Two transition states of the glycogen shunt and two steady states of gene expression support metabolic flexibility and the Warburg effect in cancer

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Abstract

Previously we suggested that the early Warburg effect can be explained by the use by cancer cells the glycogen shunt during a rapid increase in glucose concentration. In analogy to the Crabtree effect in yeast, the shunt plays a critical role in maintaining homeostasis of glycolytic intermediate levels during these transitions. We extend this analysis here, and propose that the recently appreciated flexibility of cancer cell glucose and glycogen metabolism involves 4 metabolic states that we recently identified in metabolic control analysis studies of yeast. Under stable conditions of low glucose and normal O_2 yeast, and by analogy cancer, cells are in the Respiration State in which through gene expression for oxidizing non glucose substrates. When their environment changes to high glucose with reduced O_2 levels, such as occur in tumors, they transition to the Glycolysis State due to gene expression of new glycolytic enzyme isoforms such as PKM2. These isoforms optimize metabolism to sustain the Warburg effect. When the changes in glucose and O_2 levels are rapid there may be insufficient time for gene expression to adapt. The metabolic flexibility conferred by 2 states of the glycogen shunt allow the cells to survive these transitions. The model explains experimental observations in cancer such as the function of the glycogen shunt and the frequent expression of PKM2 in cells undergoing the Warburg Effect. A surprising conclusion is that the function of PKM2 is to maintain glycolytic intermediate homeostasis rather than controlling the glycolytic flux. The glycogen shunt may also have an important role in cancer metabolic reprogramming by allowing cancer cells to survive large glucose and oxygen changes during the selection of mutations that lead to the Warburg phenotype

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Abbreviations and glossary

ATP: adenosine tri phosphate, the main molecule providing energy to drive enzymatic reactions in the cell.

Crabtree Effect: When aerobic yeast grown on acetate are fed glucose the majority is converted to ethanol and not oxidized, a process called fermentation. This process is analogous to the increase in non oxidative glycolysis during the Warburg effect in cancer.

Glycolytic intermediates: phosphorylated metabolites such as glucose-6-phosphate (G6P) that are produced by glycolysis.

Glycogen: A glucose polysaccharide that is the major storage compound for glucose in eukaryotes.

Glycogen shunt: Cyclic pathway in which G6P derived from glucose phosphorylation is first converted to glycogen and then back to G6P as opposed to directly being used for glycolysis. It differs from previous metabolic descriptions in that the rates of glycogen synthesis and breakdown are coordinated with glucose phosphorylation, glycolysis and gluconeogenesis to maintain homeostasis of glycolytic intermediates and high energy phosphates.

Metabolic flexibility - The ability of metabolism to adapt to changes in substrate and oxygen levels in the environment.

Metabolic plasticity - The range of metabolic phenotypes present in a cell or organism population.

Pyruvate Kinase: an enzyme in the glycolytic pathway that is often found in the PKM2 isoform in cancer.

PKM1, PKM2: Isoforms of pyruvate kinase expressed in mammals. PKM2 is often found in cancer cells.

PyK2, PyK1: The equivalent to PKM1 and PKM2 in yeast.

Warburg effect: A metabolic state often found in cancer in which there is an excess of glucose consumption over glucose that is oxidized. Named the Warburg effect by Racker in the 1970s, it is analogous to the Crabtree effect in yeast. It is often referred to as aerobic glycolysis or non oxidative glycolysis.

Introduction

Metabolic research on cancer usually starts with Warburg's result in the 1920's [1], described as the Warburg effect, that aerobic tumors, suddenly offered glucose, did not follow the efficient energetic pathways of oxidative phosphorylation (approximately 32 ATP molecules per glucose) but rather stopped at lactate production which only produced 2 ATPs per glucose. This led him and others [2, 3] to propose that cancer cells had impaired mitochondrial function and needed glycolytic ATP to supply energy. However, the traditional paradigm has been challenged by studies over the last 2 decades that have found that cancer cells can exhibit a Warburg Effect even when they have adequate mitochondrial capacity to meet their energetic needs [3–6]. These studies and others have led to suggestions that the enhanced glucose utilization associated with the Warburg effect, rather than being essential for energy production, may serve other functions [6-10].

We proposed [11], by analogy with the similar Crabtree effect in yeast, that the Warburg effect depends upon the activity of the glycogen shunt [12–14] during the early period after exposure to high glucose and reduced O_2 levels such as in a tumor. The glycogen shunt coordinates the flux of the glycogen synthesis pathway with the glycolytic pathway to prevent excessive accumulation of glycolytic intermediates such as glucose 6 phosphate (G6P) and the high energy phosphate ATP. Because the shunt uses glycolytic ATP an excess of lactate is produced leading to the Warburg effect. A limitation of this proposal is it did not explain why cancer cells (and yeast) sustain the Warburg effect during long term exposure to high glucose conditions, after glycogen synthesis is suppressed. Furthermore, it did not explain how cancer cells, like yeast, have the metabolic flexibility to transition from a primarily glycolytic state back to a state where they derive energy from respiration of non glucose substrates [15].

We recently extended our analysis of metabolic adaptation to high glucose in the Crabtree effect in yeast to long term glucose exposure. The analysis showed that the metabolic transition from low glucose conditions in which yeast obtain energy from oxidation of non glucose substrates (respiration) to high glucose conditions depends initially on rapid activation of the glycogen shunt and longer term on gene expression of new glycolytic enzyme isoforms [16]. The new isoforms lead to a new metabolic steady state of glycolysis in which the majority of glucose is not oxidized, analogous to the Warburg effect. The new isoforms, most importantly for pyruvate kinase, stabilize the glycolytic pathway and make it more efficient for glycolytic ATP and ethanol production than during the early transition period when the glycogen shunt is active.

In this paper we propose that these 3 states, steady state Respiration, the Glycogen Shunt, and steady state Glycolysis may also explain the similar ability of cancer cells to transition from low glucose high O_2 to high glucose low (or normal) O_2 environments. A fourth state in which the Glycogen Shunt works in the phosphorylase direction allows cells undergoing steady state glycolysis to survive while they transition back to respiration through gene expression of non glucose substrate metabolic pathways. The model provides new insight into the function of the glycogen shunt [17] and expression of the PKM2 form of pyruvate kinase [18–20] in cancer cell metabolic adaptation. We also propose a mechanism by which the metabolic flexibility provided by the glycogen shunt allows a greater glycolytic plasticity in cancer cells which may contribute to their evolving a Warburg phenotype.

Description of the 4 state model

Figure 1 shows a schematic of the 4 metabolic states we propose cancer cells undergo in transitioning from a low glucose well oxygenated conditions to high glucose reduced O_2 conditions and then back again. In the model, in analogy to yeast, there are 4 critical metabolic states that occur sequentially during this environmental transition. In the first state, which we call in analogy with yeast the *Respiration State,* cancer cells are in metabolic steady state in a well oxygenated low glucose environment as found in many tissues. A rapid change from a well oxygenated low glucose environment to a high glucose reduced O_2 environment activates glucose metabolism along with the glycogen shunt, which we call the *Glycogen Shunt Synthesis State.* This state is transient and due to gene expression is soon replaced by a new metabolic steady state we call the *Glycolysis State.* The transient and steady states of glucose metabolism are associated with the Warburg effect. When the cell returns to a low glucose well oxygenated environment it requires time for gene expression to restore its ability to metabolize non glucose substrates. During this transition period, which we call the *Glycogen Shunt Phosphorylase State,* the cell obtains ATP and redox equivalents from G6P produced by glycogenolysis while gene expression upregulates non glucose metabolic pathways and gluconeogenesis for G6P production.

We describe below the key adaptations of glucose and glycogen metabolic pathways in the 4 state model. The description starts with the steady state metabolic conditions of the Respiration and Glycolysis States. We then describe the 2 transitional Glycogen Shunt States.

Respiration state

Figure 2 *top* presents a pathway diagram of the Respiration State. Energy and redox equivalents are provided by oxidation of non glucose substrates. Despite the cells respiring on non glucose substrates they still maintain glycolytic enzyme activity, which is needed for metabolism to rapidly transition to glycolysis during the transition to high glucose and reduced O_2. The glycolytic isoforms differ from those found in the Glycolysis State due to their being insensitive to allosteric activation [16]. This insensitivity to activation allows the gluconeogenic pathway to provide G6P for the pentose phosphate shunt without being "short circuited" by glycolytic enzyme flux. For example, in yeast the dominant pyruvate kinase isoform is PyK2 in this state which has similar kinetics to of mammalian pyruvate kinase isoforms such as PKM1 found in tissue that primarily uses respiration for energy (see Table 1 and section 2.2).

Glycolysis state and the role of PKM2 in controlling glycolytic intermediate homeostasis as opposed to the glycolytic flux

Figure 2 *bottom* describes glucose metabolism in the Glycolysis State. In this state the enzyme isoforms for glycolysis, e.g. hexokinase (HK) and pyruvate kinase (PKM2), and other metabolic pathways, are optimized for glucose metabolism. Based on our MCA analysis in yeast [16] a critical factor for stabilizing this state is the expression of the PyK1 form of pyruvate kinase, which is analogous to PKM2 in cancer. In yeast this isoform replaces PyK2 which is insensitive to allosteric activation by fructose 1,6 Bisphosphate (FBP) expressed in the Respiration State. The high Hill coefficient and low Ka of PyK1 for allosteric activation by FBP stabilizes the concentration of glycolytic intermediates over the full range of glycolytic flux. Based on the similarities in the kinetics of PyK1 and PKM2 (Table 1 and references 16, 18–23) we propose that PKM2 serves a similar homeostatic role in glycolysis for cancer cells. Instead of controlling the glycolytic flux, through its high sensitivity to allosteric activation by FBP it allows the glycolytic flux to increase without large changes of the concentration of glycolytic intermediates. This proposal goes against the prevailing opinion in the field that PKM2 is expressed largely to reduce the flux from phosphoenolpyruvate to pyruvate, resulting in G6P
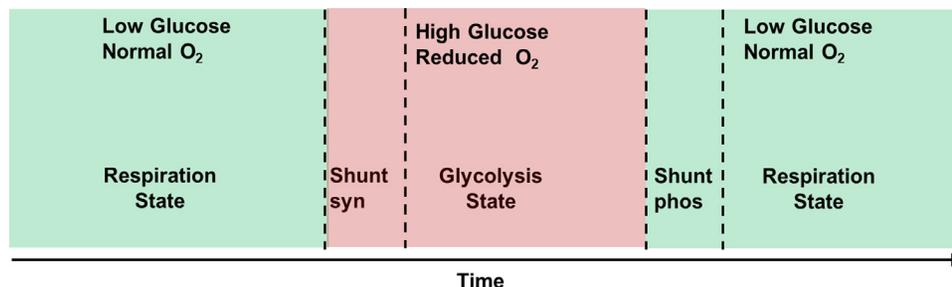
Fig. 1. Four state model of the adaptation of cancer metabolism to low and high glucose conditions

The figure shows the environmental transition a cancer cell may undergo from low glucose normal O_2 conditions to high glucose reduced O_2 conditions in tumors and then back to low glucose conditions. We propose in analogy with Crabtree yeast that there are 4 main metabolic states that the cancer cells use to adapt to these changes.

Respiration State: Under conditions of low glucose and normal O_2 conditions cell enzyme activities and isoforms are optimized to obtain energy and reducing equivalents through oxidation of non- glucose substrates (see Fig. 2). In cancer cells and yeast the respiration state can also be established under conditions of low glucose and reduced O_2.

Glycogen Shunt Synthesis State (Shunt Syn): When the environmental conditions change to high glucose and low O_2 the cells need to switch to glucose metabolism to survive. Because the enzyme isoforms and activities are not optimal for glycolysis the cells need the glycogen shunt to stabilize the glycolytic pathway (see Fig. 3). As a consequence of its homeostatic role the shunt requires glycolytic ATP which leads to increased lactate production and a resultant Warburg effect.

Glycolysis State: While the glycogen shunt is active the cells use gene expression to replace glycolytic and other enzyme isoforms in order to optimize cell metabolism for high glucose reduced O_2 conditions. Once this transition is complete the cells reach a new metabolic steady state which is referred to as the Glycolysis State during which the Warburg Effect continues (see Fig. 2).

Glycogen Shunt Phosphorylase State (Shunt Phos): If environmental conditions change back to low glucose and normal O_2, cells in the Glycolysis State are initially unable to support the cell's energy and redox needs due to downregulation of metabolic pathways for non glucose substrates in the Glycolysis State. To provide sufficient time for gene expression to restore the Respiratory State the cells activate the glycogen shunt. However, the direction of the shunt is reversed with glycogen breakdown by phosphorylase providing G6P for energy production via glycolysis and reducing equivalents via the pentose phosphate pathway (See Fig. 3).

Table 1

Comparison of kinetic properties of yeast Pyk1 and Pyk2 with mammalian PKM1 and PKM2 Table 1 compares the kinetic properties of mammalian PKM1 and PKM2 to their yeast equivalents PyK2 and PyK1 Data for cancer and normal mammalian cells is taken from reference 20 which reviews studies s in which PKM2 and PKM1 were measured under in vivo substrate and effector conditions and reference 21 which determined the Hill coefficients. The kinetic properties of yeast PyK1 and PyK2 are summarized in reference 16. As seen in the table the properties of the pyruvate kinase isoforms in the Respiration and Glycolysis States are similar between yeast and cancer cells.

Mammalian and Yeast Pyruvate Kinase Properties Under In Vivo Conditions

|	PKM1	PyK2	PKM2	PyK1
Relative total activity	1	1	>1	1.7
Allosteric activator	none	none	F1,6BP	F1,6BP
Kinetics	Hyperbolic	Hyperbolic	Sigmoidal	Sigmoidal
Hill coefficient F1,6BP	1	1	3	2

being shunted to the pentose phosphate pathway to provide redox equivalents and substrates for biosynthesis and ROS inactivation [18].

Further evidence for the homeostatic role of PKM2 is from studies, reviewed in reference 19, that found that the concentration of the glycolytic intermediates phosphoenolpyruvate and fructose 1,6 bisphosphate are similar in cancer cells and non cancer cells. In yeast homeostasis of these intermediates is also maintained when they transition from the Glycogen Shunt Synthesis State, in which they express PyK2, to the Glycolysis State. Based on the MCA, an additional prediction is that PKM2, similar to PyK1, will exert little control over the glycolytic flux. The low control coefficient is a consequence of its high sensitivity to allosteric activation which is needed to maintain glycolytic intermediate homeostasis [16]. In agreement with this

prediction studies in cancer cells and implanted tumor models using MCA and other analysis methods have found that PKM2 exerts negligible control of the glycolytic flux [21, 22, 23].

Glycogen shunt synthesis state

When cells in the Respiration State are exposed to high glucose and reduced O_2 they activate the synthesis phase of the glycogen shunt [11, 14, 16]. Figure 3 *top* describes the metabolic fluxes and ATP synthesis and breakdown steps in this state. The key functions of this state are to maintain homeostasis of glycolytic intermediate concentrations and ATP which stabilized the glycolytic pathway, which is imbalanced in the Respiration
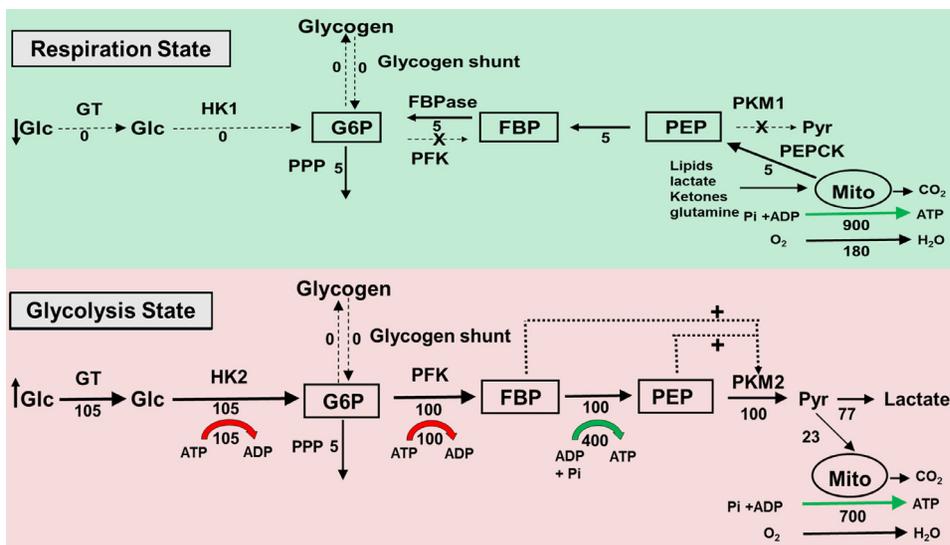
Fig. 2. Metabolic fluxes and glycolytic enzyme isoforms in the Respiration and Glycolysis States

Respiration State (low glucose, normal O_2). In the Respiration State gluconeogenic enzymes such as fructose 1, 6 bis phosphatase (FBPase) are active and supply G6P for the pentose phosphate pathway. Other metabolic pathways are optimized to obtain energy by oxidation of non glucose substrates such as blood lactate, lipids, ketones, and acetate. Although glycolysis does not take place the activities of hexokinase, phosphofructokinase and other glycolytic enzymes in the upper portion of the pathway are high. The activity of enzymes in the lower portion of the glycolytic pathway are reduced, and the expressed isoforms of pyruvate kinase (e.g., PKM1) are insensitive to allosteric activation to prevent futile cycles.

Glycolysis State (high glucose, low O_2). After extended exposure to high glucose cancer cells in the Respiration state will replace (through gene expression and post translational mechanisms) their glycolytic enzyme isoforms, such as HK1, and PKM2, with isoforms characteristic of cancer cells such as HK2, and PKM2. In addition, the glycogen shunt and FBPase are suppressed, resulting in net ATP synthesis in glycolysis at the normal 2 ATP synthesized per glucose phosphorylated ratio. The PKM2 isoform is highly sensitive to being activated allosterically by FBP, and has similar kinetics as PyK1 in yeast (Table 1). As a consequence of these isoform changes the glycolytic pathway is stabilized without the need for the glycogen shunt, as are pathways connected to it at branch points such as the pentose phosphate pathway at G6P.

V_{HK}, flux through HK; Vshunt, flux through glycogen shunt; V_{PPP}, flux through pentose phosphate pathway, V_{PFK1} flux through PFK; VFBPase, flux through FBPase, V_{PEPCK} – flux through phosphoenol pyruvate carboxykinase, V_{PKM1}, flux through pyruvate kinase M1;, V_{PKM2} flux through pyruvate kinase M2, Mito, mitochondrion.

State. This function is accomplished by coordination of the fluxes of the glycogen shunt, glycolysis, and the upper portion of gluconeogenesis pathway in order to balance production and breakdown of glycolytic intermediates and ATP. The relative fluxes are shown in the figure based upon ^{13}C and ^{31}P magnetic resonance spectroscopy studies of yeast [24 – 27], but with a higher oxidative ATP flux based on in vivo studies of tumors [4,5]. While the shunt is active state gene expression leads to new isoforms of glycolytic enzymes which are optimized for high glucose low O_2 conditions, eventually leading to the establishment of the Glycolysis State in which the shunt is suppressed.

Glycogen shunt phosphorylase state

Figure 3 *bottom* shows the role of the Glycogen Shunt in the phosphorylase direction when cells in the Glycolysis State are exposed to low glucose and normal O_2 conditions. During the early period after the conditions in the environment change glycogenolysis is activated and supplies G6P for glycolysis and the pentose phosphate pathway. The glycogenolysis flux continues until the Respiration State is reestablished by gene expression and the cell is able to obtain sufficient energy and redox equivalents from non glucose substrates.

## Evidence for the respiration and glycolysis states in cancer

Extensive studies of cancer cells both in vitro and in vivo have shown high rates of glucose consumption that exceed glucose oxidation, referred to as the

Warburg effect, which at steady state is similar to the Glycolysis State in yeast [1–11]. An additional similarity is that cancer cells express unique isoforms of glycolytic enzymes and transporters in this state [6–10, 18–22]. As described in section 2 the kinetic properties of the most common isoform of pyruvate kinase in this state (PKM2) are similar to that of the isoform of pyruvate kinase in yeast (Pyk1) [16, 21]. In vivo measurements in cancer cells expressing PKM2 have found the enzyme has minimum flux control for glycolsis, similar to what has been found in yeast expressing Pyk1 [16, 21–23].

Less evidence is available for cancer cells in the Respiration State. However recently cancer cells have been found in vivo that obtain energy primarily from respiration and undergo gluconeogenesis similar to the Respiration state in yeast [15]. This shift to respiration occurs despite the cells having previously exhibited a Warburg effect [15], providing strong evidence for their metabolic flexibility. Furthermore, tumor cells in vitro and in vivo can respire on non-glucose substrates such as glutamine [28, 29] as well as acetate and ketones [4, 5]. This evidence supports that cancer cells have the metabolic flexibility to transition from the Glycolysis state to the Respiration state when there is a decrease in glucose concentration in their environment.

Evidence for the glycogen shunt in cancer

As reviewed in reference 30, cancer cells activate glycogen synthesis when challenged with reduced O_2 levels in an environment with sufficiently high glucose concentration [30, 31]. These findings are consistent with the proposed role of the glycogen shunt in the transition from the Respiration to the Glycolysis state (Figs. 1 and 3). Further evidence for the importance of the Glycogen Shunt Synthesis
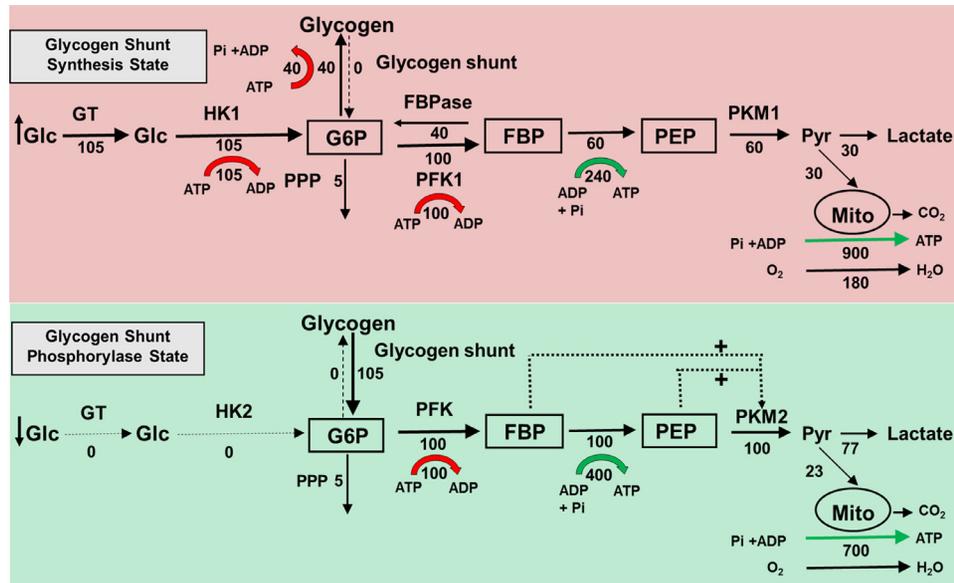
Fig. 3. Metabolic fluxes and glycolytic enzyme isoforms in the glycogen shunt synthesis and phosphorylase states.
Glycogen Shunt Synthesis State. Within minutes after exposure to high glucose cells in the Respiration State activate glycolysis to supply energy. During the transition to the Glycolysis State, which takes place over approximately 120 minutes in yeast, G6P is shunted into glycogen by the glycogen shunt where it is stored. The glycogen synthesis flux balances the fluxes in and out of the G6P, FBP, and other pools of glycolytic intermediates, ATP homeostasis is maintained by glycogen synthesis and futile cycling at the enzyme fructose 1,6 bisphosphatase (FBPase), normally a gluconeogenic enzyme) balancing ATP production in the lower portion of glycolysis (see green arrows indicating ATP synthesis and red arrows indicating ATP consumption) with ATP consumption in the upper portion. Only a small fraction of the pyruvate produced is oxidized due to the much higher ATP production per glucose from mitochondrial oxidation, and therefore net lactate (or ethanol in yeast) is produced characteristic of the Warburg effect. The relative fluxes of glycolysis, FBPase, and glycogen synthesis were determined from studies of yeast using ^{13}C and ^{31}P Nuclear Magnetic Resonance (25–28). The rate of glucose oxidation relative to total glucose phosphorylation (normalized to 100) is a hypothetical as the actual range found in tumors is large (29).
Glycogen Shunt Phosphorylase State. When cells in the Glycolysis State are exposed to conditions of low glucose and normal O_2 concentrations they need to shift to non glucose fuel sources to support their ATP and redox needs. These needs are initially met by breakdown of glycogen to G6P via glycogen phosphorylase (17) as shown in the figure. While the shunt is active gene expression restores the enzyme activities in the Respiration State and glycogen breakdown is suppressed (16).

State is the increasing number of reports of tumors with cells with high glycogen concentrations [31].

Harris and colleagues have provided evidence for the Glycogen Shunt Phosphorylase State by showing that inhibitors of glycogen phosphorylase led to reduced rates of cell growth [17]. *They also found cellular oxidative damage similar to when the pentose phosphate pathway is blocked. They attributed this finding to the need for glycogenolysis to provide G6P for the pentose phosphate pathway. Their findings, along with findings of tumor glycogen mobilization under energetic demands beyond those that can be met by glucose* [32, 33] *are consistent with the proposed role of the glycogen shunt to provide G6P during the transition from the Glycolysis to the Respiration State* (Fig. 3 *bottom*).

The metabolic flexibility provided by the glycogen shunt may provide the additional cell survival time needed for the genomic changes that lead to the Warburg effect

Studies of cancer have frequently found mutations in oncogenes and tumor suppressor genes leading to overexpression of glycolytic enzyme isoforms such as PKM2 [34, 35]. Although the specific mutations vary between lines of cancer cells they often lead towards similar metabolic traits such as the Warburg effect, a process referred to as metabolic reprogramming [36].

Although metabolic reprogramming associated with the Warburg effect and other characteristic cancer metabolic pathways (e.g., for glutamine metabolism) is well established it remains unclear how the genes involved are selected for, especially given that they will not provide an advantage for cancer cells prior to their being exposed to the high glucose low O_2 conditions often found in mature tumors. We recently proposed that the metabolic flexibility allowed by the glycogen shunt may have contributed to the evolution of the Crabtree effect in yeast [16]. The ancestor of Crabtree positive yeast did not have a Crabtree effect [37, 38]. Figure 4 shows simulations using MCA in which we modeled the impact of the glycogen shunt on the survival of non Crabtree yeast in the Respiration State exposed to several high glucose challenges. The wild type glycolytic activity was set to a low value based on yeast without a Crabtree effect [38]. As seen in the figure yeast with higher glycolytic activity (due to variations in development or gene alterations), in the absence of the glycogen shunt (C = 10 in the figure), are paradoxically more vulnerable to dying after repeated high glucose exposures. However, when the glycogen shunt is present at high activity (C <1) the entire population survives including yeast with elevated glycolytic enzyme activity. By analogy the metabolic flexibility provided by the glycogen shunt in cancer progenitor cells may allow cells with enhanced glycolysis to survive the transition to high glucose and reduced O_2 levels. The enhanced survival provides time for further mutations enhancing glycolysis to take place leading to the Warburg Effect as proposed as a general principle in evolutionary speciation by West-Eberhard [39].
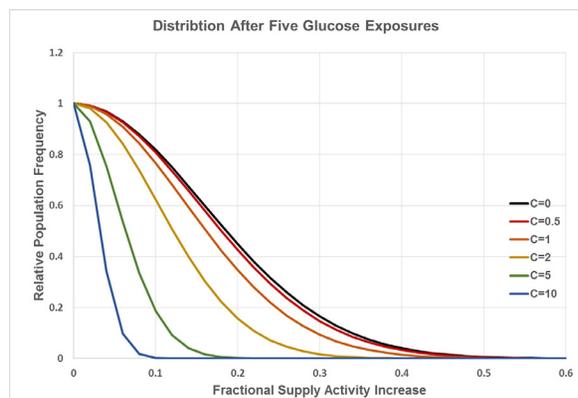
Fig. 4. Metabolic flexibility due to the glycogen shunt allows yeast cells with a greater range (plasticity) of glycolytic activity to survive repeated exposures to high glucose. The figure shows the right side of a normal distribution of yeast glycolytic enzyme activity (Fractional Supply Activity Increase) in the Respiration State after 5 successive exposures to high glucose conditions. The mean activity at wild type is normalized to 0. When the glycogen shunt is highly active (C < 1, where C is the metabolite concentration control coefficient) all the cells in the population survive the multiple glucose exposures. In contrast at progressively lower glycogen shunt activity (C > 1) a progressively smaller fraction of yeast with above wild type glycolytic activity survive.

Discussion

The 4 state model is based on the general principle that under all environmental conditions it is essential to maintain homeostasis of shared glycolytic pathway intermediate levels in order to stabilize the entire cellular metabolic network. The importance of metabolic homeostasis for cell survival and the criteria needed to achieve it was formalized by Kacser and Acerenza in their Universal Model [40]. They showed proper metabolic function depended on the stability of the concentrations of intermediates and substrates shared between pathways, such as glucose 6 phosphate and ATP between the glycolytic, glycogen synthesis and breakdown, gluconeogenic and pentose phosphate pathways (See Fig. 3). The coordinated fluxes of these pathways during the transitional glycogen shunt states are determined by these homeostatic criteria [12, 13, 14, 16]. Similarly, the enzyme isoform shifts in cancer and yeast, such as to PKM2 and PyK1, are largely for homeostastasis as opposed to flux control [16].

The importance of homeostasis as a unifying principle in metabolism, even in cancer cells, could be described as a modern rephrasing of Claude Bernard's 1865 publication of "An Introduction to the Study of Experimental Medicine" [41]. It is a tribute to the clairvoyant founder of Physiology that his proposal that living organisms need a steady internal environment to survive is true even today at the molecular level in metabolic pathways. The balance of the flux in and out of the pools of glycolytic intermediates and ATP, achieved by the shunt during transitions and at steady state by gene expression, maintains homeostasis of the chemical intermediates forming a constant interior milieu as Bernard proposed.

A limitation in the explanatory range of the 4 state model is that, while the homeostatic function of the glycogen shunt can explain the Warburg effect during the transition to high glucose reduced O_2 conditions, the model provides no explanation for why the cells continue to maintain elevated non oxidative glycolysis and lactate production, The ability to describe *how* a model predicts experimental measurements, but not *why* the cell is in a specific metabolic state such as during the Warburg effect, is a dilemma that Bernard anticipated in his founding principles of the methods of physiology.

He maintained that the physiologist is not different from the physicist in that they share' a common goal of "getting back to the immediate cause of the phenomenon they are studying" which "consists in finding the conditions necessary to the appearance of the phenomenon." Finding those conditions will tell us how the phenomenon exists but not why it exists. Similarly studies of unique phenomena in cancer metabolism, such as the Warburg effect, are plunging deeper into how a phenomenon exists but in the end why remains elusive and as Bernard proposed may not be answerable from a purely metabolic/physiological approach.

The mechanism proposed in Section 4 for how the metabolic flexibility provided by the glycogen shunt could enhance the rate of evolution of the Crabtree Effect in yeast, and by analogy the Warburg effect in cancer, is related to the general proposal of the importance of phenotypic plasticity in Evolutionary Biology made by West-Eberhart and others [39, 42]. Phenotypic plasticity, which is defined as the range of phenotypic variations (due to environmental differences during development and the range of gene isoforms) in a population, allows some of the population to survive changes in their environment that would be lethal for the average phenotype. The survivors then can survive the many generations needed for mutations that enhance their fitness for survival. For cancer cells, due to their rapid mutation rate, much less extra time is needed to allow selection for metabolic traits such as the Warburg effect. The glycogen shunt, by allowing cells to survive the transition to an environment they are not adapted also allows more time for mutations and other genome alterations that lead to the Warburg effect.

The 4 state model primarily differs from previous approaches at describing cancer metabolic reprogramming in its focus on the coordination between multiple pathways, and associated enzyme kinetics, with the target of maintaining metabolic intermediate homeostasis. For example, the glycogen shunt involves the coordinated regulation of glycolysis, gluconeogenesis, glycogen synthesis, glycogen breakdown, and the pentose phosphate pathway to maintain G6P and other glycolytic intermediate homeostasis. Although cancer metabolic reprogramming is often described as dysfunctional, it is likely that the requirement that intermediate homeostasis be maintained for proper metabolic regulation and survival [40] remains an important principle guiding principle.

Conclusions

- The need to maintain homeostasis of metabolic intermediates during adaptations to novel environments is critical for cancer as well as normal cells.
- Glucose metabolic flexibility and the support of the Warburg effect in cancer cells are potentially explained by 4 metabolic states identified by MCA analysis in Crabtree yeast
- The stable Respiration and Glycolysis States are respectively optimized for low glucose and normal O_2 levels and high glucose and reduced O_2 levels through gene expression of cancer associated glycolytic enzyme isoforms such as PKM2.
- The gene expression of PKM2 is largely to maintain glycolytic intermediate homeostasis as opposed to controlling the glycolytic flux. It is likely that the majority of enzyme isoform changes during metabolic reprogramming also serve homeostatic functions.
- The glycogen shunt is coordinated with the glycolytic, gluconeogenic, and pentose phosphate pathways to maintain G6P homeostasis and in doing so provides the short term metabolic flexibility needed for cancer cells to survive rapid transitions in glucose and O_2 levels.
- The metabolic flexibility provided by the glycogen shunt may also allow cells early in oncogenesis to survive exposure to low O_2 conditions long enough for mutations that enhance glycolysis and the Warburg Effect to be selected.

Limitations

The primary limitation of this work is that there are few quantitative flux studies performed on cancer cells that can be used to test the predictions of the model, and therefore our conclusions are tentative. To test this and other metabolic models we recommend more studies be performed using experimental paradigms based on MCA and other quantitative methods for studying metabolic control such as described in references 22 to 24. The 4 state model is limited primarily to glycolysis, gluconeogenesis and the glycogen shunt and does not directly take into account the impact on glucose metabolism of other substrates such as amino acids, and differences in the state of the cell due to the cell division and other processes. However as has been done for yeast and other microorganisms [19] the model can be extended to include the influence of these substrates and their unique metabolic pathways in cancer. Because of the rapid mutation rate of cancer cells the proposal about the potential importance of the glycogen shunt in providing the metabolic flexibility needed so that cells survive long enough for the selection of mutations that enhance glycolysis the Warburg Effect could potentially be tested.

Competing interests

Author Contribution Statement

The authors made the following contributions to the manuscript. Douglas L Rothman: Conceptualization, formal analysis, investigation, methodology, original draft, review and edit. Robert G Shulman Conceptualization: formal analysis, investigation, methodology, original draft, review and edit.

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