Published in final edited form as:
IUBMB Life. 2012 May ; 64(5): 362–369. doi:10.1002/iub.1017.

Glucose-6-Phosphate Dehydrogenase, NADPH, and Cell Survival

Robert C. Stanton
Joslin Diabetes Center, Beth Israel Deaconess Medical Center, and Harvard Medical School

Introduction

It has been assumed that the physiologic regulation and roles of glucose-6-phosphate dehydrogenase (G6PD) are established as it was first described in 1931 [1] and the classic technique for measuring its activity [1] is basically the same as used today. Most studies have since focused on G6PD deficiency (which is associated with hemolysis after eating certain foods or taking certain medications) and lipid metabolism [2, 3]. G6PD deficiency is the most common gene mutation in the world and the numerous mutations have been classified by the World Health Organization [4] according to the activity as: Class I is <1% of wild type activity; Class II is <10%; Class III is 10–60%, Class IV is 60–90% (this is considered normal G6PD activity), and Class V is >110%. It is estimated that at least 400 million people worldwide are G6PD deficient and most are Class III. There are many excellent recent reviews on G6PD deficiency a few are referenced here [3, 4]. Extensive research on G6PD deficiency has led to elucidation of the gene sequence, the protein sequence, and the crystal structure of G6PD [5]. However, it is now clear that G6PD is a critical metabolic enzyme under complex control that resides at a the center of an essential metabolic nexus that affects many physiologic processes. This brief review will provide insights into an evolving field in which alterations in G6PD expression, intracellular location, tissue expression, and posttranslational regulation play critical roles in normal physiology as well as in pathophysiology.

Biochemistry and Molecular Biology

In brief, after glucose is transported into cells, it undergoes phosphorylation by the hexokinase/glucokinase enzymes [6]. Glucose-6-phosphate (G6P) may be utilized in glycolysis to produce energy in the form of ATP and NADH, used to store energy in the form of glycogen, or used by the pentose phosphate pathway (PPP). The PPP (also called the hexose monophosphate shunt) (the figure is divided into an initial oxidative stage of which G6PD is the first and rate-limiting enzyme and a non-oxidative stage in which transketolase and transaldolase are the key enzymes [6, 7]. The major products of the PPP are ribose-5-phosphate, that is required for nucleic acid synthesis, and NADPH generated from NADP by G6PD and the next enzyme in the pathway, 6-phosphogluconate dehydrogenase (PGD). The glucose-6-phosphate utilized in the pathway may be cycled back into glycolysis as the ultimate sugars produced by the PPP are glycolytic intermediates, glyceraldehye-3-phosphate and fructose-6-phosphate.

G6PD is an X-linked gene that maps to the Xq28 region and whose sequence has been highly conserved throughout evolution [5, 8]. An octapeptide sequence that contains a lysine residue that is required for enzyme activity, RIDHYLGK, and a heptapeptide sequence for the dinucleotide binding site, GxxGDLx, have been highly conserved [5, 6]. The gene

Address: Renal Section Joslin Diabetes Center One Joslin Place Boston, MA 02215 Telephone: 617-309-2477 Fax: 617-309-2467 robert.stanton@joslin.harvard.edu.

consists of 13 exons and 12 introns, the expressed protein in mammalian cells being active as a dimer or tetramer but not as a monomer [5, 6]. The protein consists of 514 amino acids and has binding sites for NADP and G6P as well as an allosteric modifier binding site for NADP that acts to stabilize the dimer and thus keep the protein in an active conformation [5]. Reviews of G6PD molecular biology are available [3, 5, 6, 8] of which one [5] contains the crystal structure of human G6PD and elegantly discusses the structural functional relationships [5].

Regulation

It has been traditionally taught that G6PD is regulated by the NADPH/NADP ratio so that as the ratio decreases, activity increases to provide more NADPH. Indeed, G6PD is activated following exposure of cells to various extracellular oxidants [6] that lead to decrease in level of NADPH. Regulation by the NADPH/NADP ratio has been clearly demonstrated *in vitro* [9], but not *in vivo*. However other factors (i.e., non-oxidant stimuli) also regulate G6PD. Marine biologists studying fertilization in sea urchin eggs observed that upon fertilization a rapid production of ROS occursat the cortex of the egg that forms the hard fertilization coat comprised mainly of dityrosine residues [10, 11], A highly orchestrated, very rapid series of protein-protein interactions and protein translocations, occurring within seconds of attachment of the sperm to the unfertilized egg, were shown to lead to to rapid formation of this hard fertilization coat [10, 11]. Upon fertilization, some of the G6PD and likely most of the NADPH oxidase were rapidly activated and translocated to the outer surface of the egg [10, 11]. Activated NADPH oxidase provides the oxidant necessary to form the hard fertilization coat. Two very important insights are illustrated by these findings: 1) activation of G6PD may simultaneously serve multiple roles within a cell, and 2) regulation of G6PD involves both activation and intracellular translocation of multiple targets. Thus in fertilization G6PD provides NADPH at the egg outer membrane to the NADPH oxidase that produces the ROS required for formation of the hard fertilization coat and within the egg provides NADPH for the antioxidant enzyme systems to maintain redox balance. Hence increased G6PD activity has an antioxidant effect and a prooxidant effect within the egg.

Many studies over the past 25 years have shown that G6PD is highly regulated at the level of transcription, translation, post-translation, and intracellular location so that G6PD is the downstream target of many signaling pathways (Table 1). Of major significance is the postrranslational regulation of G6PD by such factors as phosphorylation and direct protein-protein binding as discussed below with reference to ataxia telangiectasia mutated (ATM) protein and p53 [12, 13]. The first demonstration that G6PD undergoes posttranslational modification (phosphorylation) and that growth factors such as platelet-derived growth factor and epidermal growth factor stimulate G6PD activation and intracellular translocation in mammalian cells (fibroblasts and kidney cortical cells) came from our laboratory [14]. similarly for the finding that phosphatidylinositol-3-kinase (PI-3K), phospholipase C-γ, and Ras-GTPases regulate the growth factor-induced stimulation of translocation of G6PD [15] came from our laboratory. In addition our work showed that G6PD was localized around the nucleus in quiescent cells (fibroblasts) and was translocated to the plasma membrane on stimulation by growth factors. In endothelial cells, also vascular endothelial cell growth factor stimulated activation and translocation of G6PD to the plasma membrane that were regulated by the nonreceptor tyrosine kinase Src [16]. It is now clear that G6PD is regulated by an expanding list of positive and negative regulators. Positive regulators include vitamin D, insulin, PI3-K, AKT, mTOR (mammalian target of rapamacycin), and S6 kinase [17–19]. The gene is also positively regulated by the major lipid transcription factor sterol responsive element binding protein (SREBP) under conditions of increased need for NADPH for biosynthesis of lipids [2], and by transcription factor Nrf2 that regulates several antioxidant response element genes [20]. The tumor suppressor p53 called TIGAR (TP53-induced

glycolysis and apoptosis regulator) has been reported to be a downstream signal [21]. TIGAR, activated by p53, led to a decrease in ROS that was mediated by activation of G6PD and PPP by TIGAR. Although TIGAR upregulates G6PD, p53 can also inhibit G6PD activity [13]. Thus the ROS level and cellular phenotype that follow activation of p53 are dependent, in part, on the balance of G6PD stimulatory versus G6PD inhibitory signals downstream of p53. A very intriguing finding connects ataxia telangiectasia (AT), in which patients suffer from neurological defects, cancer, and increased sensitivity to DNA double strand breaks from radiation [12]. AT patients have defects in the ATM protein and mouse models of AT and cells from patients with AT have abnormally high levels of ROS and low levels of antioxidants that are dependent on NADPH. Using multiple techniques the authors showed that ATM activated G6PD via the association of Hsp27 with ATM. SINCE mammalian G6PD is inactive in a monomer form, they speculated that Hsp27 maintains the dimer/tetramer forms of G6PD. Hence the phenotype of AT may be due, in part, to increased ROS from decreased G6PD activity.

There are also major negative regulators of G6PD. Thus, activation of G6PD in macrophages has been shown to be inhibited by an increase in cyclic AMP (cAMP) and cAMP-dependent protein kinase A (PKA) [22]. Our laboratory has confirmed these findings. In addition we determined that PKA leads to an increase in serine/threonine phosphorylation of G6PD that likely plays a role in the downregulation of G6PD activity [23, 24]. Although we have determined that PKA can directly decrease G6PD activity *in vitro*, it is not yet known if the *in vivo* effects of G6PD are direct or indirect. In collaborative work, we have also shown that the cAMP response element modulator (CREM) decreased transcription of the G6PD gene [25]. Thus cAMP may decrease G6PD activity both by decreasing G6PD gene transcription and by posttranslational modification of existing protein. Arachidonic acid counteracts the stimulation of G6PD by insulin that is mediated by AMP kinase and p38 MAP kinase [26]. Tumor necrosis factor alpha also decreases G6PD activity [20] as does, as already mentioned, the critical transcriptional regulator, p53. In a colon cancer cell line p53 was shown to bind directly to G6PD in the cytoplasm) and decrease G6PD activity possibly through disruption of the dimer/tetramer formation [13]. This mechanism of inhibition is far from certain since only 10% of the G6PD appeared to be bound to p53.

It is clear from the above that there are multiple interacting signals that regulate G6PD activity, location, and protein-protein interactions from the level of the gene to the expressed protein that ultimately leads to the final cellular phenotype.

G6PD is Essential for Cell Survival

G6PD activity has long been known to be increased in cells undergoing normal or neoplastic cell growth [6, 27–30]. In experiments to determine if increased G6PD activity *per se* was an essential component of normal cell growth [30], we used several cell types and showed that G6PD activity correlated directly with cell growth, that inhibition of G6PD activity prevented cell growth, and that overexpression of G6PD alone stimulated cell growth. We also showed that increased G6PD activity was essential for preventing ROS mediated cell death and serum starvation[31]. It had already been shown that G6PD activity in embryonic stem cells with a knockout of G6PD were exquisitively sensitive to the deleterious effects of external oxidants [32]. Indeed a mammalian knockout of G6PD has been shown to be embryonically lethal [33]. The importance of G6PD to cell growth and to cell death has also been shown in other work, e.g., on NIH3T3 fibroblasts, in which overexpression of G6PD stimulated cell growth [34], and on G6PD-deficient fibroblasts, in which increased ROS accelerated cellular senescence [35].

It has been stated in many review articles about G6PD and cell growth that the reason for increased G6PD activity under normal or neoplastic cell growth is to provide ribose-5-phosphate for nucleic acid synthesis. We have evaluated whether ribose-5-phosphate is the ultimate critical product of G6PD activation using a fibroblast cell lines in which G6PD activity had been inhibited and found that addition of RNA (a well-utilized physiological source of ribose-5-phosphate), did not restore cell growth [30]. Thus it is possible that lack of NADPH was the essential missing factor required for cell growth. Although, it is likely that ribose-5-phosphate and NADPH are both important products of G6PD required for cell growth.

Roles of NADPH

NADPH is mainly produced by four enzymes in mammalian cells, G6PD, PGD, malic enzyme, and isocitrate dehydrogenase. All have been studied extensively and play critical cellular roles. G6PD appears to be of unique importance to many cellular processes that utilize NADPH, as inhibition of G6PD impairs many cellular processes that are dependent on NADPH. Hence the other enzymes do not provide a sufficient amount of NAPDH to maintain many of these proeesses at normal levels. There are many enzymes that use NADPH for reducing power in the cell but due to the limited scope of this review, I will focus on a few major pathways. (Table 2 – Enzymes dependent on NADPH)

The antioxidant system depends on production of NADPH for proper function. Its three major components in cells are the glutathione system, catalase, and superoxide dismutase [36]. The first is dependent on production of reduced glutathione by glutathione reductase that depends on NADPH. Catalase does not need NADPH to convert hydrogen peroxide to water but has an allosteric binding site for NADPH that maintains catalase in its active conformation. Superoxide dismutase does not use NADPH to convert superoxide to hydrogen peroxide but if this is not adequately reduced chemically by catalase or glutathione the increased hydrogen peroxide levels will quantitatively increase and inhibit the superoxide dismutase. Hence the entire antioxidant system depends on NADPH. As previously described, the need for NADPH from G6PD in embryonic stem cells with a complete knockout of G6PD made them highly sensitive to diamide (a potent oxidant) and led to more cell death than in cells with wild type G6PD activity [32]. This indicates that other cellular enzymes did not supply enough NADPH to protect the cells from cell death.

Another set of enzymes that are dependent on NADPH are the nitric oxide synthases [37, 38]. NADPH provides reducing power such that nitric oxide synthase (NOS) is able to convert arginine to citrulline and nitric oxide (NO). NOS is dependent on NADPH, tetrahydrobioptein, and on FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide). In collaborative work, we have determined that endothelial NOS (eNOS) activity was dependent on G6PD-derived NADPH [37, 38]. Basal production of nitric oxide by eNOS and stimulation of eNOS to increased nitric oxide production was significantly lower in endothelial cells when G6PD activity was inhibited using an antisense oligonucleotide to G6PD illustrating, as seen in the antioxidant system, that eNOS is dependent on NADPH produced by G6PD. Interestingly, dihydrofolate reductase (the main source of tetrahydrobiopterin) is a NADPH-dependent enzyme. Hence a decrease in G6PD with the resultant decrease in NADPH likely led to decreased NO production due to lower levels of both NADPH and tetrahydrobiopterin.

Another system that is dependent on NADPH from G6PD activity is the family of enzymes called NADPH oxidases. These enzymes have many essential physiological roles (e.g normal cell growth, white blood cell function) and many pathophysiologic ones have been determined (e.g, in diabetes, cardiovascular disease) as a major source of ROS [39] .

Already mentioned above, rapid activation of NADPH oxidase in sea urchin eggs was associated with rapid activation of G6PD and colocalization of G6PD with NADPH oxidase [10, 11]. A similar process occurs upon activation of granulocytes and macrophages by bacteria or by such chemical stimuli such as f-met-leu-phe [40] that is known as the `respiratory burst'. Thus both altered enzyme activity as well as altered cellular translocation are essential aspects of G6PD regulation.

There are many other systems dependent on NAPDH including enzymes involved in lipid production such as 3-hydroxy-3-methylglutaryl-CoA reductase and the NADPH-cytochrome P450 oxidoreductase that is required for drug metabolism, steroid biosynthesis, and many other functions [41].

Pathophysiologic Roles for Altered G6PD Activity

Numerous observations have demonstrated highly significant decreases in G6PD activity due to hyperglycemia or diabetes in liver, kidney, brain, endothelial cells, red blood cells, and other cells and tissues (see this paper for references [36]). Bovine aortic endothelial cells exposed to glucose in the medium (normal, 5.5 mM; increased, 10 mM to 25 mM) showed G6PD activity to be decreased within minutes and that was mediated, in significant part, by an increase in cAMP and cAMP-dependent protein kinase A [24]. Streptozotocin-induced diabetes in rats was associated with increased urine albumin excretion (a marker of kidney damage) and decreased G6PD activity in kidney cortical cells that correlated with decreased NADPH and reduced glutathione and may have contributed to kidney cell damage [23]. Moreover there was increased PKA activity and increased phosphorylation of G6PD on serine residues that was inversely correlated with G6PD activity. There were also decreased expression of G6PD over the 20 week period of the study. This work was confirmed and expanded upone when another research group showed that hepatocyte growth factor (HGF) rescued kidney mesangial cells from oxidative stress by inhibiting PKA activity and activating G6PD [42]. In that study cGMP dependent protein kinase G was also activated by HGF and was a positive regulator of G6PD. Kidneys from diabetic mice had decreased G6PD activity and decreases in the mitochondrial enzymes, succinate dehydrogenase, malate dehydrogenase, and glutamate dehydrogenase [43].

To determine if decreased G6PD activity *per se* could lead to cellular damage our laboratory studied mice that were deficient in G6PD activity. These mice were originally produced by Walter Pretsch by chemical mutagenesis [44] and have reduced G6PD activity as follows (hemizgotes – 15–20% of wild type; homozygotes- 15–20% of wild type; and heterozygotes – 40–60% of wild type). We determined that the decrease in G6PD activity was inversely correlated with increasing urine albumin level suggesting that G6PD activity *per se* could lead to kidney cell damage [45]. Also kidney cells from these mice also showed elevated markers of inflammation and of proapoptotic proteins.

Decreased G6PD activity has also been observed in other cells and tissues from diabetic animals. For example neutrophils exposed to increased glucose had impaired neutrophil function associated with decreased G6PD activity [46]. This result suggested that the high glucose-mediated decrease in G6PD led to decreased NADPH that was needed for proper NADPH oxidase activity in neutrophils. Moreover studies on liver and pancreatic tissue from diabetic animals had lower activity of G6PD [47]. And in a mouse model of diabetes, the animals had decreased G6PD activity in cardiac muscle and benfotiamine improved recovery from myocardial infarction by increasing G6PD activity [48]. Increasing G6PD activity is thus potentially an important therapeutic approach to prevention and treatment of diabetic complications.

It should be noted that all animal models of diabetes have significant limitations (see this reference for a recent review [49]). Those for type 1 diabetes involve administration of streptozotocin or alloxan to poison pancreatic beta cells. Since these chemicals can affect other cell types results from such animals may reflect chemical damage more than a disease process. Genetic models include NOD mice for type 1 diabetes and db/db and ob/ob mice for type 2 diabetes [49]. Human diabetes is a complex, polygenic disease in that many genes have been linked to the development of diabetes and to date, no animal clearly reflects human disease. Efforts to develop better models for human diabetes and its complications include the establishment of the Animal Models of Diabetes Complications Consortium under the auspices of the National Institute of Diabetes, Digestive, and Kidney Disease. Nevertheless animal models remain a necessary starting point from which human studies can be designed.

Recently our laboratory studied the role of G6PD in pancreatic beta cell survival and function. Intriguingly, pancreatic islets have been shown to have low levels of antioxidants such as glutathione peroxidase, catalase, and superoxide dismutase [36], thus we hypothesized that a decrease in G6PD activity may be especially detrimental to pancreatic islets since these cells already have low antioxidant function that depends on the NAPDH from G6PD. In these studies, we determined that wild type mouse islets normally have low G6PD activity as compared to non-islet pancreatic cells [36]. Islets from the G6PD deficient mice were much smaller than those of wild type mice implying that decreasing G6PD activity leads to impaired pancreatic islet cell growth [36]. We next hypothesized that a rise in glucose level would lead to a decrease in G6PD activity in the islet cells with decrease of NADPH and subsequent death of the cells [36]. In a beta cell line in culture and in isolated mouse and human islets, increased glucose in the medium decreased G6PD activity and also decreased beta cell survival. Inhibition of G6PD with antisense oligonucleotides to G6PD also produced beta cell death while overexpression OF G6PD rescued the cells from the increase in ROS caused by increased medium glucose [36]. Increased glucose in the medium also led to impaired glucose-stimulated insulin secretion that was rescued by overexpression of G6PD. Thus these results suggested that loss of beta cells over time (a hallmark of both type 1 and type 2 diabetes) may be due, at least in part, to decreased G6PD activity. Similar effects of increased glucose level on pancreatic G6PD activity have also been reported [47].

Some studies have shown that increased glucose in the medium may increase G6PD activity under certain conditions. For example, researchers have reported that elevations in blood glucose has caused elevation in G6PD activity in cardiac muscle and aorta [50, 51]. The researchers reported higher G6PD activity that their studies showed was used to provide NADPH for NADPH oxidase and that led to higher levels of ROS with cell damage or cell death. It is not clear whether these results reflect cell heterogeneity of response or differences in animal models compared to the studies discussed previously. In addition in one study, increased glucose in the medium increased G6PD activity in pancreatic islet and beta cells [52] and the authors suggested that the increase in G6PD was detrimental to these cells These results are different from those reported by our laboratory and other laboratories [36, 47]. Ultimately evaluation of human pancreatic tissue from normal and from diabetic individuals are required before definitive conclusions can be drawn.

Another interesting pathophysiologic role was discovered when it was hypothesized that aldosterone causes endothelial dysfunction via inhibition of G6PD. Studies done on cultured endothelial cells and on animals using inhibitors of G6PD, overexpression of G6PD, and inhibitors of aldosterone then showed that aldosterone decreased G6PD protein expression and nitric oxide (NO) production. Moreover preventing a decrease in G6PD activity led to restoration of aldosterone induced NO production [25].

It has been known for many years that increased G6PD is a hallmark of many cancers [27–29]. Indeed, overexpression of G6PD can initiate neoplastic transformation as illustrated by the experiment in which nude mice were injected with fibroblasts overexpressing G6PD and the mice developed tumors [34]. Another example comes from a mouse model of cardiomyopathy [53] in which increasing the G6PD activity worsened the myopathy induced by alpha b-crystallin while decreasing G6PD activity protected against the development of the myopathy. This deleterious effect of increased activity of G6PD was called reductive stress.

Decreased G6PD Activity and Human Disease

The association of human G6PD deficiency has been mainly focused on the associated hemolysis or on the relationship to protection from malaria. But more studies are correlating G6PD activity and other diseases. For example, small studies from the Middle East are suggestive that decreased G6PD activity may predispose to development of diabetes on the basis of highly significant correlations of increased frequency of diabetes mellitus in G6PD deficient subjects compared to subjects from the same population who have wild type G6PD activity [54, 55]. The authors have proposed that patients with diabetes should be screened for G6PD deficiency (or vice versa). In another study, researchers found a inverse relationship of G6PD activity and the presence of proliferative diabetic retinopathy in humans in that as G6PD activity declined the increase in diabetic retinopathy was more prevalent [56].

I am often asked, “how about all of those G6PD deficient people in the world?” [3]. Are all of those people susceptible to the development of various diseases due to their decreased G6PD activity? These questions have not yet been answered. Interestingly, an association of G6PD deficiency with predisposition to disease development has been proposed in the past in that Gaskin et al proposed a relationship between G6PD deficiency, diabetes, and hypertension [57]. Although there have been some small studies over the years, to date there are no large-scale studies addressing the role of G6PD deficiency in the development of many diseases. These studies have potentially major health implications. As already noted, over 400 million people have G6PD deficiency (Class I, II, or III mutations) when deficiency is defined, by convention, as <60% of wild type activity. Yet this number may significantly underestimate the number of people in the world with a significant enough decrease in G6PD activity to make them susceptible to the development of many diseases. In many of the studies reported in this review, only a relatively modest decrease in G6PD activity (such that activity was 60–90% of wild type) was enough to cause significant impairment in cell function and cell survival. These results suggest that a relatively small decrease in G6PD activity may have significant deleterious effects on cellular physiology and cellular survival. Thus large-scale studies on disease susceptibility need to also include those people with Class IV mutations (as defined as having 60–90% of normal G6PD activity) that would greatly increase the population that might be at risk. It is not likely that mild G6PD deficiency alone is sufficient to cause disease but rather that G6PD deficiency is a very important predisposing factor.

CONCLUSION

In conclusion, G6PD is at the nexus of many essential metabolic pathways. We are only at the beginning of understanding G6PD, NADPH and their interrelationships with cellular systems. A full understanding of G6PD, its role as a metabolic nexus for many cellular systems, and how it is regulated should provide critical insights into many intracellular processes and disease mechanisms.

References

1. Kornberg A, Horecker BL. Glucose-6-Phosphate Dehydrogenase. Method Enzymol. 1956; 1:323.
2. Horton JD. Sterol regulatory element-binding proteins: transcriptional activators of lipid synthesis. Biochem Soc Trans. 2002; 30:1091–1095. [PubMed: 12440980]
3. Nkhoma ET, Poole C, Vannappagari V, Hall SA, Beutler E. The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. Blood Cells Mol Dis. 2009; 42:267–278. [PubMed: 19233695]
4. Minucci A, Giardina B, Zuppi C, Capoluongo E. Glucose-6-phosphate laboratory assay: How, when, and why? IUBMB Life. 2009; 61:27–34. [PubMed: 18942156]
5. Au SW, Gover S, Lam VM, Adams MJ. Human glucose-6-phosphate dehydrogenase: the crystal structure reveals a structural NADP(+) molecule and provides insights into enzyme deficiency. Structure. 2000; 8:293–303. [PubMed: 10745013]
6. Kletzien RF, Harris PK, Foellmi LA. Glucose-6-phosphate dehydrogenase: a “housekeeping” enzyme subject to tissue-specific regulation by hormones, nutrients, and oxidant stress. Faseb J. 1994; 8:174–181. [PubMed: 8119488]
7. Wood T. Physiological functions of the pentose phosphate pathway. Cell Biochem Funct. 1986; 4:241–247. [PubMed: 3539386]
8. Ho HY, Cheng ML, Chiu DT. G6PD--an old bottle with new wine. Chang Gung Med J. 2005; 28:606–612. [PubMed: 16323551]
9. Holten D, Procsal D, Chang HL. Regulation of pentose phosphate pathway dehydrogenases by NADP+/NADPH ratios. Biochem Biophys Res Commun. 1976; 68:436–441. [PubMed: 3177]
10. Shapiro BM. The control of oxidant stress at fertilization. Science. 1991; 252:533–536. [PubMed: 1850548]
11. Swezey RR, Epel D. Enzyme stimulation upon fertilization is revealed in electrically permeabilized sea urchin eggs. Proc Natl Acad Sci U S A. 1988; 85:812–816. [PubMed: 3422463]
12. Cosentino C, Grieco D, Costanzo V. ATM activates the pentose phosphate pathway promoting anti-oxidant defence and DNA repair. Embo J. 2011; 30:546–555. [PubMed: 21157431]
13. Jiang P, Du W, Wang X, Mancuso A, Gao X, Wu M, Yang X. p53 regulates biosynthesis through direct inactivation of glucose-6-phosphate dehydrogenase. Nat Cell Biol. 2011; 13:310–316. [PubMed: 21336310]
14. Stanton RC, Seifter JL, Boxer DC, Zimmerman E, Cantley LC. Rapid release of bound glucose-6-phosphate dehydrogenase by growth factors. Correlation with increased enzymatic activity. J Biol Chem. 1991; 266:12442–12448. [PubMed: 2061319]
15. Tian WN, Pignatare JN, Stanton RC. Signal transduction proteins that associate with the platelet-derived growth factor (PDGF) receptor mediate the PDGF-induced release of glucose-6-phosphate dehydrogenase from permeabilized cells. J Biol Chem. 1994; 269:14798–14805. [PubMed: 8182086]
16. Pan S, World CJ, Kovacs CJ, Berk BC. Glucose 6-phosphate dehydrogenase is regulated through c-Src-mediated tyrosine phosphorylation in endothelial cells. Arterioscler Thromb Vasc Biol. 2009; 29:895–901. [PubMed: 19359662]
17. Bao BY, Ting HJ, Hsu JW, Lee YF. Protective role of 1 alpha, 25-dihydroxyvitamin D3 against oxidative stress in nonmalignant human prostate epithelial cells. Int J Cancer. 2008; 122:2699–2706. [PubMed: 18348143]
18. Duvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, Triantafellow E, Ma Q, Gorski R, Cleaver S, Vander Heiden MG, MacKeigan JP, Finan PM, Clish CB, Murphy LO, Manning BD. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. Mol Cell. 2010; 39:171–183. [PubMed: 20670887]
19. Wagle A, Jivraj S, Garlock GL, Stapleton SR. Insulin regulation of glucose-6-phosphate dehydrogenase gene expression is rapamycin-sensitive and requires phosphatidylinositol 3-kinase. J Biol Chem. 1998; 273:14968–14974. [PubMed: 9614103]
20. Zhang HS, Wang SQ. Nrf2 is involved in the effect of tanshinone IIA on intracellular redox status in human aortic smooth muscle cells. Biochem Pharmacol. 2007; 73:1358–1366. [PubMed: 17303087]

21. Bensaad K, Tsuruta A, Selak MA, Vidal MN, Nakano K, Bartrons R, Gottlieb E, Vousden KH. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell. 2006; 126:107–120. [PubMed: 16839880]
22. Costa Rosa LF, Curi R, Murphy C, Newsholme P. Effect of adrenaline and phorbol myristate acetate or bacterial lipopolysaccharide on stimulation of pathways of macrophage glucose, glutamine and O2 metabolism. Evidence for cyclic AMP-dependent protein kinase mediated inhibition of glucose-6-phosphate dehydrogenase and activation of NADP+-dependent `malic' enzyme. Biochem J. 1995; 310(Pt 2):709–714. [PubMed: 7654215]
23. Xu Y, Osborne BW, Stanton RC. Diabetes causes inhibition of glucose-6-phosphate dehydrogenase via activation of PKA, which contributes to oxidative stress in rat kidney cortex. Am J Physiol Renal Physiol. 2005; 289:F1040–1047. [PubMed: 15956780]
24. Zhang Z, Apse K, Pang J, Stanton RC. High glucose inhibits glucose-6-phosphate dehydrogenase via cAMP in aortic endothelial cells. J Biol Chem. 2000; 275:40042–40047. [PubMed: 11007790]
25. Leopold JA, Dam A, Maron BA, Scribner AW, Liao R, Handy DE, Stanton RC, Pitt B, Loscalzo J. Aldosterone impairs vascular reactivity by decreasing glucose-6-phosphate dehydrogenase activity. Nat Med. 2007; 13:189–197. [PubMed: 17273168]
26. Talukdar I, Szeszel-Fedorowicz W, Salati LM. Arachidonic acid inhibits the insulin induction of glucose-6-phosphate dehydrogenase via p38 MAP kinase. J Biol Chem. 2005; 280:40660–40667. [PubMed: 16210322]
27. Boros LG, Brandes JL, Yusuf FI, Cascante M, Williams RD, Schirmer WJ. Inhibition of the oxidative and nonoxidative pentose phosphate pathways by somatostatin: a possible mechanism of antitumor action. Med Hypotheses. 1998; 50:501–506. [PubMed: 9710324]
28. Frederiks WM, Bosch KS, Hoeben KA, van Marle J, Langbein S. Renal cell carcinoma and oxidative stress: The lack of peroxisomes. Acta Histochem. 2010; 112:364–371. [PubMed: 19500819]
29. Frederiks WM, Vizan P, Bosch KS, Vreeling-Sindelarova H, Boren J, Cascante M. Elevated activity of the oxidative and non-oxidative pentose phosphate pathway in (pre)neoplastic lesions in rat liver. Int J Exp Pathol. 2008; 89:232–240. [PubMed: 18422600]
30. Tian WN, Braunstein LD, Pang J, Stuhlmeier KM, Xi QC, Tian X, Stanton RC. Importance of glucose-6-phosphate dehydrogenase activity for cell growth. J Biol Chem. 1998; 273:10609–10617. [PubMed: 9553122]
31. Tian WN, Braunstein LD, Apse K, Pang J, Rose M, Tian X, Stanton RC. Importance of glucose-6-phosphate dehydrogenase activity in cell death. Am J Physiol. 1999; 276:C1121–1131. [PubMed: 10329961]
32. Pandolfi PP, Sonati F, Rivi R, Mason P, Grosveld F, Luzzatto L. Targeted disruption of the housekeeping gene encoding glucose 6-phosphate dehydrogenase (G6PD): G6PD is dispensable for pentose synthesis but essential for defense against oxidative stress. Embo J. 1995; 14:5209–5215. [PubMed: 7489710]
33. Longo L, Vanegas OC, Patel M, Rosti V, Li H, Waka J, Merghoub T, Pandolfi PP, Notaro R, Manova K, Luzzatto L. Maternally transmitted severe glucose 6-phosphate dehydrogenase deficiency is an embryonic lethal. Embo J. 2002; 21:4229–4239. [PubMed: 12169625]
34. Kuo W, Lin J, Tang TK. Human glucose-6-phosphate dehydrogenase (G6PD) gene transforms NIH 3T3 cells and induces tumors in nude mice. Int J Cancer. 2000; 85:857–864. [PubMed: 10709108]
35. Ho HY, Cheng ML, Lu FJ, Chou YH, Stern A, Liang CM, Chiu DT. Enhanced oxidative stress and accelerated cellular senescence in glucose-6-phosphate dehydrogenase (G6PD)-deficient human fibroblasts. Free Radic Biol Med. 2000; 29:156–169. [PubMed: 10980404]
36. Zhang Z, Liew CW, Handy DE, Zhang Y, Leopold JA, Hu J, Guo L, Kulkarni RN, Loscalzo J, Stanton RC. High glucose inhibits glucose-6-phosphate dehydrogenase, leading to increased oxidative stress and beta-cell apoptosis. Faseb J. 2010; 24:1497–1505. [PubMed: 20032314]
37. Leopold JA, Walker J, Scribner AW, Voetsch B, Zhang YY, Loscalzo AJ, Stanton RC, Loscalzo J. Glucose-6-phosphate dehydrogenase modulates vascular endothelial growth factor-mediated angiogenesis. J Biol Chem. 2003; 278:32100–32106. [PubMed: 12777375]

38. Leopold JA, Zhang YY, Scribner AW, Stanton RC, Loscalzo J. Glucose-6-phosphate dehydrogenase overexpression decreases endothelial cell oxidant stress and increases bioavailable nitric oxide. Arterioscler Thromb Vasc Biol. 2003; 23:411–417. [PubMed: 12615686]
39. Guzik TJ, Griendling KK. NADPH oxidases: molecular understanding finally reaching the clinical level? Antioxid Redox Signal. 2009; 11:2365–2370. [PubMed: 19358633]
40. Forman HJ, Torres M. Reactive oxygen species and cell signaling: respiratory burst in macrophage signaling. Am J Respir Crit Care Med. 2002; 166:S4–8. [PubMed: 12471082]
41. Iyanagi T. Molecular mechanism of phase I and phase II drug-metabolizing enzymes: implications for detoxification. Int Rev Cytol. 2007; 260:35–112. [PubMed: 17482904]
42. Hui L, Hong Y, Jingjing Z, Yuan H, Qi C, Nong Z. HGF suppresses high glucose-mediated oxidative stress in mesangial cells by activation of PKG and inhibition of PKA. Free Radic Biol Med. 2010; 49:467–473. [PubMed: 20466058]
43. Ramudu SK, Korivi M, Kesireddy N, Lee LC, Cheng IS, Kuo CH, Kesireddy SR. Nephro-protective effects of a ginger extract on cytosolic and mitochondrial enzymes against streptozotocin (STZ)-induced diabetic complications in rats. Chin J Physiol. 2011; 54:79–86. [PubMed: 21789888]
44. Sanders S, Smith DP, Thomas GA, Williams ED. A glucose-6-phosphate dehydrogenase (G6PD) splice site consensus sequence mutation associated with G6PD enzyme deficiency. Mutat Res. 1997; 374:79–87. [PubMed: 9067418]
45. Xu Y, Zhang Z, Hu J, Stillman IE, Leopold JA, Handy DE, Loscalzo J, Stanton RC. Glucose-6-phosphate dehydrogenase-deficient mice have increased renal oxidative stress and increased albuminuria. Faseb J. 2010; 24:609–616. [PubMed: 19805580]
46. Perner A, Nielsen SE, Rask-Madsen J. High glucose impairs superoxide production from isolated blood neutrophils. Intensive Care Med. 2003; 29:642–645. [PubMed: 12552364]
47. Dıaz-Flores M, Ibanez-Hernandez MA, Galvan RE, Gutierrez M, Duran-Reyes G, Medina-Navarro R, Pascoe-Lira D, Ortega-Camarillo C, Vilar-Rojas C, Cruz M, Baiza-Gutman LA. Glucose-6-phosphate dehydrogenase activity and NADPH/NADP+ ratio in liver and pancreas are dependent on the severity of hyperglycemia in rat. Life Sci. 2006; 78:2601–2607. [PubMed: 16325866]
48. Katare R, Caporali A, Emanueli C, Madeddu P. Benfotiamine improves functional recovery of the infarcted heart via activation of pro-survival G6PD/Akt signaling pathway and modulation of neurohormonal response. J Mol Cell Cardiol. 2010; 49:625–638. [PubMed: 20542491]
49. Rees DA, Alcolado JC. Animal models of diabetes mellitus. Diabet Med. 2005; 22:359–370. [PubMed: 15787657]
50. Serpillon S, Floyd BC, Gupte RS, George S, Kozicky M, Neito V, Recchia F, Stanley W, Wolin MS, Gupte SA. Superoxide production by NAD(P)H oxidase and mitochondria is increased in genetically obese and hyperglycemic rat heart and aorta before the development of cardiac dysfunction. The role of glucose-6-phosphate dehydrogenase-derived NADPH. Am J Physiol Heart Circ Physiol. 2009; 297:H153–162. [PubMed: 19429815]
51. Gupte RS, Floyd BC, Kozicky M, George S, Ungvari ZI, Neito V, Wolin MS, Gupte SA. Synergistic activation of glucose-6-phosphate dehydrogenase and NAD(P)H oxidase by Src kinase elevates superoxide in type 2 diabetic, Zucker fa/fa, rat liver. Free Radic Biol Med. 2009; 47:219–228. [PubMed: 19230846]
52. Lee JW, Choi AH, Ham M, Kim JW, Choe SS, Park J, Lee GY, Yoon KH, Kim JB. G6PD up-regulation promotes pancreatic beta-cell dysfunction. Endocrinology. 2011; 152:793–803. [PubMed: 21248143]
53. Rajasekaran NS, Connell P, Christians ES, Yan LJ, Taylor RP, Orosz A, Zhang XQ, Stevenson TJ, Peshock RM, Leopold JA, Barry WH, Loscalzo J, Odelberg SJ, Benjamin IJ. Human alpha B-crystallin mutation causes oxido-reductive stress and protein aggregation cardiomyopathy in mice. Cell. 2007; 130:427–439. [PubMed: 17693254]
54. Niazi GA. Glucose-6-phosphate dehydrogenase deficiency and diabetes mellitus. Int J Hematol. 1991; 54:295–298. [PubMed: 1777604]
55. Saeed TK, Hamamy HA, Alwan AA. Association of glucose-6-phosphate dehydrogenase deficiency with diabetes mellitus. Diabet Med. 1985; 2:110–112. [PubMed: 2952393]

56. Cappai G, Songini M, Doria A, Cavallerano JD, Lorenzi M. Increased prevalence of proliferative retinopathy in patients with type 1 diabetes who are deficient in glucose-6-phosphate dehydrogenase. Diabetologia. 2011; 54:1539–1542. [PubMed: 21380594]

57. Gaskin RS, Estwick D, Peddi R. G6PD deficiency:its role in the high prevalence of hypertension and diabetes mellitus. Ethnicity and Disease. 2001; 11:749–754. [PubMed: 11763298]

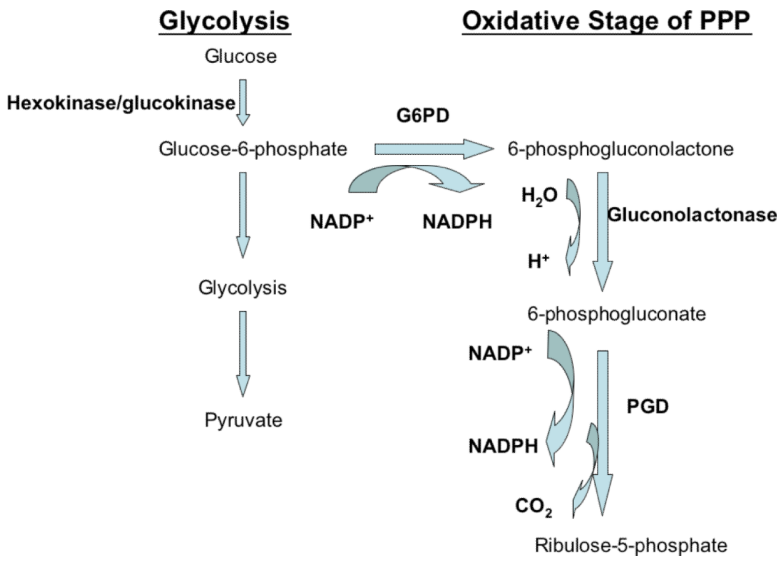

Figure 1A.
Oxidative Branch of the Pentose Phosphate Pathway (PPP). Abbreviations: G6PD – glucose-6-phosphate dehydrogenase; PGD - 6-phosphogluconate dehydrogenase.

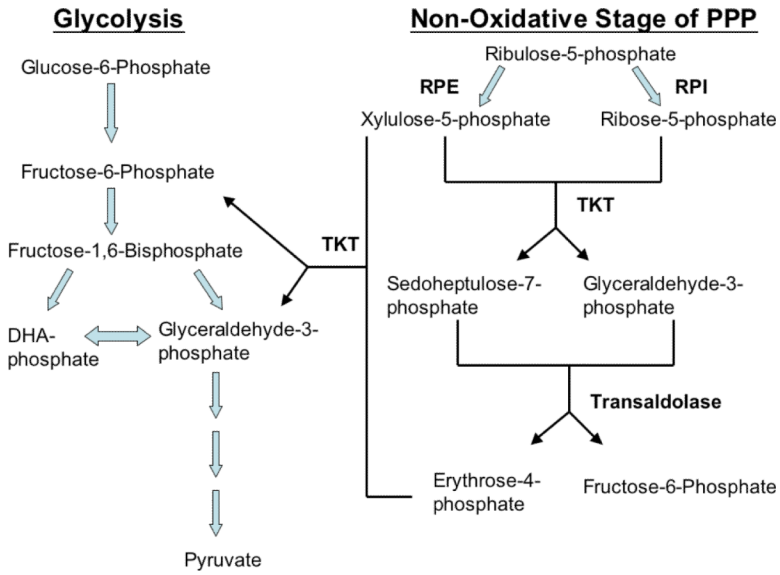

Figure 1B.

Non-oxidative Branch of the Pentose Phosphate Pathway (PPP). Abbreviations: RPE – ribulose-5-phosphate 3-epimerase; RPI - ribulose-5-phosphate isomerase; TKT-transketolase.

Table 1

Table 1: Signals Regulating G6PD Activity/Location

Positive Regulators	Negative Regulators
PDGF	Aldosterone
EGF	cAMP
VEGF	cAMP-dependent PKA
HGF	CREM
Insulin	Arachidonic Acid
Vitamin D	p38 MAP kinase
PI3-Kinase	p53
Phospholipase C-γ	TNF α
Ras-GTPase	AMP Kinase
cGMP-dependent PKG	
mTOR	
S6 kinase	
Src	
TIGAR	
Hsp27	
ATM	
SREBP	
Nrf2	

Abbreviations: PDGF- platelet derived growth factor; EGF-epidermal growth factor; VEGF- vascular endothelial cell growth factor; HGF-hepatocyte growth factor; PI-3K- phosphatidylinositol-3-kinase; PKG- protein kinase G; mTOR- mammalian target of rapamycin; TIGAR- TP53-induced glycolysis and apoptosis regulator; Hsp27- heat shock protein 27; ATM- ataxia telangiectasia mutated; SREBP- sterol response element binding protein; PKA-protein kinase A; CREM-cyclic AMP response element modulator; Nrf2- nuclear-factor-E2-related factor; TNF α- tumor necrosis factor alpha; AMPK-5' adenosine monophosphateactivated protein kinase

Table 2

Partial List of Cellular Systems Dependent on NADPH

Antioxidant Enzymes (Glutathione Reductase and Catalase)
Nitric Oxide Synthase
Dihudrofolate Reductase
NADPH Oxidase
Cytochrome p450 oxidoreductase
Lipid Synthesis Enzymes (HMG CoA Reductase and others)