failing myocardium (32, 35). Specifically, G6PD expression and activity were increased in failing myocardium in dogs and humans (Fig. 4) (32, 35). The increase in G6PD corresponded with an increase in NADPH levels and superoxide production, and pharmacological inhibition of G6PD in vitro decreases NADPH levels and ROS production.

Similar evidence for a role of G6PD in deriving NADPH-dependent ROS was observed in a number of other experimental models with different cell and tissue types (2, 36, 51, 78–80). These include a decrease in aortic superoxide production in apolipoprotein E-deficient atherosclerotic mice that are crossed with G6PDX mice (63). G6PD deficiency also decreased aortic dihydroxyethidium and nitrotyrosine and lowered the hypertensive response to angiotensin-II infusion (62). Superoxide production is increased in the aorta, heart, and liver in genetically obese Zucker rats, and in vitro pharmacological inhibition of G6PD decreased the generation of superoxide in these tissues (31, 97). Recently, G6PD was reported to decrease superoxide production by Nox4 in isolated liver nuclei (100). Overall, the production of superoxide may be increased when G6PD activity is increased or decreased when there is limited G6PD activity.

Suppression of myocardial ROS generation in response to pharmacological G6PD inhibition suggests that inhibiting G6PD may also decrease the generation of ROS in vivo (32, 35) and thus suggests a potential therapeutic approach for

![Figure 4. G6PD inhibition decreases NADPH levels and O2•− production in failing myocardium. Myocardium was obtained from failing and nonfailing patients undergoing cardiac surgery. NADPH levels and O2•− production were assessed in myocardial homogenates in the presence or absence of the G6PD inhibitor 6-aminonicotinamide. *P < 0.05 vs. normal; #P < 0.04 vs. heart failure. Reprinted with permission from Gupte et al. (32) with modifications.](http://ajpheart.physiology.org/)

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In fact, superoxide is a signaling molecule that is necessary for vascular function, and Nox4 deficiency, which decreases superoxide levels, decreased angiogenesis and accelerated the development of heart failure (1, 26, 77, 95, 113). In light of the recent finding that Nox4-derived superoxide is necessary for vascular growth (89, 95, 113), others found that G6PD may fuel Nox4-derived superoxide production (100). Furthermore, we found decreased superoxide and capillary density in failing G6PD-deficient myocardium (39). Overall, the finding that limiting G6PD activity also decreases superoxide production does not necessarily mean that this is a beneficial effect of G6PD deficiency.

**G6PD deficiency and cardiac ischemia-reperfusion.** Because G6PD deficiency decreases myocardial antioxidant capacity, it may exacerbate the adverse cardiac effects of acute oxidative stress such as with ischemia-reperfusion injury (92). Isolated perfused hearts from G6PDX mice displayed a greater impairment in relaxation and pressure development after injury compared with WT mice (Fig. 5) (45). In these experiments, acute ischemia-reperfusion injury increased G6PD activity in WT mice, whereas G6PDX-deficient myocardium was unable to compensate for the increased oxidative stress as indicated by a decreased GSH/GSSG ratio in these hearts (45). The exacerbated impairments in relaxation and pressure development in G6PDX mice were rescued by antioxidant treatment, but not by ribose supplementation, demonstrating that the deficit was due to a deficiency in antioxidant defenses rather than a ribose deficiency resultant from the nonoxidative phase of the pentose phosphate pathway. Thus G6PD deficiency increases the sensitivity of the myocardium for ROS and exacerbates contractile dysfunction in the face of oxidative injury.

**Heart failure and G6PD deficiency.** We recently tested the effects of G6PD deficiency on the development of heart failure and found that G6PDX deficiency exacerbated cardiac remodeling after chronic stress (39). G6PDX mice were subjected to 6 wk of pressure overload or to myocardial infarction with 12 wk of follow-up. In these experiments, G6PD deficiency worsened left ventricular dilation (Fig. 6) without affecting the overall heart mass or diastolic or systolic function. We further stressed pressure-overloaded mice with a high fructose diet and found that in this case, G6PD deficiency exacerbated cardiac remodeling, fetal gene expression, and hypertrophy among high fructose-fed mice. Thus G6PD deficiency adversely affected the
development of left ventricular chamber expansion and heart failure.

Interestingly, in these studies NADPH levels were increased in G6PD-deficient myocardium in response to either infarction or pressure overload (39), suggesting that there is a compensatory mechanism that increases NADPH levels in G6PD deficiency. This compensatory increase in NADPH levels could be due to an increase in mitochondrial NADPH-producing enzymes and may not affect the cytoplasmic pool of NADPH levels. In this regard, superoxide production was decreased in G6PDX mice, possibly due to decreased cytoplasmic availability of NADPH for use by Nox. However, despite the compensatory increase in NADPH levels and the decrease in superoxide production, G6PD-deficient myocardium had increased oxidative stress in response to pressure overload as indicated by a decrease in GSH, indicating an adverse effect of G6PD deficiency.

In the myocardial infarction model of heart failure described above, there was no effect of G6PD deficiency on superoxide production, but there was an increase in lipid peroxidation products in G6PDX mice, indicating increased oxidative stress in this heart failure model as well (39). A possible explanation for the increased oxidative stress despite there being an increase in NADPH levels and no overall change in superoxide generation in this case is that G6PD-deficient myocardium are unable to fully alleviate oxidative stress. Here it should be considered that the measured level of NADPH does not necessarily reflect the flux through G6PD or the rate of production of NADPH but simply reflects the concentration of NADPH at equilibrium. Also, measures of G6PD activity are generally reflective only of the amount of enzyme, rather than the activity within the cell, because at physiological concentrations of NADPH, G6PD is almost completely inhibited (22). Thus activation of G6PD is a matter of de-repression of the enzyme. In the 1970s, Eggleston and Krebs found that GSSG de-represses G6PD through the activation of some cofactor (22, 76). Although their data have been challenged, the activation of G6PD by GSSG remains an intriguing possibility (56). If GSSG activates G6PD, then it may take high levels of GSSG to sufficiently activate G6PD to counterbalance oxidative stress in G6PD-deficient myocardium. This would result in greater oxidative stress as observed by a decreased GSH/GSSG ratio, but not necessarily an increase in the production of ROS. This change in the levels of GSH/GSSG (and NADPH) may result in aberrant signaling and the adverse changes that we observed with G6PD deficiency with heart failure (11, 14, 16, 60, 85, 106).

**Beneficial effects of pharmacologic G6PD activation in heart failure.** Benfotiamine is a vitamin B1 analog that activates the pentose phosphate pathway by increasing the activity of G6PD and transketolase (46). A recent investigation in a mouse model of myocardial infarction found administration of benfotiamine for 4 wk before coronary ligation increased G6PD activity; decreased infarct size and oxidative stress; and improved survival, capillary density, blood flow, and functional parameters in the heart (46). Other studies found benfotiamine reduced diastolic dysfunction associated with diabetes (13, 46, 47). In cardiomyocytes exposed to hypoxia, silencing of G6PD prevented antiapoptotic signaling in response to benfotiamine, indicating that the beneficial effects of benfotiamine on cardiomyocytes may be due to increased activation of G6PD. Overall, these studies show that the activation of G6PD may be beneficial in heart failure.

**G6PD deficiency and vascular dysfunction.** The pro- and antioxidant effects of G6PD also play an important role in vascular redox homeostasis and vascular reactivity. In the vascular endothelium, G6PD deficiency increases oxidant stress and decreases bioavailable nitric oxide (52). In vitro studies in human coronary artery endothelial cells and in vivo studies in G6PDX mice have shown that this occurs as a result of uncoupling of endothelial nitric oxide synthase (eNOS) to increase ROS formation and is associated with a decrease in GSH levels and the GSH/GSSG ratio (52, 53). G6PD deficiency is also associated with a decrease in nitric oxide levels owing to increased consumption by ROS as well as a decrease in eNOS activity owing to limited NADPH stores (52). In contrast, overexpression of G6PD limits endothelial oxidant stress and increases nitric oxide levels (55). With the use of intravital videomicroscopy, G6PDX mice were shown to have an impaired vasodilator response to the endothelium-dependent vasodilator acetylcholine (53). These findings were supported in a small study of G6PD-deficient individuals that examined endothelial function using brachial artery vascular reactivity. When compared with age-matched controls, G6PD-deficient subjects demonstrated impaired forearm blood flow responses, and this was associated with increased levels of 8-isoprostanes, a marker of systemic oxidant stress (25).

Elevated levels of aldosterone, which is a steroid hormone that is structurally similar to DHEA, decrease endothelial G6PD activity leading to endothelial dysfunction (53). This acquired G6PD-deficient state is associated with eNOS uncoupling and eNOS-derived ROS formation, decreased GSH levels and GSH/GSSG ratio, and an increase in peroxynitrite formation. Aldosterone increased endothelium-dependent vasodilation in WT mice but had no further effect on G6PDX mice and G6PD activity, and vasodilator responses were restored with vascular gene transfer of G6PD (53). The increase in ROS associated with this acquired G6PD-deficient state was also found to inhibit cGMP formation through a mechanism that involved oxidative posttranslational modification of guanylyl cyclase (61). Taken together, these findings indicate that G6PD deficiency has important consequences for nitric oxide-mediated vasodilator responses.

Vascular contraction with potassium chloride or amphotericin B leads to an increase in G6PD activity that occurs in a protein kinase C-dependent manner (30). Under these conditions, inhibition of G6PD decreased ROS and contractility. Aortas from G6PDX mice were also found to have a decrease

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**Fig. 7. Effects of changes in NADPH levels.** Increasing NADPH fuels superoxide production by NADPH oxidase or may contribute to reductive stress. Decreasing NADPH may limit cholesterol synthesis but also decreases antioxidant capacity.
in potassium chloride-evoked force compared with WT mice (30). Further work revealed that G6PD deficiency also modulates calcium stores to promote precontracted vascular smooth muscle relaxation by decreasing calcium influx and increasing sequestration as well as inhibiting Rho kinase (2). Thus, in the contractile state, G6PD deficiency decreases oxidative stress and promotes vascular relaxation.

Clinical Implications and Future Directions

The clinical effects of G6PD deficiency on the heart remain largely unexplored despite it being the most common known enzyme deficiency in the world (12). It is important to gain further insight of G6PD deficiency in the context of human cardiovascular health and disease. Although studies suggested that G6PD deficiency may increase superoxide production in failing myocardium and that G6PD deficiency may decrease the risk of developing coronary heart disease (67), our recent studies in mice indicate increased oxidative stress in G6PD-deficient failing myocardium and that G6PD deficiency adversely affects the development of heart failure (39).

Because G6PD deficiency increases oxidative stress and adversely affects the development of heart failure in mice (39, 45), mechanistic studies should be performed in humans to assess the effects of deficiency on indexes of ROS in heart failure patients. Failing myocardium from tissue banks and screened for G6PD deficiency may be easily identified by common alleles such as the G6PD A− allele or the Med allele. A relatively minor amount of myocardial tissue is required to assess the production of ROS and oxidative stress (32). G6PD activity and NADPH levels can also be assessed in a relatively small amount of myocardial tissue. Thus one could determine the effect of G6PD deficiency on NADPH levels, ROS production, and oxidative stress in failing human myocardium.

The effects of G6PD deficiency on the development and progression of heart failure in human patients could be explored by screening hypertensive patients for G6PD deficiency and then following these patients over an extended period to see whether G6PD deficiency affects the development of heart failure in these patients. Another study could examine G6PD-deficient patients who have already developed heart failure to determine whether G6PD deficiency positively or negatively affects prognosis. Thus the development of heart failure should be examined in G6PD-deficient patients.

Summary and Conclusions

Overall, G6PD deficiency may decrease the rate of cardiovascular disease development among humans through its effect on atherogenesis (15, 63, 67, 70). However, these conclusions come from limited data. Furthermore, it appears that in response to stress, G6PD deficiency sensitizes the myocardium to an allowance for increased levels of oxidative damage and may thus lead to worsened disease outcomes (Fig. 7) (39, 44–46, 54). More population studies in humans are needed to better elucidate the effects of G6PD deficiency on the pathophysiology of cardiovascular disease.

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