Opinion

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# The consequences of enhanced cell-autonomous glucose metabolism

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**The intake and metabolism of carbohydrates for the generation of energy and biomass is evolutionarily conserved, down to the most primitive of cells. Although a basal rate of glucose metabolism occurs in all cells, the processing rates of glucose can become dramatically enhanced when cells acquire malignant properties, or remain undifferentiated. This article investigates the consequences of how increased glucose metabolism affects cellular physiology by altering the physicochemical properties of the whole metabolic network. As a result, enhanced lactate production in the presence of oxygen (the Warburg effect) is required, and metabolism is consequently reconfigured, through multiple mechanisms, to confer numerous physiological and possibly regulatory properties to cells.**

## Enhanced glucose metabolism

Coordinated by insulin action and growth factor stimulation, glucose metabolism is subject to fundamental systemic regulation in multicellular organisms, and its hyperactivation is a common feature of growing cells. In addition, the components of the insulin and insulin-like growth factor signal transduction pathways are aberrantly regulated in pathophysiologic cell growth [1–3]. Thus, growth signaling together with adaptation to altered metabolic environments leads to enhanced glucose processing. As a prominent example, hyperactivation of the insulin/PI3K/mTOR signaling pathway is found in approximately three-quarters of all human cancers [4]. Indeed, nearly all *bona fide* tumor-suppressor genes and oncogenes associated with cancer now have established metabolic roles [5]. These include commonly studied cancer-associated genes such as *KRAS*, *MYC*, and *TP53*.

A major consequence of altered metabolism induced by growth signaling is the increased expression of carbohydrate transporters, upregulation of gene expression, and enhancement of enzyme activities involved in glycolytic metabolism [6]. These effects include increased expression of glycolytic enzymes, post-translational modifications (PTMs) that increase their activity, and the selective expression of isoforms such as LDH-A, that confer additional regulatory properties to this metabolism [7,8]. As a result, cellular biochemistry adapts to allow for large increases in glucose uptake and rates of glucose metabolism. In this article I discuss several consequences of dramatically enhanced glucose uptake and glycolytic metabolism, and propose that cellular physiology through multiple mechanisms is fundamentally altered when cells, such as cancer cells or embryonic stem cells, absorb abnormally large amounts of glucose.

## Consequences of enhanced glucose uptake

Known for over 80 years, the Warburg effect (see Glossary) has recently been the subject of intense interest in cancer biology [6,9]. This renewed interest has arisen in part from the astonishing generality of the phenomenon, whereas the cancer-causing mutations are highly context-specific. Although no two tumors contain an identical genetic makeup, most share this common altered metabolism. This common feature could lend itself to creating more effective pharmacological interventions, thus reaching a broader number of patients. In its definition, the preferential conversion of glucose to lactate that occurs even in the presence of oxygen has been emphasized [10]. In addition to the fermentative metabolism of glucose observed in respiring tumor tissue, an additional observation reported by Warburg and his colleagues was that the quantity of glucose being consumed by the tumor tissue was markedly increased. To compute systemic glucose consumption, Warburg and colleagues measured the difference in the amount of glucose entering capillaries and exiting veins within different regions of the circulatory system [11]. When comparing these numbers to the values obtained from

## Glossary

**Gibbs free energy**: a form of energy associated with work required for maintaining a configuration of reactive chemicals. It is also referred to as a chemical potential. A value of zero results in a system in chemical equilibrium. Positive values result in a net flux in the forward direction. Negative values result in a net flux in the backward direction.

**Michaelis constant ($K_m$)**: a term in the denominator of the Michaelis–Menten equation that describes the rate of enzymatic kinetics. The value of $K_m$, measured in units of chemical concentration, is a measure of relative enzyme saturation in comparison to the substrate concentration of the enzyme. Values of $K_m$ for physiologically relevant enzyme chemistry typically are in the micromolar range.

**Post-translation modifications (PTMs)**: reversible chemical modifications carried out enzymatically on proteins, in which the relative levels on any given protein may be susceptible to metabolic flux.

**Warburg effect**: a phenomenon relating to glucose metabolism in tumors. Tumors metabolize glucose through fermentation, resulting in lactate product that occurs even in the presence of oxygen (aerobic glycolysis). In addition, tumors carrying out aerobic glycolysis also take up and process large amounts of glucose. This effect occurs even in the presence of fully functioning mitochondria.

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the corresponding measurement in multiple tumors, it was found that the amount of glucose being consumed by tumors was larger than the amount consumed by entire regions of the circulatory system [11]. Thus, the amount of processed glucose per cell, per unit time, could be estimated to be many orders of magnitude larger than the amount processed in peripheral tissue.

Such a dramatically enhanced rate of glucose processing affects cellular physiology. The most notable effect is that the physicochemical constraints imposed by enhanced rages of glucose metabolism require fermentation (aerobic glycolysis) [12]. This fermentative metabolism occurs even in the presence of oxygen and fully functioning mitochondria. Under these conditions, intracellular metabolite concentrations could vary by orders of magnitude. In addition, metabolic fluxes are substantially altered because chemical reaction rates increase monotonically as a function of metabolite concentration. Examples how these changes affect cellular physiology are discussed below.

## Balance of redox potential

### *NADPH/NADP$^+$ redox balance*

The NADPH/NADP$^+$ redox potential favors the reductive direction in cells [13] (i.e., the ratio of NADPH to NADP$^+$ is much greater than one). This form of cellular energetics confers several advantages. As a result, anabolic metabolism involving reductive biosynthesis is energetically favored. For example, *de novo* lipid biosynthesis leading to the synthesis of membrane components can require as many as fourteen molecules of NADPH for every molecule of lipid synthesized [14]. Other biosynthetic processes such as nucleotide metabolism also require NADPH. In addition, NADPH regenerates the reduced form of glutathione that can then be utilized to scavenge reactive oxygen species (ROS), and preserve the ratio of oxidized to reduced cysteine in cells [15,16].

This redox potential involving the NADPH/NADP$^+$ ratio depends on the relative rates of NADPH generation and consumption. The most commonly recognized pathway leading to NADPH production, involves the oxidative branch of the pentose phosphate pathway [17]. This branch point, stemming from glycolysis, involves the oxidation of glucose-6-phosphate and, through a series of steps, generates ribose-5-phosphate for synthesis of nucleotide precursors. Upon carbon flowing through this pathway, there is a yield of NADPH for every molecule of glucose that passes through these oxidative chemical reactions. Additional metabolic reactions also generate NADPH – such as that from malic enzyme that oxidizes malate to form pyruvate, and the oxidative decarboxylation of isocitrate by isocitrate dehydrogenase.

When the rates of glucose uptake and processing increase, flux through the oxidative branch of the pentose phosphate pathway could reach saturation [18,19]. Thus, the amount of NADPH generated from the oxidative branch of the pentose phosphate pathway exceeds the amount required for biosynthesis and regeneration of glutathione. It is therefore speculatively plausible that, under these conditions, the synthesis of NADPH is not limiting for biosynthesis or glutathione maintenance for ROS detoxification. As a result, the flux of NADPH generated through the oxidative pentose phosphate pathway would be maintained at a constant rate [18,19]. For example, a testable hypothesis is that this phenomenon is evident in a recent study in which the metabolism of KRAS-driven inducible pancreatic ductal adenocarcinoma cells was examined in the presence or absence of a mutant *KRAS* transgene [20]. In this study, as has been shown previously [21–23], glucose uptake markedly diminishes in the absence of *KRAS* oncogene expression. However, under these circumstances, it was observed that there were no changes in the flux through the oxidative pentose phosphate pathway. Consequently, changes in carry-over flux from the non-oxidative branch of the pentose phosphate were observed, as previously reported in transformed cells [24–26].

Such findings raise many interesting questions. It has been proposed that cells require an optimal amount of ROS for growth and survival [27]. In this model, excess ROS leads to genotoxic stress including irreversible DNA damage [28]. However, some basal level of ROS is required to carry out signal transduction [29]. Cell signaling through ROS is extensive and often occurs through the oxidation of cysteine residues that are positioned near the catalytic site of enzymes [30,31]. Oxidation of cysteine affects the rate of enzyme catalysis. Examples of ROS-mediated signaling involve the oxidation of PTEN, a phosphatase that negatively regulates the PI3K/AKT/mTOR pathway [32,33]. This inactivation leads to increased pathway activity that confers survival and metabolic functions to cells [34]. Another example involves the glycolytic enzyme pyruvate kinase M2 (PKM2). PKM2 catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate, as the final step in glycolysis [35]. It has recently been shown that cysteine residues in the catalytic site of PKM2 are subject to oxidation that renders the enzyme inactive [16,36]. This oxidation event functions to reroute glycolytic flux [16,36]. Transcription factors are also under the direct biochemical regulation through ROS [37]. Thus, insufficient ROS could plausibly lead to insufficient basal, housekeeping signal transduction, and metabolic regulation within glycolysis.

As a result of this requirement for ROS homeostasis, cells may require an optimal NADPH/NADP$^+$ ratio. In the context of high glucose-processing rates, the requirement for an optimal redox potential involving the NADPH/NADP$^+$ ratio would put multiple constraints on how fluxes surrounding glycolysis can be configured. Such constraints could create dependencies on pathways such as the non-oxidative pentose phosphate pathway. In the case of tumors that exhibit enhanced rates of glucose processing, targeting such dependencies is currently a subject of intense study [38,39].

### *NADH/NAD$^+$ redox balance*

The other redox potential essential for maintaining cellular physiology is the ratio of NADH/NAD$^+$ [12]. It is thought that this ratio is much less than one, thus favoring NAD$^+$-mediated redox reactions occurring in the oxidative direction. Glycolysis, the metabolism of glucose to pyruvate, however, utilizes NAD$^+$ and is overall an oxidative process. However, this ratio shifts toward the reductive direction (ratio of NADH/NAD$^+$) upon cell transformation

or activation of growth factor-mediated signaling pathways [40]. Thus, in addition to the ATP-dependence of glycolytic flux [13,41], sufficient substrates upstream of the $NAD^+$-mediated oxidation step are also required to drive the overall glycolytic reaction forward.

For each molecule of glucose that enters and is captured in cells, its metabolism through glycolysis eventually reaches an oxidative step at the sixth reaction within glycolysis. In turn, $NAD^+$ is oxidized to form NADH. When cells process glucose at high rates, the glycolytic flux involving glucose oxidation generates two stoichiometric equivalents of NADH. If the rate of glycolysis is sufficiently low, this redox imbalance is counteracted by the transport of substrates, such as malate and aspartate, through redox-coupled mitochondrial shuttles. This process results in net-zero changes in biomaterial, but in a net reduction of one molecule of NADH to $NAD^+$ for each molecule transported through the mitochondria [12]. When the rate of glycolysis is larger than the external rate of $NAD^+$ regeneration, fermentation of pyruvate through lactate dehydrogenase to regenerate $NAD^+$ is necessary.

It is tempting to speculate that this shift in NAD-mediated redox potential will affect the activity of NAD-dependent enzymes such as those in the Sirtuin family and PARP-dependent ADP ribosyltransferases. Sirtuins have multiple physiological roles, and the shift of the NAD-mediated redox potential in favor of a more reductive environment probably exerts a multitude of effects, by influencing the substrates of the Sirtuins such as the deacetylation of p53 and histones [42].

**Box 1. Enhanced glucose uptake, changes in nonequilibrium thermodynamics, and differential pathway regulation**

Free energy or chemical potential (ΔG) changes upon moving through glycolysis (Figure I). At intermediate and lower regions of the pathway, glycolysis is thought to be in equilibrium, implying that ΔG is zero. However, upon high rates of glycolysis, changes in the relative concentrations of glycolytic intermediates drive the system out of equilibrium. As a result, free-energy gradients that provide a thermodynamic driving force can be established to drive metabolic flux in the forward or backward direction. These chemical potential differences could allow for specific anabolic metabolism – such as the diversion of glycolytic intermediates through 3-phosphoglycerate into *de novo* serine and glycine metabolism. The red shaded region indicates possible ranges of potential energy differences at each point in lower and intermediate glycolysis. A positive value drives flux in the backward direction. A negative value would drive flux in the forward direction. The blue arrows denote possible upper and lower bounds on the free energy at each point in glycolysis. As a result, metabolic flux can be directed specifically to different metabolic pathways. If the value of ΔG is sufficiently large at any point, that point is thought to be rate-limiting. Abbreviations: G-6-P, glucose-6-Phosphate; F,1,6BP fructose-1,6-bisphosphate; G-3-P, glyceraldehyde-3-phosphate, DHAP, dihydroxyacetone phosphate; Gly-1,3-DP, 1,3-diphosphoglycerate; 3-P-Gly, 3-phosphoglycerate, 2-P-Gly, 2-phosphoglycerate; PEP, phosphoenolpyruvate

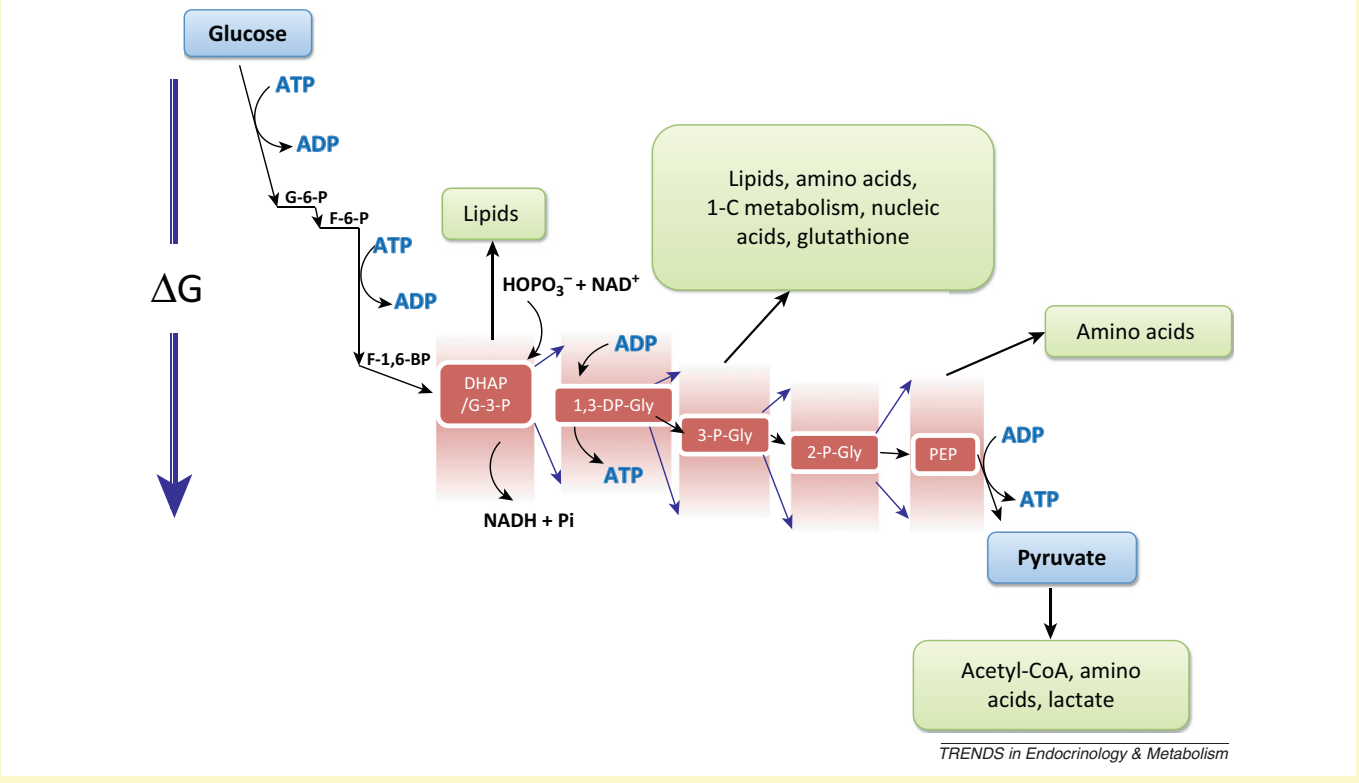


**Figure I**. Free-energy changes upon glucose uptake and processing. Glucose is captured by cells and metabolized to pyruvate through a series of intermediate steps referred to as glycolysis. Reactive intermediates in glycolysis can be metabolized alternatively to generate various components contributing to cellular biomass, including lipids, amino acids, 1-carbon units for 1-carbon metabolism, nucleic acids, and glutathione. Some reactions are coupled to cofactors such as ATP and $NAD^+$. The relative height of each metabolite in the figure denotes its free energy (ΔG). Decreasing heights imply that the reaction is thermodynamically favored, with a net flux in the corresponding direction. It is hypothesized that the region within intermediary glycolysis, historically thought to be in equilibrium (i.e., no change in ΔG upon carrying out a forward or backward reaction), would exhibit a variation within the shaded region (blue arrows) when the rate of glycolysis increases. As a result, free-energy gradients are predicted to exist in regions of glycolysis that would otherwise be in equilibrium. These gradients would then serve to drive metabolic flux into different pathways. This phenomenon results from higher concentrations of intermediate metabolites that would result from enhanced rates of glycolysis.

### Nonequilibrium thermodynamics and flux changes in glycolysis – diversion of glycolytic intermediates

The thermodynamic driving force that occurs in response to changes in metabolic flux is the gradient of metabolite concentrations upon moving from one point to another in the metabolic network. When the concentration of substrate is large relative to the concentration of the product reaction, this situation sets a chemical potential or free-energy difference that induces a flux in the direction of the product reaction [43]. This principle of nonequilibrium thermodynamics can be applied to understanding what happens to glycolysis, when the rate of glucose uptake changes (Box 1).

One consequence of high rates of glucose uptake is that the concentration of intercellular metabolites increases monotonically with increasing rates of glycolysis. As a result, larger concentration gradients can be established from one point to another along any metabolic pathway. Historically, the rate-limiting steps in glycolysis have been associated with points along the pathway in which large changes in free energy occur [13]. These steps involve the enzymes hexokinase, phosphofructokinase, and pyruvate kinase. In each case, there is an ATP-coupled reaction that serves to thermodynamically drive the chemical reaction. These enzymes are thought to be the points of regulation in glycolysis. However, when differences in the relative concentrations of metabolites exist, when going from one point to the next in the pathway, these concentration gradients serve equally well in providing a thermodynamic driving force [44–46].

Therefore, under conditions of high glucose uptake, one function of enhanced glycolysis might be to provide additional rate-limiting steps along the glycolytic pathway. For example, intermediary glycolysis is thought to be in chemical equilibrium with respect to the Gibbs free energy ($\Delta G \sim 0$). However, it is possible that concentration gradients would drive the pathway out of equilibrium and create net fluxes [43]. Enhanced glycolysis would accentuate this effect by increasing the overall concentration of intermediates in the pathway.

A consequence of these concentration gradients in glycolysis is that they can provide a driving force for the diversion of glycolytic flux into alternative pathways. If there is an energy barrier to proceeding via glycolysis, then a competing alternative pathway for a given glycolytic intermediate would have greater activity by virtue of substrate competition. For example, an anabolic pathway originating from 3-phosphoglycerate (3PG), the seventh intermediate along the glycolytic pathway, can be initiated through the oxidation of 3PG to form 3-phosphohydroxypyruvate [47,48]. This reaction occurs enzymatically via the catalytic activity of phosphoglycerate dehydrogenase that utilizes the cofactor $NAD^+$ as an oxidizing agent. Genetic evidence has been presented that the gene encoding phosphoglycerate dehydrogenase, *PHGDH*, is the focus of recurrent amplification in solid tumors [49,50]. As a result of increased copy number, there was increased expression of the enzyme, leading to enhanced flux through the pathway. In addition, it has been shown that increased pathway activity is sufficient in some instances to promote features of tumorigenesis [50].

In other contexts, this flux diversion might occur at other branch-points along glycolysis, such as those emanating from dihydroxyacetone phosphate (DHAP), PEP, and pyruvate [51,52]. Thus, by changing the nonequilibrium thermodynamics of intermediate glycolysis, enhanced rates of cell-autonomous glucose metabolism could lead to the specific diversion of glycolytic flux into multiple pathways. These pathways could provide contextual building blocks for biosynthesis and control the redox potential in cells mediated both by NADPH and NADH. In addition, these increased fluxes could also initiate signal transduction mechanisms through metabolic intermediates that serve as the substrate of protein PTMs.

### Enhanced glucose metabolism and its signaling crosstalk to other biological processes

Signal transduction in eukaryotes occurs predominantly through the propagation of secondary messenger molecules, or the reversible PTM of proteins. Protein phosphorylation has historically been the most common PTM studied in the context of cell signaling. Elaborate protein kinase–substrate networks have been characterized and these networks are known to regulate diverse aspects of cellular physiology [53]. However, recent advances in mass spectrometry have unearthed widespread usage of PTMs in addition to phosphorylation [54]. Examples of these modifications include acetylation, methylation, succinylation, GLCNACylation, malonylation, crotonylation, among many others. Relative to protein phosphorylation, their functions are less well known. Often occurring at lysine residues, these modifications involve multiple substrates that are intermediates in numerous metabolic pathways [12].

These modifications are thought to be reversible, and are thus dynamically regulated [55–57]. The enzymatic properties of the proteins that carry out these reactions, although poorly understood, are nevertheless constrained to follow general properties of enzyme kinetics. The values of their Michaelis constants ($K_m$) are in the micromolar range, similar to those observed for metabolic enzymes. However, the concentrations of key metabolic sensors, such as acetyl-CoA and *S*-adenosyl-methionine, can vary on orders of magnitudes depending on the cellular environment [58]. As such, the forward rate of catalysis by the corresponding enzymes can vary substantially [59]. Such variation in the rate of enzymatic protein biochemistry alters the levels of PTMs of their protein targets. Therefore, alterations in the metabolic fluxes that then affect the concentrations of these metabolites would have enormous consequences on the downstream cellular communication that ensues (Box 2). Several instances of these altered rates affecting cellular physiology have been identified [28,59].

For example, the epigenetic state of a cell is determined in large part by the collection of PTMs that are assembled on histones that comprise chromatin [56,57]. It has been proposed that these PTMs that mediate epigenetic status are under the control of metabolism [60,61]. Commonly studied modifications on histones include methylation, acetylation, and phosphorylation [56]. Other less-studied modifications probably have a substantial regulatory role

**Box 2. Metabolic signal transduction and glucose metabolism as its effector**

Signal transduction by PTM involves an enzyme, protein target, and metabolic substrate (Figure I). Metabolic substrates involve metabolites such as acetyl-CoA, *S*-adenosyl-methionine, and UDP-GLCNAC. Depending on the surrounding metabolic flux, metabolite concentrations can vary on orders of magnitude (ΔS shown in red). As a result, the reaction rate that determines the relative levels of PTMs on proteins can vary substantially. These intercellular metabolite concentrations are thought to be dependent on the amount of glucose taken up by cells. Therefore, the concentration can vary to values well below or above the $K_m$ of the enzyme. As a result, the rate at which PTMs occur can vary dramatically. Changing the rates at which these metabolic substrates are transferred onto their protein targets can have dramatic consequences on cellular physiology.

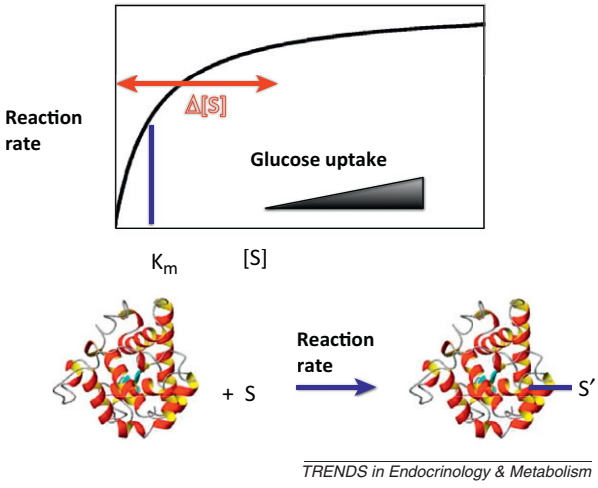


**Figure I**. Metabolic signal transduction. How enhanced rates of glycolysis might affect signal transduction. An enzymatic chemical reaction that involves the post-translational modification (PTM) of a protein through a substrate, S (that becomes covalently modified to the protein as S′), occurs at a particular reaction rate (bottom). As an example, if the enzyme follows Michaelis–Menten kinetics (top), the value of $K_m$ (blue line) is determined by the substrate concentration ([S]) at which the reaction rates reaches half of its maximum value. One hypothesis is that the substrate concentration of a metabolite that contributes to an enzymatic PTM is a function of the rate of glycolysis (or other forms of altered metabolism associated with malignancy). If the substrate concentration is near the value of the $K_m$, and increases with increasing rates of glycolysis, then glycolysis would be predicted to have dramatic effects on the signal transduction that involves these forms of PTM.

[28,62,63]. Together, the combined statuses of these 'marks' at defined residues determine much of the expression of the genome. Given that these marks occur through enzymatic modifications, the relative levels of these marks could be under metabolic control through alterations in glucose metabolism, and the consequent changes in intercellular concentrations of metabolites.

## Concluding remarks

Despite these intriguing connections between glucose metabolism and metabolic rewiring, many questions remain (Box 3). The extent to which enhanced glucose metabolism leads to changes in redox potential, metabolic rewiring of carbon metabolism, and differential kinetics of PTM, is poorly understood. Additional work utilizing quantitative approaches such as NMR, mass spectrometry, and computational modeling will be required to elaborate these mechanisms. For example, combining these techniques with isotope labeling can quantify the absolute concentrations and rates of metabolic fluxes in defined genetic backgrounds, in environmental conditions of altered nutrient availability, and under conditions of pharmacological perturbation. Protein-based mass spectrometry that can quantitate PTMs under these same conditions would provide additional understanding of the metabolic regulation of these signal transduction events [55]. Such data could provide the basis for quantitative models for the interaction of glucose metabolism, its subsequent alteration in downstream fluxes, and the signaling events that it regulates.

Ultimately, it is hoped that translating this knowledge of altered glucose metabolism can inform new therapeutic opportunities in cancer and other diseases in which malignant cells consume excess glucose [64]. This translation has many difficulties resulting from the requirements of glucose for metabolism in all healthy cells. The resulting toxicities that arise from targeting glucose metabolism have been difficult to control [65]. By obtaining a more fundamental understanding of how glycolysis is regulated at the biochemical and biophysical level, one would hope to obtain some predictive capabilities that define the efficacy and toxicity of cancer therapies. Modeling efforts have been successfully applied to simpler organisms [66], and hold promise for more complex organisms [67,68]. Such information is particularly relevant in guiding clinical studies that involve the development of agents that target specific points in glycolysis and its peripheral pathways.

**Box 3. Outstanding questions**

- Does the Warburg effect alter the redox potential (both NAPH/$NADP^+$ and NADH/$NAD^+$) in cells?
- Can these redox potentials be measured accurately in physiological settings?
- Does increased glucose uptake affect intracellular metabolite concentrations quantitatively?
- Does enhanced glucose metabolism affect the enzymatic rates of PTMs and, if so, what affect does this have on cellular physiology?
- Do other alterations in nutrient availability affect the quantitative rates of signaling reactions in cells?
- If so, do these changes have affects on cellular physiology – such as epigenetic status? – cell growth control?
- Can quantitative metabolomics and mathematical modeling provide insights into the physiology affected by alterations in cell-autonomous glucose metabolism

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