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Review

Glucose as a Major Antioxidant: When, What for and Why It Fails?

Andriy Cherkas [1,*], Serhii Holota [2,3], Tamaz Mdzinarashvili [4], Rosita Gabbianelli [5] and Neven Zarkovic [6]

[1] Department of Internal Medicine #1, Lviv National Medical University, Lviv, Ukraine; cherkasandriy@yahoo.com;
[2] Department of Pharmaceutical, Organic and Bioorganic Chemistry, Lviv National Medical University, Lviv, Ukraine; golota_serg@yahoo.com;
[3] Department of Organic Chemistry and Pharmacy, Lesya Ukrainka Eastern European National University, Lutsk, Ukraine; golota_serg@yahoo.com;
[4] Institute of Medical and Applied Biophysics, I. Javakhishvili Tbilisi State University, Georgia; tamaz.mdzinarashvili@tsu.ge;
[5] Unit of Molecular Biology, School of Pharmacy, University of Camerino, Camerino, Italy; rosita.gabbianelli@unicam.it;
[2] Laboratory for Oxidative Stress (LabOS), Institute "Rudjer Boskovic", HR-10000 Zagreb, Croatia; zarkovic@irb.hr;
* Correspondence: cherkasandriy@yahoo.com (A.C.)

Abstract: A human organism depends on stable glucose blood levels in order to maintain the metabolic needs. Glucose is considered as the most important energy source and glycolysis is postulated as a backbone pathway. However, when glucose supply is limited, ketone bodies and amino acids can be used to produce enough ATP. In contrast, for the functioning of pentose phosphate pathway (PPP) glucose is essential and cannot be substituted for by other metabolites. PPP generates and maintains levels of NADPH needed for reduction of oxidized glutathione and protein thiols, synthesis of lipids and DNA as well as for xenobiotics detoxification, regulatory redox signaling and counteracting infections. Flux of glucose into a PPP, particularly under extreme oxidative and toxic challenges is critical for survival, whereas the glycolytic pathway is primarily activated when glucose is abundant, and there is lack of $NADP^+$ that is required for activation of glucose-6 phosphate dehydrogenase. An important role of glycogen stores in resistance to oxidative challenges is discussed. Current evidence explains disruptive metabolic effects and detrimental health consequences of chronic nutritional carbohydrate overload and provides new insights into positive metabolic effects of intermittent fasting, caloric restriction, exercise, and ketogenic diet through modulation of redox homeostasis.

Keywords: glucose; pentose phosphate pathway; NADPH; redox balance; glycogen; glycolysis; stress resistance; insulin resistance

1. Introduction

The glucose level in blood is one of the most important homeostatic parameters and is strictly regulated [1]. A complex interplay of signals from central and autonomic branches of the nervous system, impact of multiple hormones and cytokines all maintain coordinated glucose flows within the body according to the actual needs and availability and its concentration is maintained in a narrow range [2]. Short-time high glucose levels can cause certain degree of damage due to increased rate of non-enzymatic glycation of proteins but are usually not life-threatening. However, low blood concentrations can cause severe brain damage and potentially death in relatively short periods of

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time [3]. Brain and particularly neurons are the most sensitive to glucose deprivation, while other tissues and cells show a wide divergence in resistance to hypoglycemia [1] that is very much dependent on their function, peculiarities blood flow and capability to store glucose in the form of glycogen.

It is well known that most of the glucose in human metabolism is utilized intracellularly in glycolytic pathway with further degradation of products in the tricarboxylic acid (TCA) cycle in order to produce NADH and ATP [4]. Glycolysis is effectively activated by insulin in conditions of glucose abundance and a number of intermediates are also used for synthesis of needed amino and fatty acids as well as other important metabolites [2]. However, in conditions of limited glucose supply and/or excessive metabolic needs there are numerous alternative ways to generate enough NADH and ATP, for example by oxidation of fatty acids, utilization of ketone bodies etc. Flexibility and interchangeability of cellular energy supply provides sustainable and at the same time variable flow of metabolites that is capable to accumulate them when the nutrients are in abundance and consume them in a most effective way when there is their deficit. In the periods of starvation or glucose deficit activation of catabolic programs is capable to maintain energy production in most of the organs [5]. Since neurons do not accumulate glycogen and total accumulation of glycogen in central nervous system being extraordinarily low is limited to astrocytes, neurons rely on glucose supply from the bloodstream [6]. Interestingly, in the periods of starvation the brain can effectively use ketone bodies as a primary fuel accounting for more than 75% of its energetic needs, pointing out the possibility that glucose may be used for other purposes in this case [7]. This is further confirmed by the observation that glycolysis in neurons is actively downregulated by proteasomal degradation of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3, preventing utilization of glucose for bioenergetics purposes. This mechanism, as suggested by authors, spares glucose in neurons for maintaining antioxidant status, especially in conditions of limited glucose supply [8].

The importance of other major pathway of glucose metabolism, which to certain degree is an alternative or parallel to upper glycolysis, a pentose phosphate pathway (PPP) is also long known. It is believed that its major function is generation of reducing equivalents in the form of NADPH needed for de novo lipogenesis, synthesis of DNA and aromatic amino acid [9]. Indeed, proliferating cells use most of the NADPH for DNA and fatty acid synthesis [10]. The other major functions of NADPH are the reduction of oxidized thiols and glutathione, generation of superoxide anion and hydrogen peroxide during respiratory burst to fight infections and to provide redox signals to regulate cell functions. In addition, it is also needed for detoxification of xenobiotics [11]. A growing number of publications point out rerouting of glucose into a PPP as a major protective mechanism employed to counteract acute and severe oxidative stress [9,12,13]. According to the calculations, full oxidation of one molecule of glucose in PPP yields 12 molecules of NADPH reduced from $NADP^+$ [14]. This highlights extraordinary efficiency and prompt responsiveness of this mechanism in balancing redox homeostasis in conditions of acute oxidative challenge. Indeed, activation of redox-sensitive transcription factors such as Nrf2 or FOXOs, in response to oxidative stress will result in induction of antioxidant enzymes within hours [15], while rerouting of glucose into PPP to generate reducing power for antioxidant enzymes takes place almost immediately [14]. With the use of13C flux analysis in neurons it was recently shown, that glucose metabolism through PPP may be much more significant than it was estimated earlier [16]. Moreover, authors demonstrated that about 73% of produced labeled pyruvate was exported from neurons as lactate16. This may indicate that neurons remove glucose that cannot be fully utilized in the TCA away from the cells. In case of increased functional activity, oxidative stress or glucose deficit during starvation, most of the glucose flux may be redirected into PPP.

PPP is a major source of NADPH, however, not the only one. Substantial amounts of NADPH are generated in folate-dependent NADPH-producing pathway [10] as well as by cytosolic isocitrate dehydrogenase and malic enzyme11. However, these sources are often coupled with synthetic pathways, for example isocitrate is in abundance when glycolysis is activated and contributes to fatty acid synthesis, therefore it is difficult to expect their substantial contribution to regeneration of NADPH in case of oxidative stress. To certain degree metabolism of amino acids can compensate

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functional lack of glucose and contribute to maintenance of NADPH, but this seems to be the mechanism with limited power under extreme exposures. Noteworthy, recently it was shown, that malic enzyme and 6-phosphogluconate dehydrogenase (6PGD) form a hetero-oligomer to promote activity of 6PGD, independently on activity of malic enzyme [17]. It is likely that the other structural and functional interactions may exist in the cells in order to couple synergistic metabolic processes in response to oxidative stress. Activity of alternative pathways provides robustness of NADPH supply and to some extent compensates deficit of PPP flux in patients with glucose-6 phosphate dehydrogenase (G6PD) deficiency, the most common genetic disease in humans [18]. Patients with G6PD deficiency generally have no symptoms and their lifespan is not affected by disease, but it was shown that in addition to increased hemolysis they are less resistant to some poisonings [18] and have higher risk of diabetes and metabolic syndrome [19]. Glucose-6 phosphate (G6P) is an exclusive substrate for G6PD, a rate limiting enzyme of PPP and in human organism can be supplied from extracellular space in the form of glucose, then phosphorylated by hexokinase. Alternatively, glucose-1 phosphate released from glycogen, if the latter is available in the cell, is converted by phosphoglucomutase to G6P. Some tissues, namely liver, kidneys or intestine [7], and to some extent glial cells can generate glucose via gluconeogenesis. Also, tumor cells may reverse glycolysis in order to maintain their biosynthesis in glucose-free conditions [20]. Noteworthy, expression of most of the enzymes in the PPP is controlled by Nrf2, a redox sensitive transcription factor involved in upregulation of antioxidant and detoxifying genes, degradation of damaged proteins and metabolic reprogramming during stress [21] pointing out tight conjugation of redox balance maintenance and glucose metabolism. In other words, on the cellular and organism´s levels response to local or systemic oxidative stress is associated with increased glucose release/production by liver and subsequently hyperglycemia, which may be physiological adaptive response in healthy subjects and may also take place as a chronic metabolic deterioration in patients.

2. Oxidative PPP is thermodynamically more favorable compared to upper glycolysis under conditions of limited glucose supply

In physiological conditions (without metabolic/oxidative stress) ratio of reduced and oxidized forms of this coenzyme NADPH/NADP+ is very high (in the range of approximately 100/1 but is highly tissue-dependent) and the lack of free NADP+ prevents G6P from entering PPP [14]. However, as soon as NADPH is oxidized (e.g. in conditions of oxidative stress) availability of NADP+ immediately redirects metabolic flow to PPP and suppress further steps of glycolysis and downstream utilization of glucose metabolites in TCA [8, 13]. In other words, cells prioritize metabolism of glucose through PPP over standard reactions of upper glycolysis in order to maintain a sufficient NADPH/NADP+ ratio needed for counteraction of acute oxidative challenge (prompt enzymatic reduction of glutathione and other oxidized thiols), biosynthesis and/or generation of superoxide during immune responses or as physiological redox signaling (Figure 1). Glucose availability for PPP, either from extracellular space, or from intracellular glycogen stores, is essential for acute antioxidant responses providing survival of cells and organism in extreme conditions. Stable and robust liver glucose output in case of severe stress (including oxidative stress), infection, starvation and extreme exercise is protected by the development of insulin resistance in order to provide sufficient glucose flow to balance redox homeostasis. In absolute quantities, especially at rest, glucose flow into PPP may be low compared to standard upper glycolysis, but under conditions when the NADPH/NADP+ ratio drops, amounts of glucose entering PPP will correspond to the degree of NADPH depletion.

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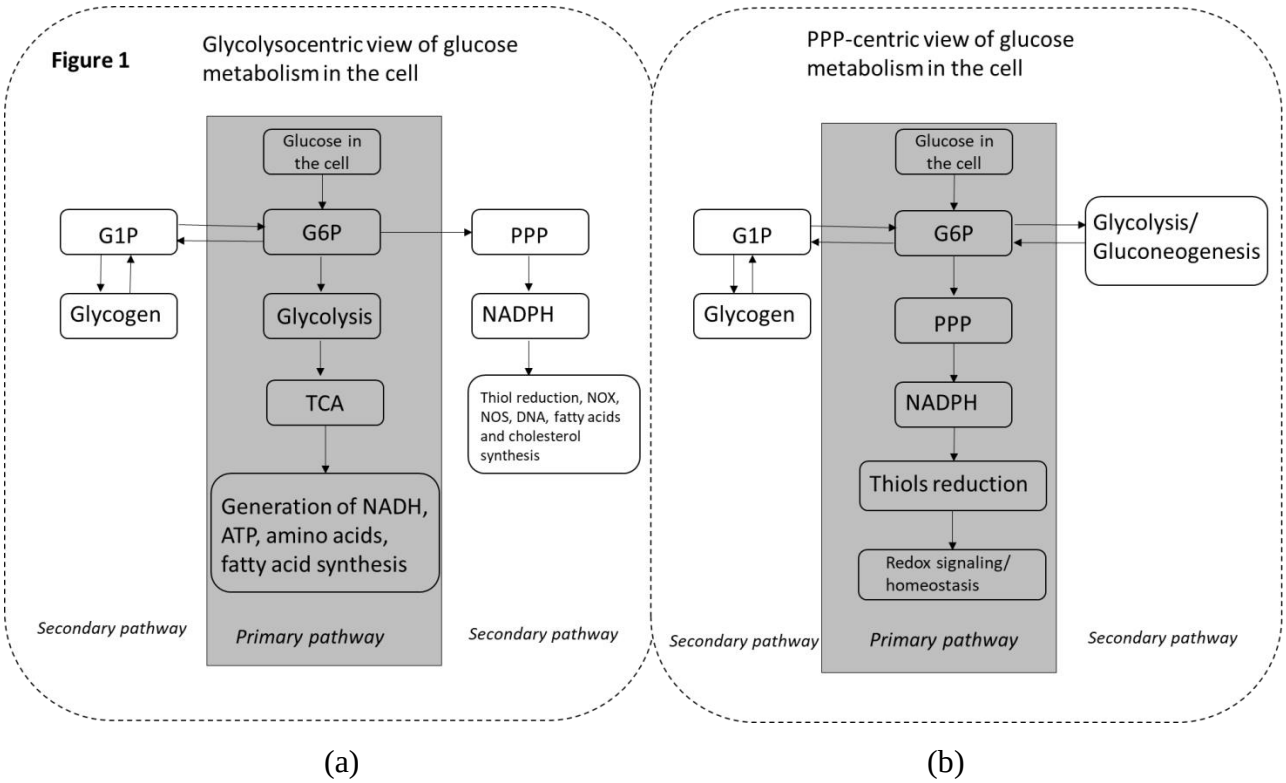

(a) (b)

Figure 1. Schematic presentation of the conventional (a) and pentose phosphate pathway-centric (b) views of glucose metabolism. Abbreviations: G1P – glucose 1 phosphate, G6P – glucose 6 phosphate, PPP – pentose phosphate pathway, TCA – tricarboxylic acid cycle, NOX – NADPH oxidase, NOS – nitric oxide synthase.

A key factor leading G6P into PPP is the presence of NADP+. The first reactions of PPP as well as other reactions producing NADPH are energetically very favorable and are basically irreversible [22]. In contrast, most of the reactions of glycolysis are reversible. Activation of glucose utilization through glycolysis in physiological conditions takes place, when the NADPH/NADP+ ratio is high, glucose is relatively abundant and insulin signaling is not compromised. Glycolysis serves as a source of pyruvate for TCA cycle and a number of anabolic intermediates in conditions of high carbohydrate availability and is supposed to be fully activated only occasionally under physiological conditions often switching to oxidation of fatty acids when glucose availability is limited. This shift takes place in concert with activation of transcription factors involved in antioxidant defense (Nrf2, FOXOs, etc.) as a part of systemic antioxidant response aimed to balance redox homeostasis, where gluconeogenesis and activation of PPP are fundamental parts of it (Figure 2). A sedentary lifestyle plus an abundance of carbohydrates in food further exacerbates the metabolic effects of nutritional glucose overload and causes metabolic syndrome, diabetes type 2 and contributes to increases in the incidence of cancer.

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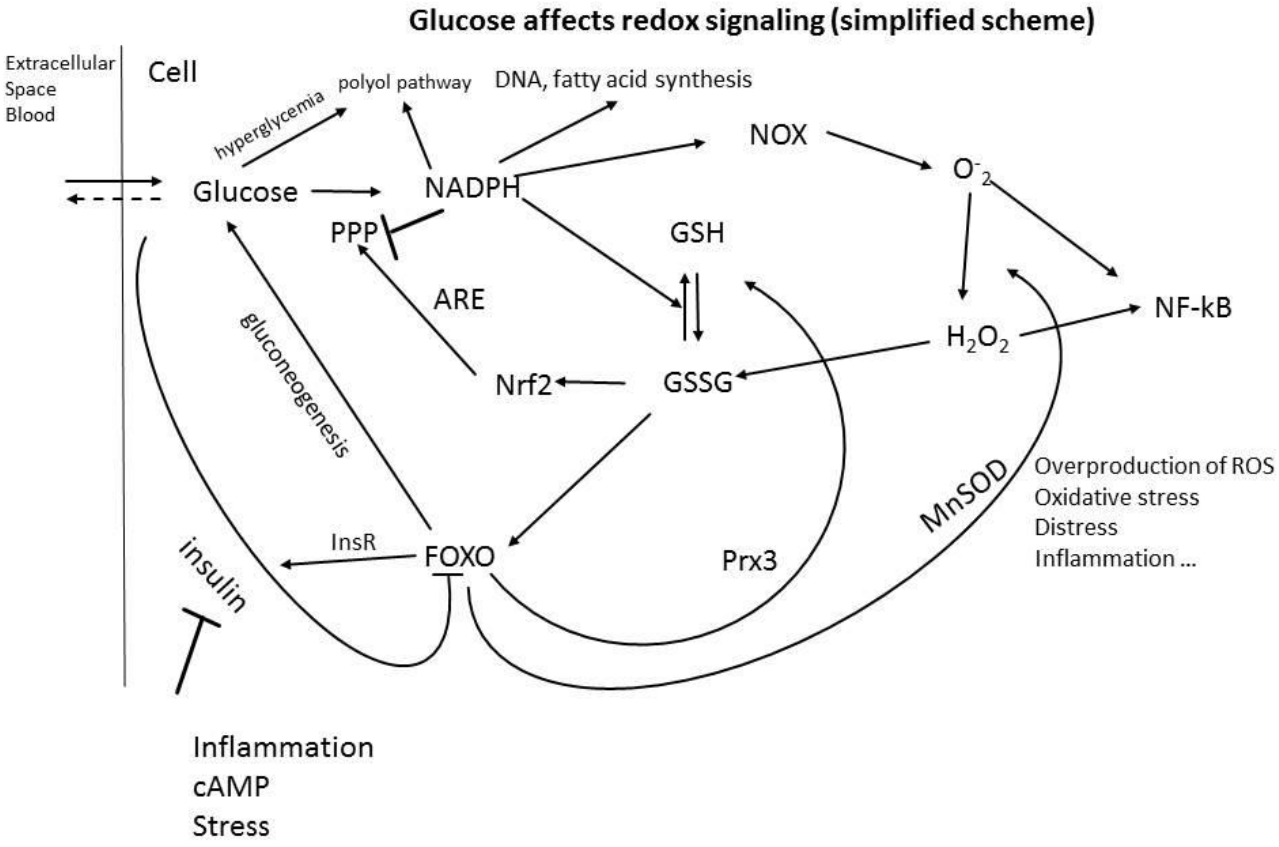

Figure 2. Glucose availability is a major factor in maintenance of redox homeostasis through reduction of oxidized NADP+, which is used for reduction of oxidized glutathione and thiols. At the same time NADPH is used for synthesis of DNA and fatty acid synthesis and is needed for activities of NADPH oxidases and NO-synthase activities and other processes. Abbreviations: PPP – pentose phosphate pathway, NOX – NADPH oxidase, NOS – nitric oxide synthase, GSH – glutathione, GSSG – glutathione disulfide, ARE – antioxidant response element, InsR – insulin receptor, cAMP – cyclic adenosine monophosphate, FOXO – forheadkbox O transcription factors, Prx3 – peroxiredoxin 3, Nrf2 – Nuclear factor (erythroid-derived 2)-like 2 transcription factor, MnSOD – manganese superoxide dismutase, nF-kB - nuclear factor kappa-light-chain-enhancer of activated B cells.

3. The evidence of pandemic glucose toxicity in human population on global scale

As currently observed, massive nutritional carbohydrate overload associated with dramatic decreases in physical activity caused an epidemic of noninfectious diseases such as obesity, metabolic syndrome, type 2 diabetes, atherosclerosis, hypertension and cancer [23]. A recent large-scale epidemiological cohort study pointed out that high carbohydrate consumption is a major factor for all-cause mortality, while total fat and different types of fat were not associated with increased mortality. Thus, authors question current dietary guidelines suggesting an urgent need for their reconsideration [24]. Together with other evidence, including numerous animal studies, a serious demand for strategies of how to counteract carbohydrate overload is indicated. Different approaches are increasingly discussed in the literature [25].

In fact, abundance of carbohydrates in food during human evolution was rather rare and a kind of short-term luxury. Human neonates grow very rapidly and their need in carbohydrate supply is probably higher than in adults and in other species due to relatively large brain and rapid

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development of nervous system, however, human breast milk contains "only" about 6.7 g per 100 ml of lactose accounting for up to 40% of calories, highest compared to other mammals[26, 27]. It is not likely, that adults need more carbohydrates than babies as a percent of calories intake in usual conditions without high physical activity. Excessive consumption of carbohydrates and low physical activity are major contributors to increasing rates of metabolic syndrome and obesity in children and adolescents [28].

At high growth rate, intensive physical activity as well as strong immunity needed for resistance to infections and occasional poisonings requires maintenance of sufficient levels of glucose in blood despite prolonged periods of carbohydrate deficit. Dietary glucose as well as other carbohydrates were precious food components promoting survival. Therefore, an evolutionary sweet taste developed to detect sources of digestible carbohydrates [29]. Only development of agriculture about 10000 years ago enabled higher consumption of grain, increasing the share of carbohydrate in the diet. Even though carbohydrates became more available, any possible overload would rather not take place considering high physical activity of most of the people at that time. Thus, gradual increase in basic food availability and elimination of physical work created a massive nutritional carbohydrate overload at the organism's level causing respective health consequences [24]. Consistent with this, recently a metabolic core model was used to evaluate how increased glycolytic utilization of glucose together with glutamine-dependent lactate production promotes cancer growth [30]. There is a growing evidence, both mechanistic and epidemiological, that confirms previous predictions of interrelationships between risk of cardiovascular/metabolic diseases and cancer risks [31-33].

Thus, from the evolutionary point of view human organism was rather not used to high consumption of glucose and have had to optimize metabolism in order to be able to produce sufficient its amounts accordingly to metabolic needs. PPP importance evolved in order to provide resistance to oxidative challenges that is crucial for the survival in acute extreme conditions in multicellular organisms. Upregulation of PPP under stress is tightly coupled with enhanced glucose output from glycogen stores and/or stimulation of gluconeogenesis. Glucose 6-phosphate is a specific substrate for PPP that makes glucose so important and strictly regulated in maintaining redox homeostasis in human organism [1].

Consistent with this is recent data generated on C. elegance model regarding integration of stress induced responses of nervous system with metabolic adaptations [34]. It was shown that the flight response mediated by tyramine (worm analog of catecholamines) in the end leads to stimulation of insulin-IGF-1 signaling and have the opposite effect to longevity-promoting stress responses to heat, starvation, glucose restriction or exercise [35-37]. If hypothetically translated to modern humans it can provide accurate mechanistical explanations why emotional stress accompanied with sedentary behaviors may have detrimental health consequences associated with both excessive insulin and adrenaline signaling causing atherosclerosis [23], contributing among the other factors to insulin resistance and accelerated aging [38] but can be reversed by exercise or fasting [39].

4. Glycogen protects against (not only) oxidative stress

According to our hypothesis, availability of intracellular glycogen is supposed to be protective against oxidative stress, and vice-versa; its absence exposes cells to higher risk. Indeed, neurons, which are unable to accumulate glycogen appear to be among the most sensitive cells to oxidative stress and they apply sophisticated mechanisms to direct the flow of glucose into the PPP in order to protect themselves [8]. A recent C. elegans study demonstrated the crucial role of glycogen stores in resistance to acute oxidative stress [40]. Moreover, excessive accumulation of glycogen from a high glucose diet and with impaired glycogen degradation resulted in decreased lifespan of the worms [40]. Insecticide poisonings causing oxidative stress in the fruit-eating bat *Artibeus lituratus* causes glycogen stores depletion [41]. It was recently shown that hawkmoths, who have one of the highest metabolic rates among known animals, use nectar sugar directed through PPP to counteract oxidative

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damage resulting from flight [42]. In humans, inability to deplete muscular glycogen in patients with glycogen phosphorylase deficiency (McArdle disease) is associated with severe exercise induced oxidative stress and a risk of rhabdomyolysis [43]. This points out the possibility that the function of glycogen in muscles is not only an energy store during periods of intensive contraction, but also for counteracting oxidative challenges associated with exercise.

It was noted that main life- and health-span promoting interventions such as caloric restriction, intermittent fasting and exercise have in common that the depletion of glycogen stores [39], thus reducing the protective capacity of glycogen and exposing the cells to moderate hormetic oxidative stress. Glycogen stores are not simply an intracellular source of glucose, they also have an important signaling function [44] and are protective against a number of stressful situations, namely hyper/hypo osmotic stress [45], anoxia/hypoxia [46] etc. In addition, growing evidence indicates that a metabolic switch from utilization of glucose, which is abundant in western diets, to ketone bodies use derived from fatty acids is an evolutionarily conserved trigger-point responsible for health effects from intermittent fasting, caloric restriction and exercise [47]. Also, a complex interplay of hormones including insulin, glucagon, leptin, adiponectin and others regulate metabolic adjustments in conditions of food abundance and deficit to provide needed glucose levels and energy in the organism [48-50].

5. Epigenetics and posttranslational protein modification modulate oxidative stress responses

Epigenetics regulates gene expression modifying DNA methylation and chromatin structure. This regulatory mechanism works differently in each tissue to guarantee the regulation of specific genetic responses to environmental factors (i.e. nutrition, chemicals, stress, etc.), without any changes in the sequence of nucleotides [51, 52]. Epigenetics plays a key role starting from early life, where it is the master director of cell differentiation, X-inactivation and programming of adult health [53]; epigenetic changes can be transferred to the progenies and, sometimes, they can be reverted [54].

DNA methylation consists in the methylation of Cytosine at CpG islands in the promoter region of genes which has been associated with gene silencing, while different responses (activation or inhibition of gene expression) derives from methylation of CpG islands located in the regulatory regions of genes. Histone modifications are changes that are more complex, because functional groups (i.e. acetyl, methyl, P, etc.) deriving from oxidation of nutrients, can be added to histones' amino acidic residues, thus remodeling chromatin. The final result of histone modification is chromatin remodeling at specific genes, leading to increased/decreased gene expression associated with healthy or unhealthy regulatory responses [53].

Oxidative stress related with metabolic responses linked to high glucose intake can enhance DNA methylation interfering with S-adenosyl-L-methionine (SAM), the key methyl donor for DNA methyltransferases (DNMTs) which catalyze CpG methylation [55]. However, oxidation at the level of guanine leading to 8-hydroxydeoxyguanosine (8-OHdG) in CpG islands, can also decrease cytosine methylation and reduce the binding of transcription factors to the promoter region. Oxidation of 5-methylcytosine (5mC) due to Ten-Eleven Translocation (TET) proteins leads to 5-hydroxymethylcytosine (5hmC) formation which is deaminated to 5-hydroxymethyluracil and then replaced with unmethylated cytosine [56]. Oxidative stress can also inhibit the NAD+ dependent deacetylase SIRT1 that controls inflammatory responses, lipid storage, telomerase activity, mitochondrial respiration and ROS production [57, 58]. In this context, a high fat/glucose diet that decreases NAD+ content can negatively regulate Sirtuin activity.

Regulation of responses to oxidative stress is complex and include many mechanisms [59] including oxidative modifications of macromolecules by reactive oxygen species [60], signaling through lipid peroxidation and their products [61] and involvement of different transcription factors (i.e. mentioned above FOXOs and Nrf2)[62, 63]. Considering ubiquitous expression of these transcription

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factors as well as their crucial cellular functions it is very difficult to modulate them by pharmacological interventions [60, 64]. Similarly, lipid peroxidation products play important physiological functions, for example in gastrointestinal tract [65]. Considering significance of regulatory functions of 4-hydroxynonenal and other lipid peroxidation products they also start attracting interest as a target for pharmacological interventions in major stress-associated disorders [66].

6. Glucose-6 phosphate dehydrogenase deficiency

G6PD deficiency is the most common genetic human disease [18]. Since G6PD is a gateway to such an important metabolic pathway as PPP, dramatic consequences to the patients could be expected. However, it is not the case. As it was already mentioned in the introduction section, patients have few or no symptoms with generally positive prognosis and their expected lifespan is not different compared to the general population [18]. There are two basic explanations for this evidence: first, most of the patients have moderate degree of G6PD deficiency and PPP is still functioning at some level and also alternative pathways generate sufficient amounts of NADPH; second, humans in modern lifestyle are exposed to relatively low intensity stressors and there is simply no need for acute responses to stress. In contrast, severe G6PD deficiency indeed has a detrimental effect on the immune system and causes higher susceptibility to infections [67]. In addition, G6PDH deficient athletes and patients with this genetic defect may have severe hemolytic crises after physical exertion [68], however, severity of susceptibility of individual subjects may vary widely [69]. Complete G6PD knockout in mammals is incompatible with life, but in mouse embryonic stem cells it led to severe susceptibility of cells to oxidative stress induced by H2O2 or diamide and reduced cloning efficiency. However, the later was restored when the oxygen concentration was reduced [70]. Therefore, in conditions of substantial oxidative challenge proper function of PPP and generation of NADPH are essential for survival [71]. Conversely, G6PD overexpression may be expected to increase resistance to oxidative stress. Indeed, recent reports indicate that G6PD overexpression extends the lifespan of Drosophila melanogaster [72], which is consistent with some of the results obtained using a G6PD overexpressing mouse model, where it leads to the extension of health-span of mice and increased resistance to oxidative damage [73].

7. PPP involvement in pancreatic regulation of blood glucose levels

A growing body of evidence indicates that release of insulin from pancreatic β-cells depends on a functioning of PPP. It was shown that insulin levels of G6PD deficient patients are lower compared to unaffected controls and patients have significantly reduced insulin response to elevation of blood glucose [74]. More recently, with the use of metabolomics approach it was shown that insulin release is controlled by direct implication of PPP [75]. According to a recent review, among the most important amplifiers/regulators of insulin secretion by β-cells are high levels of NADPH and glutathione [76]. So, insulin release is taking place in conditions of "metabolic welfare" and oxidative stress may reduce the ability of β-cells to release insulin [77]. α-cells also have their intrinsic mechanisms of glucose sensing relying on intracellular redox balance, but they are activated by pro-oxidant situations [78]. Interestingly, under physiological conditions β-cells do not accumulate glycogen but are able to do so under prolonged hyperglycemia and may prolong insulin secretion even after normalization of glucose concentration. In contrast, α-cells do not accumulate glycogen, so when the concentration of blood glucose drops, they can be quickly activated without delay [79], which is extremely important in case of emergencies.

One may argue, that there are many other mechanisms of regulation of insulin and glucagon that can either enhance or inhibit respective secretion, including paracrine δ-cells secreting somatostatin[80, 81], effects of glucagon-like peptide-1 (GLP-1)[82], glucose-dependent insulinotropic peptide (GIP)[83], leptin/adiponectin axis[84], autonomic nervous system[85, 86] etc. However, the effects of

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all these regulators are integrated at the level of α- and β-cells and their metabolism resulting shifts of redox potential [75, 76, 78].

8. Role of PPP in inflammation and insulin resistance

NADPH produced by PPP or by other pathways is also used by NADPH oxidases and nitric oxide synthase to produce superoxide anion. This is important for proper functioning of the immune system and for redox regulation of multiple processes in the tissues including endothelial function [87]. It was shown, that pro-inflammatory interleukin 1β enhances glucose uptake under hyperglycemic conditions in cultured human aortic smooth muscle cells. It also activates PPP and promotes production of superoxide by NADPH oxidase contributing to vascular damage [88] pointing out a particularly dangerous combination of inflammation and hyperglycemia. Since immune cells require glucose for their function, they send regulatory signals, for example TNF-α [89] or microRNAs [90] to the liver to enhance hepatic glucose output and thus chronic inflammatory conditions may cause insulin resistance [91]. The opposite effects are mediated by anti-inflammatory interleukin-10[92] and the spleen plays a particularly important role in these regulatory interactions [93]. The autonomic nervous system may also be involved in regulation of interactions of local inflammatory conditions and oxidative stress [94]. Autonomic output is actively involved in regulation of glucose homeostasis and can adjust the rates of glucose production and utilization independently of hormonal influences [95]. Healing of oxidative stress associated conditions, therefore, may improve autonomic balance [96]. Chronic carbohydrate overload and reduced physical activity cause obesity and metabolic syndrome and for these conditions insulin resistance is very typical [97]. Thus, there exist complex multilevel regulatory interactions to provide sufficient flow of glucose to tissues in order to maintain redox balance. Prompt adjustments of redox homeostasis are critical for the immune defense where the PPP plays a major role. Insulin resistance developing in this case seems to be adaptive and to some extent a protective mechanism [98], but, if dysregulated, it leads to detrimental health consequences.

9. Glucose – "oxidant" or "antioxidant" after all? Sola dosis facit venenum.

There is a certain degree of confusion in the literature concerning the role of glucose in maintenance of redox homeostasis. It was long known, that diabetes and hyperglycemia obviously cause redox dysregulation, oxidative stress and accelerated ageing [99]. On the other hand, glycogen clearly protects against the oxidative stress and at the same time decreases lifespan and healthspan[40, 100]. Starvation and stress induced gluconeogenesis clearly support survival and improve health- and life-spans [100, 101], but are associated with increase generation of reactive oxygen species [35]. Thus, healthy aging requires certain degree of oxidative stress that leads to metabolic shift to catabolism and activate endogenous protective mechanisms that include enhanced protein quality control, stimulation of endogenous antioxidant defense including gluconeogenesis [47, 99, 102].

The other interesting aspect of glucose involvement in redox homeostasis is that high concentrations of glucose in the cells actually may lead to increased production of hydrogen peroxide by mitochondria through inhibition of mitochondria-bound hexokinase [103]. Similarly, chronically high levels of NADPH may contribute to enhanced generation of superoxide and/or hydrogen peroxide by NADPH-oxidases as well as enhances reduction of glucose in polyol pathway (Figure 2) [87].

Diabetes, both type 1 and 2 are well documented diseases associated with oxidative stress. The fundamental feature of diabetes is chronic hyperglycemia due to excessive glucose production and/or impaired its utilization by the tissues. There are several mechanisms contributing to oxidative stress in conditions of hyperglycemia that include but are not limited to non-enzymatic glycation and formation of advanced glycation products, activation of polyol pathway that results in depletion of NADPH (decreased rate of enzymatic reduction of oxidized glutathione and thiol groups of proteins),

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increase in NADH and depletion of NAD+ (increased superoxide anion production in mitochondria), inhibition of histone deacylation and excessive histone acetylation due to accumulation of acetyl-CoA, as well as generation of excessive amounts of sorbitol and fructose [104].

Taken together, the evidence indicates that there exists a delicate balance between protective and damaging redox effects of glucose and chronic dietary carbohydrate overload may affect the regulatory mechanisms that developed during evolution to maintain redox homeostasis (Figure 3). Bell-shaped antioxidant activity of glucose explains well necessity to regulate it strictly within the narrow range, while both high and low levels of glucose/carbohydrates [105] lead to oxidative and metabolic stress that quickly becomes damaging. Dysregulation of glucose metabolism that takes place in diabetes closes vicious cycle further exacerbating redox dysregulation that was actually meant to be fixed by induction of hyperglycemia].

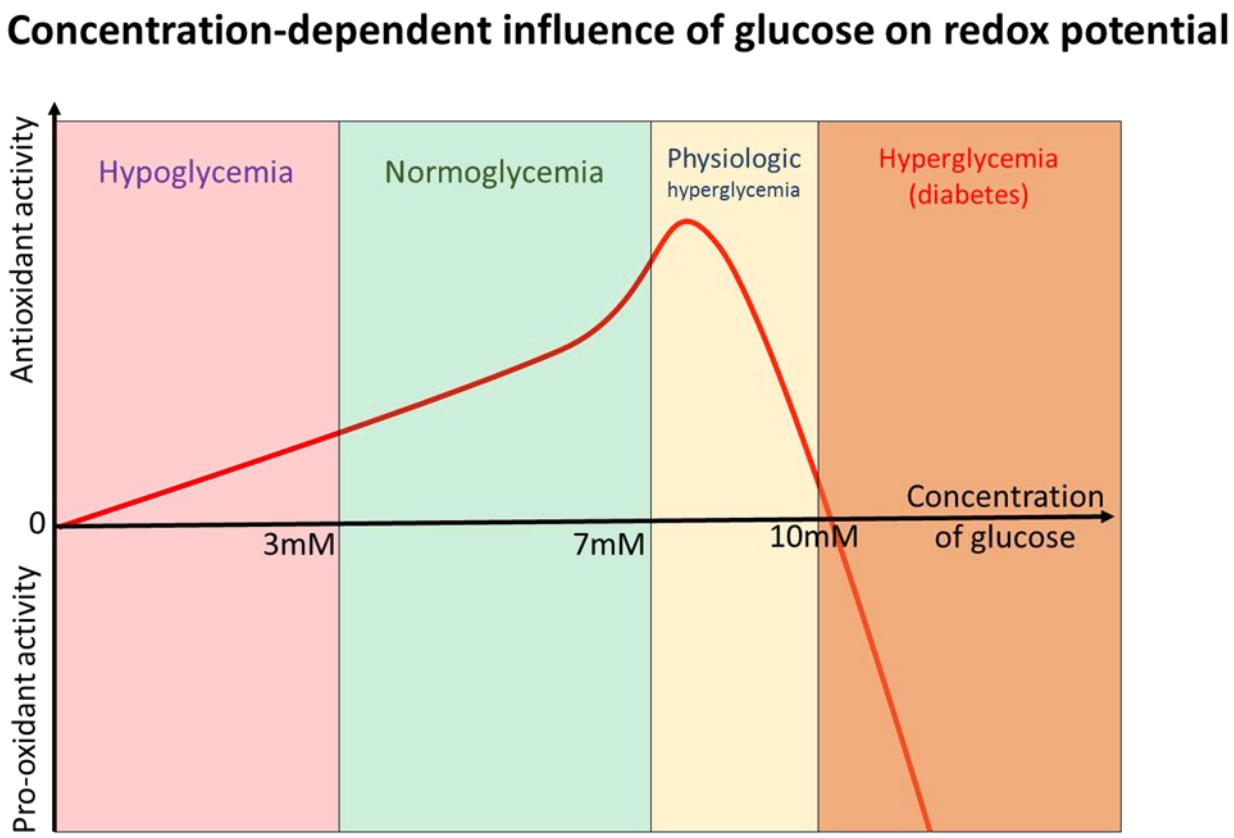

Figure 3. Dependence of redox effects of glucose on its concentration (hypothetic relationship). Hypothetical simplified model describing influence of blood concentration of glucose on redox potential in human organism. Multiple additional factors influencing redox potential such as concentration of oxygen, availability of amino and fatty acids, type of cells and effects of either hormones (insulin, glucagon etc.) or cytokines are not considered. Concentrations of glucose as well as the shape of the curve are roughly estimated and not confirmed by actual experiments and may significantly vary depending on conditions and tissue type.

10. Important implications

Glucose is a central metabolite and depending on its availability and metabolic need can play different roles in the organism. Glucose flows within the human body are strictly regulate and promptly adjustable in order to maintain metabolic flexibility and provides robust resistance to different types of stressing factors (Figure 4). The role of glucose is not limited to merely generate enough ATP (which is the case in conditions of glucose abundance and low stress), but more

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importantly, it is responsible for emergency mechanisms of maintenance of redox potential that is essential for the survival in extreme situations (fight or flight reactions, infections, poisoning etc.).

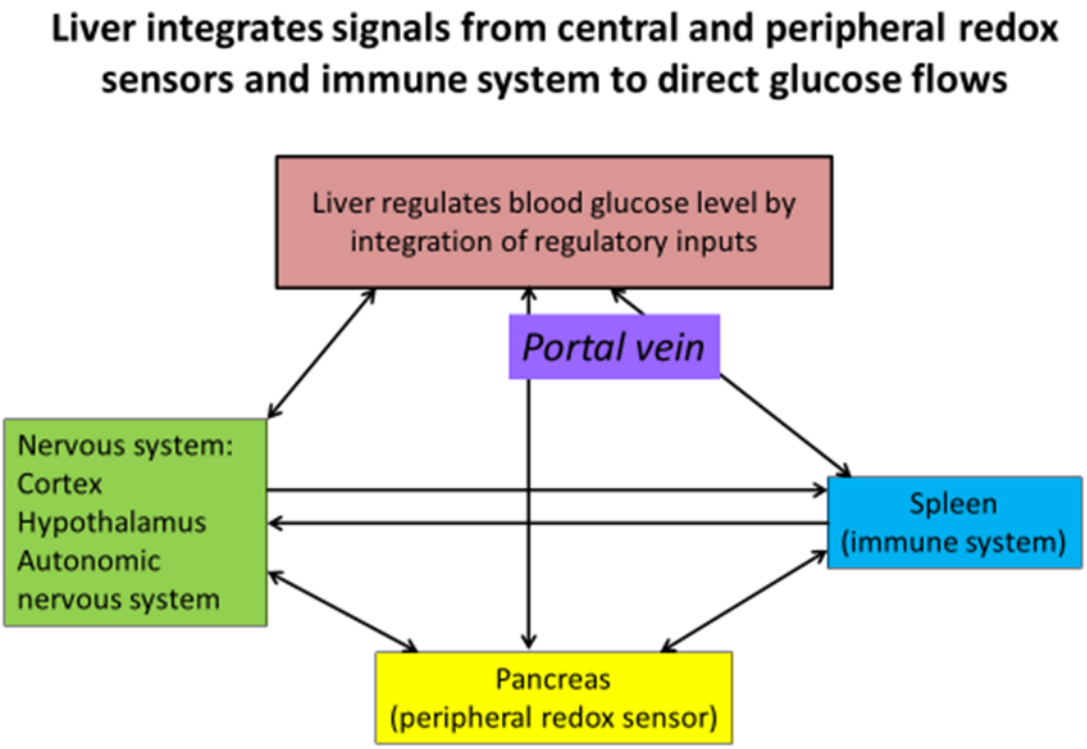

Figure 4. Role of redox sensors (pancreatic α and β cells), immune system and central nervous system in regulation of blood glucose concentration by liver. Glucose release or absorption by liver integrates signals from nervous and immune systems, and peripheral redox sensors. System is highly flexible and tunable providing redox modulation that is dependent on actual needs. Failure of feedback loops and distorted signaling either from CNS (stress), immune system (inflammation) or malfunction of peripheral sensors lead to excessive uncontrolled (poorly controlled) glucose release and or activation of gluconeogenesis leading to diabetes.

It is possible that utilization of glucose by glycolysis from the evolutionary point of view was rather a smart way of redirecting excess glucose into energy production and biosynthesis (growth, proliferation, hypertrophy etc.), when the primary life-supporting and life preserving needs ensured by glucose have already been met. Proposed principle is helpful for understanding of the regulation of glucose metabolism aimed primarily to maintain redox balance, especially in acute extreme conditions requiring prompt and massive antioxidant responses. Oxidative stress, infections/inflammation, starvation, exercise, aging and many other pathological conditions or processes decrease GSH/GSSG and NADPH/NADP+ ratios. Sensors sense these changes and drive glucose flows to compensate for these metabolic disturbances at the organismic level. In this regard insulin resistance develops as a protective "antioxidant" adaptive mechanism that stimulates glucose production and prevents its waste in order to cope with increased needs. When dysregulated and chronically over-activated, though, this leads to detrimental consequences caused by hyperglycemia [98].

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The other important issue, which is often not taken into consideration by researchers, is that the presence of glucose stores in the form of glycogen or extracellular glucose availability provide enhanced resistance to oxidative stress. It is possible also, that glucose by strengthening of the reductive power of NADPH-dependent antioxidant enzymes prevents activation of redox sensitive regulatory factors such as Nrf2. That means that glucose nutritional overload affects physiological redox signaling, and chronic over-activation of insulin signaling causes metabolic diseases, as described in details [23]. On the other hand, severe glucose overload itself can serve as a source of oxidative stress in the cells by participation of glucose in polyol pathway, over-activation of NADPH oxidases and increased production of ROS by mitochondria. Exercise, caloric restriction, intermittent fasting, a ketogenic diet and some drugs have in common that they deplete organism's glycogen stores [39], reduce glucose availability for the cells, restore physiological redox signaling suppressed by chronic excessive glucose consumption and leads to dramatic improvement of life- and health-span in model organisms and humans (Figure 2). Metabolic changes caused by carbohydrate overload in the general population often take place far before clinically significant changes occur [38]. That is why relevant and sensitive instruments for early detection of these metabolic shifts are needed.

11. Limitations of the analysis and directions of further research

In this review we presented mainly general principles of glucose metabolism in the cells and its importance for maintenance of redox balance. However, different cells in different tissues have their metabolic pattern, specific functions, and own physiological peculiarities. Glucose metabolism may differ substantially depending on the specifics of tissues and cells, proliferation activity, redox balance required to maintain the functions, expression and activities of involved enzymes. Nevertheless, convincing literature evidence indicate that glucose flows in the organism are primarily targeted to maintain redox homeostasis and counteract possible oxidative challenges.

It may be argued that the actual flow through PPP is relatively low in the brain (as it has been shown by Gaitonde M. et al. [106]) and it increases substantially to approximately 20% of total glucose utilization by neurons only in case of severe oxidative stress induced by hydrogen peroxide [107] or during experimental brain injury [108]. However, a closer look into the methods used in the studies reveals that non-physiologically high concentrations of glucose were used, namely 22.3 mM in the medium and 50mM for perfusion in [107] and 23.9-26.9 mM plasma glucose after infusion in [108]). Flow of glucose into PPP is inhibited in conditions of high NADPH/NADP+ ratio, therefore as soon as the levels of NADPH are restored glucose is redirected into glycolysis or glycogen storage.

Our reconsiderations may provide better understanding of physiology of glucose regulation in health and diseases and shift of general paradigm of glucose induced oxidative stress, which is, however true for hyperglycemia and diabetes, towards to understanding of redox effects of glucose in concentration-dependent manner. In other words, glucose maintenance at physiological levels is fundamental mechanism of counteracting excessive oxidation due to its involvement in the PPP and the NADPH production. Endogenous antioxidant systems using glucose provide sufficient antioxidant defense in physiological conditions. Moreover, redox modulating agents that have some health benefits when supplemented often turn to be rather prooxidant than antioxidant and lead to stimulation of endogenous antioxidant defense and improvement of glucose metabolism. Besides, therapeutic approaches to apply antioxidant substances in order to reduce oxidative damage and any administration of compounds with pure purpose to provide reducing equivalents appears weak [109] in contrast to often high in vitro activities [110] in comparison to existing endogenous antioxidant mechanisms, based on glucose as a source of reducing power for generation of NADPH and recycling oxidized glutathione and thiols and can in some cases be deleterious since they may interfere with redox sensors, for example protein thiol groups and cause dysregulations.

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The other important issue is the lack of convenient, informative, sensitive and specific ways of glucose and/or glycogen determination in tissues for research and clinical use. As we suggested earlier, glycogen determination, preferably in a simple, affordable and noninvasive way could be potentially a good biomarker for redox biomedicine [39]. Unfortunately, there are too many technical issues preventing development of such equipment for clinical use so far, but some efforts have been made to enable label-free glycogen estimation in C. elegans, an important animal model for metabolic and aging research [111].

12. Conclusions

Our critical analysis of current literature unveiled significant controversies and insufficient general understanding of metabolic role of glucose, particularly regarding redox homeostasis. An importance of glucose metabolism in the PPP for maintenance of redox homeostasis is very often underestimated. In the light of recent epidemiological evidence and advancements in the field of redox biology it is hypothesized, that a specific physiological function of glucose is its metabolism in the PPP to provide stress resistance to unfavorable factors by reduction of NADP+ to NADPH in order to maintain redox homeostasis and functioning of immune cells. Meanwhile the glycolysis takes place in favorable redox conditions when glucose is in abundance. This approach and interpretation in a simple way explains adverse metabolic effects and detrimental health consequences of nutritional carbohydrate overload and provides new details in explaining the positive metabolic effects of intermittent fasting, caloric restriction, exercise, and ketogenic diet. Better understanding the evolutionary adaptations and biological role of glucose may serve as an important theoretical background for future experimental and clinical studies related to glucose metabolism, aging, diabetes as well as other adjacent fields.

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