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Targeting the Pentose Phosphate Pathway in Syndrome X-related Cardiovascular Complications

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Abstract

Syndrome X is a combination or co-occurrence of several known cardiovascular risk factors (including central obesity, dyslipidemias, fatty liver disease, hyperinsulinemia, insulin resistance, and hypertension) that affects at least one in five people in developed countries. Syndrome X shortens life and increases morbidity by contributing to the development of both diabetes and cardiovascular disease. Type 1 or 2 diabetes affects approximately 170 million people globally and these numbers are rapidly rising. In patients with diabetes, vascular diseases develop early and progress at an accelerated rate. It has recently become evident that glucose-6-phosphate dehydrogenase (G6PD), the rate limiting enzyme in the pentose-phosphate pathway and its reaction products play key roles in regulating vascular function. Epidemiological studies have also shown that G6PD deficiency markedly reduces retinopathy and mortality due to cardiovascular diseases in males from certain Mediterranean regions. Conversely, G6PD expression and activity are upregulated in rat and mouse models of obesity, hyperglycemia and hyperinsulinemia, and a role for G6PD in the development of insulin resistance in type 2 diabetes has been proposed. Unfortunately, there are no selective drugs available to validate the hypothesis that G6PD and its products are involved in the development of Syndrome X in humans. This review discusses the potential mechanisms by which G6PD could be implicated in vascular diseases in Syndrome X and the need to develop new approaches, including new drugs and molecular tools, to ameliorate diabetes-induced vascular dysfunction and vasculopathies.

Keywords

Syndrome X; Hyperglycemia; Hyperinsulinemia; Diabetes; Obesity; Hyperlipidemia; Glucose-6-Phosphate dehydrogenase; Pentose Phosphate Pathway; Reactive Oxygen Species

INTRODUCTION

Syndrome X, also known as metabolic syndrome, currently affects millions of individuals world-wide, and that incidence is growing (Wild et al. 2004). Several known cardiovascular risk factors, including insulin resistance, visceral obesity, atherogenic dyslipidemia and hypertension, occur concurrently in Syndrome X, and according to the World Health Organization (WHO) definition, the first two factors are absolutely required for expression of the metabolic syndrome phenotype. In insulin resistance, adipose, skeletal muscle and liver cells do not respond appropriately to insulin, causing circulating glucose levels to be abnormally high, leading to type 2 diabetes (T2D). Atherogenic dyslipidemia, defined as high levels of serum triglyceride (TG), low levels of high-density lipoprotein (HDL) and

endothelial dysfunction, results from T2D and obesity, and both contribute to the development of atherosclerosis and cardiovascular disease (CVD). As patients with T2D develop vascular diseases early which progress at an accelerated rate, T2D is now widely recognized as an independent risk factor for CVD (Hu et al. 2002). Interestingly, T2D and CVD share underlying mediators, mechanisms and pathways. For example, severely altered glucose metabolism and up-regulated generation of reactive oxygen species (ROS) are associated with both ailments. It is now apparent that glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme in the pentose phosphate pathway (PPP) and hexose monophosphate (HMP) shunt, and its reaction products play a significant role in up-regulating ROS generation and evoking vascular dysfunction in models of Syndrome X (Park et al. 2005; Park et al. 2006; Gupte et al. 2009; Serpillon et al. 2009). G6PD-derived NADPH regulates fatty acid metabolism, energy metabolism, oxidoreductase activity and RNA and DNA synthesis in several cell types (Fig. 1). In addition, hexose-6-phosphate dehydrogenase is believed to supply NADPH for the reductase activity of 11 β -hydroxysteroid dehydrogenase, which has been implicated in the development of visceral obesity, insulin resistance and T2D (Zhang et al. 2009). Consequently, changes in glucose metabolism through the PPP can have a significant impact on organ function in T2D. In this review article the potential role played by G6PD in Syndrome X and the mechanisms by which G6PD may contribute to the development of CVD will be discussed in anticipation of stimulating interest in the development of pharmacological agents to inhibit G6PD in humans to validate the role of G6PD in Syndrome X and to potentially treat diabetes-induced CVD.

G6PD AND INSULIN RESISTANCE

Insulin stimulates glucose use differently in different tissues. In skeletal muscle and liver, insulin stimulates the synthesis of glycogen from glucose and inhibits glycogenolysis. In the liver, it also reduces hepatic gluconeogenesis, further diminishing influx of glucose into the bloodstream. In adipose tissue, insulin inhibits lipolysis, stimulates glucose uptake, and regulates glucose metabolism through the PPP. In both liver and adipocytes, insulin increases G6PD-derived NADPH, which facilitates lipid metabolism (including fatty acid and triglyceride synthesis and catabolism) and regulates several oxidoreductase enzymes. Insulin resistance occurs when there is a decrease in the responsiveness of peripheral tissues (skeletal muscle, fat and liver) to its effects. Although the understanding of how insulin resistance occurs is lacking, recent studies have suggested that overexpression of G6PD in adipocytes contributes to defective hormone (e.g., adiponectin and leptin) release from adipose tissue, dyslipidemia and insulin resistance (Park et al. 2005). In these studies siRNA-induced knockdown of G6PD in preadipocytes (3T3-L1 cells) cultured under high glucose conditions attenuated adipocyte differentiation and lipid accumulation. Inhibition of G6PD using 17-ketosteroids or dihydroepiandrosterone also blocked the conversion of 3T3-L1 and 3T3-F442A preadipocyte clones into the adipocyte phenotype (Gordon et al. 1987; Shantz et al. 1989). Conversely, overexpression of G6PD stimulated the expression of the adipocyte marker genes, TNF α and resistin; decreased synthesis of adiponectin, elevated lipid accumulation; impaired insulin signaling; and suppressed insulin-dependent glucose uptake into adipocytes. Although the mechanism by which G6PD evokes insulin resistance remains unclear, it has been suggested that overexpression G6PD promotes the expression of pro-oxidative enzymes such as iNOS and NADPH oxidases (p22^{phox}, p47^{phox}, p40^{phox}, p67^{phox} and gp91^{phox}) and pro-inflammatory cytokines (Park et al. 2006), and that the resultant oxidative stress and pro-inflammatory signaling ultimately alters the insulin response in adipocytes (Fig. 2). From these findings it may be concluded that G6PD is an important mediator of insulin resistance.

G6PD AND OBESITY

The incidence of obesity is increasing, and its role in the metabolic phenotype is evident. Nonetheless, the molecular mechanisms underlying obesity and its role in metabolic disorders, including T2D, atherosclerosis and hypertension, remains unclear. That said, recent studies indicate that G6PD expression is upregulated in adipocytes in obese (including *db/db*, *ob/ob*, and diet-induced obesity) mice, and in adipocytes and stromal-vascular cells in diabetic *db/db* mice (Park et al. 2005; Park et al. 2006). Consistent with those findings, high levels of G6PD expression and activity occur in liver and epididymal fat from genetically obese but normotensive rats and in the liver of obese Zucker diabetic fatty rats (Ellwood et al. 1985; Gupte et al. 2009). Moreover, studies demonstrating that dihydroepiandrosterone treatment reduces obesity in male and female Zucker diabetic fatty rats (Cleary and Zisk 1986), support the concept that G6PD could be a modulator of obesity. G6PD thus appears to be a modulator or mediator of two (i.e., insulin resistance and obesity) of the four factors used to define Syndrome X. It is therefore reasonable to predict that G6PD plays a key role in increasing fat metabolism in adipocytes and liver, inducing fatty liver and establishing Syndrome X in humans (Fig. 3). Development of target-specific drugs to regulate G6PD would therefore seem warranted and would likely be of benefit in the treatment of Syndrome X.

G6PD IS ACTIVATED VIA SRC KINASE-DEPENDENT PATHWAYS IN HYPERINSULINEMIC AND OBESE ANIMALS

AS G6PD appears to modulate insulin resistance, identification and characterization of the mechanisms that regulate G6PD and/or PPP activity in Syndrome X would be prerequisite for development of drugs that target G6PD. In this section, therefore, an overview of the pathways involved in insulin signaling under physiological and pathophysiological conditions is presented.

Insulin activates two parallel pathways: the phosphoinositide 3-kinase (PI3K) pathway and the mitogen-activated protein (MAP) kinase pathway. Tyrosine phosphorylation of insulin receptor substrates (IRS) activates PI3K, leading to activation of 3-phosphoinositide-dependent protein kinase 1 (PDK1) kinase and Akt kinase. The PI3K-Akt pathway is then responsible for many of the downstream metabolic effects of insulin. In skeletal muscle and adipose tissue, Akt kinase stimulates translocation of the insulin-responsive glucose transporter, GLUT4, to the cell surface, thereby increasing glucose uptake. In hepatocytes, PI3K-dependent signaling increases G6PD expression and glucose metabolism through the PPP (Wagle et al. 1998). Inhibition of the PI3K-Akt pathway due to insulin resistance reduces GLUT4 translocation, thereby reducing glucose uptake into skeletal muscle and fat. By contrast, activation of *Src* kinases stimulates hepatic G6PD activity in hyperinsulinemic and obese Zucker diabetic fatty rats (Gupte et al. 2009). Notably, *Src* kinases and *Src*-dependent phosphatases are activated in liver, fat and vascular smooth muscle in the obese and insulin-resistant *db/db* mouse and in humans (Jiang and Zhang 2002; Reddy et al. 2006; Sasaoka et al. 2006; Walcher et al. 2006). This in turn leads to activation of the overexpressed G6PD in adipocytes in obese (including *db/db*, *ob/ob* and diet-induced obesity) mice, and in adipocytes and stromal-vascular cells in diabetic *db/db* mice. (Park et al. 2005; Park et al. 2006). As illustrated in Fig. 4, these findings, suggest that pathways closely associated with insulin signaling are likely involved in activating G6PD and the PPP in Syndrome X.

G6PD AND DYSLIPIDEMIA

The defining features of dyslipidemia are high plasma TG levels, low HDL levels and high LDL levels. Insulin resistance and visceral obesity are both associated with dyslipidemia and insulin normally suppresses lipolysis in adipocytes, but in insulin resistance, impaired insulin signaling leads to increased lipolysis, which in turn leads to increased plasma FFA levels. In the liver, FFAs are a substrate for TG synthesis and also stabilize the production of apoB, the major protein component of very-low-density lipoprotein (VLDL) particles, resulting in upregulated VLDL production. Alternatively, insulin resistance may prevent degradation of apoB by reducing the activity of lipoprotein lipase, the rate-limiting and major mediator of VLDL clearance, thereby increasing VLDL levels.

Under normal conditions, the PPP is under strict hormonal and nutritional control (Gupte, 2008). In liver, adipose tissue and other metabolically active organs, PPP activity varies as a function of the demand for the NADPH needed for cholesterol and fatty acid metabolism. In adipocytes from obese mice, G6PD overexpression or activation impairs insulin signaling and increases both FFA and TG levels. Conversely, G6PD-deficiency reduces lipid metabolism in humans (Gupte 2008). In peripheral blood lymphomononuclear cells from G6PD-deficient subjects, for example, cholesterol synthesis and esterification, as well as expression of HMG-CoA and LDL receptor, are lower than in normal subjects (Batetta et al. 2002). In addition, G6PD deficiency significantly reduces HMG-CoA reductase activity (Meloni et al. 2008), and the G6PD inhibitor, dehydroepiandrosterone reduces HMG-CoA expression (Matsuzaki and Honda 2006). Taken together, these findings suggest that G6PD plays a key role in regulating lipid metabolism and insulin resistance, which directly control lipolysis in adipose tissue and lipid metabolism in liver.

OVEREXPRESSION OF G6PD AND OXIDATIVE STRESS IN HYPERINSULINEMIA- AND OBESITYINDUCED VASCULAR DYSFUNCTION

In vascular endothelial cells, Akt kinase phosphorylates and activates endothelial nitric oxide synthase (eNOS) (McCabe et al. 2000). Insulin increases local blood flow in tissues through activation of eNOS. This vascular effect of insulin couples glucose homeostasis to blood flow and at physiological concentrations contributes to glucose metabolism. Consequently, pharmacological inhibition of NO production reduces glucose disposal by about 40%. In T2D, however, eNOS is uncoupled and NO is inactivated by ROS generated via NADPH-associated pathways (Toth et al. 2007), thereby blunting the beneficial effects of insulin.

In Zucker diabetic fatty rats, the PKC pathway is activated (Brownlee 2001) leading to stimulation of G6PD activity in the heart and vascular tissue (Serpillon et al. 2009). Similarly, G6PD is overexpressed in stromal-vascular cells of diabetic *db/db* mice (Park et al. 2005; Park et al. 2006) suggesting that G6PD and the PPP are up-regulated in vascular tissue of insulin-resistant and obese animals. G6PD-derived NADPH acts as a co-factor with eNOS and several oxido-reductases (including glutathione reductase, thioredoxin reductase, hemeoxygenase, and NADPH oxidase). In T2D, the expression and activity of a number antioxidants, including NADPH-dependent glutathione reductase and hemeoxygenase, are downregulated (Abraham et al. 2004; Gupte et al. 2009; Serpillon et al. 2009), while pro-oxidant systems like the NADPH oxidases are upregulated or activated, increasing ROS production (Park et al. 2006; Gupte et al. 2009). In diabetic BB rats, uncoupled eNOS stops producing NO, despite elevated endothelial NADPH levels (Meininger et al. 2000). In heart and aortic tissue in Zucker diabetic fatty rats, antioxidants are downregulated and increased levels of G6PD-derived NADPH stimulates NADPH oxidase activity, exacerbating oxidative stress (Fig 5). Upregulation of ROS reduces endothelium-dependent vascular

dilation (endothelial dysfunction) and presumably induces diabetes-related cardiac failure (Serpillon et al. 2009). Consistent with those findings, overexpression of G6PD reportedly promotes expression of various pro-oxidative enzymes (e.g., NADPH oxidases) as well as NF κ B, which mediates pro-inflammatory signaling in obese and insulin resistant subjects (Park et al. 2006). Notably, expression of NADPH oxidases (e.g., Nox4 & gp91phox) is increased in both T2D and CVD (Guzik et al. 2002; Li and Shah 2004; Guzik et al. 2006). These oxidases are major sources of ROS in vascular tissue, and G6PD-derived NADPH is a substrate for NADPH oxidase that fuels NADPH oxidase-derived generation of ROS (Gupte et al. 2005; Matsui et al. 2005; Gupte et al. 2006; Gupte et al. 2007). G6PD deficiency completely abrogates Ang II-induced ROS generation, preventing development of Ang II-induced hypertension and vascular smooth muscle cells hypertrophy (Matsui et al. 2005). Moreover, low G6PD activity reduces ROS generation and development of atherosclerotic lesions in apolipoprotein E^{-/-} mice (Matsui et al. 2006). Similarly, some males from certain Mediterranean regions (e.g., Sardinia) deficient in G6PD protein rarely develop atherosclerosis and other vascular complications leading to a decreased mortality rate from CVD (Gupte 2008; Meloni et al. 2008). By contrast, expression of isoform A of G6PD is increased in atherosclerotic plaques (Pearson et al. 1977).

In summary, overexpression of G6PD can alter ion channel function, promote cell proliferation, enhance cholesterol and fatty acid synthesis, modulate immune system function and increase oxidation, all factors involved in the development of vascular disease. Additionally, under conditions of insulin resistance, the MAP kinase pathway continuously stimulates ET-1 production; elevates expression of the vascular cell adhesion molecules, VCAM-1 and E-selectin, enhancing leukocyte-endothelial interactions; and stimulates growth and mitogenesis. Thus, altered glucose metabolism through the PPP, along with upregulated activity in the MAP kinase pathway substantively alters vascular smooth muscle cell growth and function. In this manner, insulin resistance leads to vascular abnormalities that predispose to atherosclerosis. Insulin signaling apparently coordinately affects peripheral glucose use, vascular tone and blood flow. In addition hyperglycemia, advanced glycation products, FFA toxicity, obesity, dyslipidemia and other proinflammatory conditions (Fig. 4) contribute to and follow from insulin resistance and also can affect vascular function.

BENEFICIAL EFFECTS OF G6PD AND WHY WE NEED TO DEVELOP SECOND GENERATION DRUGS TO TARGET G6PD IN SYNDROME X

Having discussed the contribution of G6PD in the various mechanisms potentially involved in the pathogenesis of Syndrome X, it would be prudent to also consider the beneficial effects of G6PD. G6PD and its reaction products play key roles in reducing oxidative stress in endothelial cells, vascular smooth muscle cells and cardiac myocytes by activating glutathione reductase, which increases reductive potential by restoring or maintaining stores of reduced glutathione (Leopold and Loscalzo 2000; Leopold et al. 2003). In type 1 (streptozotocin and alloxan treated) diabetic rats, G6PD is inhibited in islet cells (Akpan et al. 1982), Leydig cells (Calvo et al. 1979), kidney (Xu et al. 2005), lens (Hothersall et al. 1992), heart (Tarach 1978) and liver (Aragno et al. 1999). It is therefore presumed that the level of oxidative stress may be increased in the cells and tissues in type 1 diabetes. In addition, measurements of G6PD activity in mononuclear leukocytes and RBCs from diabetic and control patients suggest that low G6PD activity is a risk factor for diabetes (Kher and Grover 1974; Muggeo et al. 1993; Wan et al. 2002) and G6PD deficiency can be a cause of hemolysis in diabetic ketoacidosis (Goudar et al. 2005). From these studies it may be concluded that G6PD plays a multifaceted role in Syndrome X. Thus, depending on the tissues in which G6PD is overexpressed and the extent to which anti-oxidants are downregulated, G6PD may either promote or prevent the symptoms of metabolic disorders,

including obesity and diabetes. Thus, targeting G6PD ubiquitously with nonselective antagonists may not be an effective approach to the treatment of Syndrome X.

There are currently two classes of compounds that can inhibit G6PD *in vivo*: analogs of NADP⁺ (e.g., 6-aminonicotinamide; 6AN), which competitively block G6PD and 17-ketosteroids (e.g., *epi*-androsterone and dihydroepiandrosterone), that noncompetitively inhibit the enzyme. Each of these antagonists has its merits and disadvantages. 6AN is specific G6PD inhibitor but is neurotoxic and has severe side effects, while the steroids *epi*-androsterone and dihydroepiandrosterone are nonspecific in their actions and likely to exert other steroid-related effects in addition to inhibiting G6PD. Currently, analogs of these steroids are being developed to minimize the steroidal side effects. In addition, *in silico* and conventional screening approaches identified gallated catechins as NADP⁺-competitive inhibitors of G6PD and other enzymes that use NADP⁺ as a coenzyme (Shin et al. 2008). Unfortunately, all of the compounds tested to date have nonspecific actions and ubiquitously inhibit G6PD. To improve pharmacological treatment it will be necessary to: 1] confirm the role played by G6PD in the manifestation of Syndrome X and CVD in humans; 2] determine where in the body G6PD is overexpressed in these diseases; and 3] determine the mechanisms that modulate the expression and activities of G6PD and, in turn, the PPP. Pharmacological agents that will specifically downregulate G6PD expression or more selectively inhibit enzyme activity in T2D, are urgently required to reduce activity to normal physiological levels in adipocytes, liver and vascular smooth muscle, without perturbing its action in endothelial or blood cells.

SUMMARY

Syndrome X is characterized in part by an imbalance in the redox status resulting in elevated oxidative stress and increased activity in pro-inflammatory pathways (see Fig. 5). Under those conditions, overexpression of G6PD promotes development of both T2D and CVD. This makes G6PD a potentially useful therapeutic target for the treatment of Syndrome X, which has become a leading cause of mortality and morbidity worldwide.

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Figure 1. Function of glucose-6-phosphate dehydrogenase

Glucose-6-phosphate (G6P) is metabolized by glucose-6-phosphate dehydrogenase (G6PD) to 6-phospho-gluconolactone, which is further metabolized by phospho-6-gluconolactone dehydrogenase (PG6D) to riboses. In this process two molecules of NADPH are generated. NADPH is a coenzyme in many synthetic pathways and cofactor for oxido-reductases.

Figure 2. A putative function for glucose-6-phosphate dehydrogenase in insulin resistance development

In adipocytes, overexpression of glucose-6-phosphate dehydrogenase (G6PD) increases free fatty acid (FFA) and triglyceride (TG) synthesis, decreases adiponectin and leptin synthesis, and upregulates expression of NADPH oxidases, $\text{TNF}\alpha$ and pro-inflammatory cytokines. Collectively, these actions induce insulin resistance.

Figure 3. Schematic diagram illustrating the role of glucose-6-phosphate dehydrogenase overexpression in the development of obesity

Overexpression and activation of glucose-6-phosphate dehydrogenase (G6PD) in adipocytes and liver of obese rats and mice enhances signaling in several pathways that presumably feedback to stimulate food intake (solid arrows) and induce hyperinsulinemia and hyperglycemia. The increased insulin and glucose levels putatively activate G6PD, thereby increasing food intake via the aforementioned feedback loop (broken arrows).

Figure 4. Signaling pathways involved in mediating overexpression and activation of glucose-6-phosphate dehydrogenase in obese, hyperinsulinemic and hyperglycemic models

Current evidence suggests that Src kinases and protein kinase C (PKC) are activated in various tissues and organs in obese, hyperinsulinemic and hyperglycemic rats and mice. Once activated, Src kinases and PKC activate G6PD, which increases oxidative stress and inflammation. When this occurs in the vasculature, it can lead to endothelial dysfunction, coronary artery disease and heart failure.

Figure 5. An overall picture of Syndrome X

The metabolic syndrome, Syndrome X, is a manifestation of the co-occurrence of several risk factors for cardiovascular disease. In Syndrome X, anti-oxidants are significantly downregulated in the liver and cardiovascular system. Under such conditions overexpression and activation of G6PD promotes dyslipidemia and ROS generation, which induces inflammation. All of these factors predispose an individual to T2D and CVD.