The Warburg effect – aerobic glycolysis in proliferating cells

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Abstract

The metabolic phenotype of proliferating cells is characterized by enhanced glycolysis uncoupled from pyruvate oxidation, but instead coupled to increased lactate release even in the presence of oxygen (Warburg effect/aerobic glycolysis). Alterations in the pathways regulating glycolysis and the oxidation of glucose-derived carbon are responsible for maintaining this phenotype. This article reviews the molecular mechanisms inherent to the intermediary metabolism of glucose contributing to the Warburg effect.

Keywords: cellular proliferation; glycolysis; glucose oxidation; cellular biosynthesis

■ Heart Metab. (2011) 51:34–38

Introduction

In the 1920s Otto Warburg demonstrated that rapidly proliferating mouse ascites tumor cells preferentially converted glucose to lactate even in the presence of oxygen (Warburg effect /aerobic glycolysis) at a much greater rate than that observed in quiescent, differentiated cells [1]. These observations were later extrapolated to proliferating primary lymphocytes [2], demonstrating that the metabolic phenotype of cell proliferation is attended by specific alterations in the intermediary metabolism of glucose. Although glycolysis generates only 7–10% of the adenosine-5'-triphosphate (ATP) compared to the complete mitochondrial oxidation of glucose-derived carbon, it does, nonetheless, efficiently generate substrates that meet the biosynthetic requirements of cellular proliferation [3]. This article provides an overview of mechanisms intrinsic to the intermediary metabolism of glucose that contribute to the Warburg effect.

Cellular glucose metabolism in differentiated versus proliferative tissues

Differentiated tissue such as cardiac muscle derives greater than 90% of its ATP requirements via mitochondrial oxidative phosphorylation [4]. The reduced forms of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) generated during the catabolic degradation of glucose and fatty acids, as well as in the tricarboxylic acid (TCA) cycle deliver protons and electrons to the electron transport chain, reducing molecular oxygen to water, and generating ATP. With regards to carbohydrate metabolism, the catabolism of glucose in differentiated tissues occurs via two separate, but coupled processes—glycolysis and subsequent glucose (i.e., pyruvate) oxidation. In sharp contrast, cellular metabolism in proliferative cells is characterized by accelerated rates of glycolysis that are uncoupled from the glucose oxidation.

Glycolysis, localized to the cytosolic compartment, converts glucose to pyruvate, or lactate, in the presence or absence of oxygen, respectively, and generates 2 mol ATP/1 mol glucose. Glycolysis consists of an ATP utilizing stage and a subsequent ATP generating stage (Fig. 1). The first enzymatic reaction committing glucose to catabolism by glycolysis, catalyzed by 6-phosphofructo-1-kinase (PFK-1), is subject to allosteric inhibition by ATP, citrate, and protons

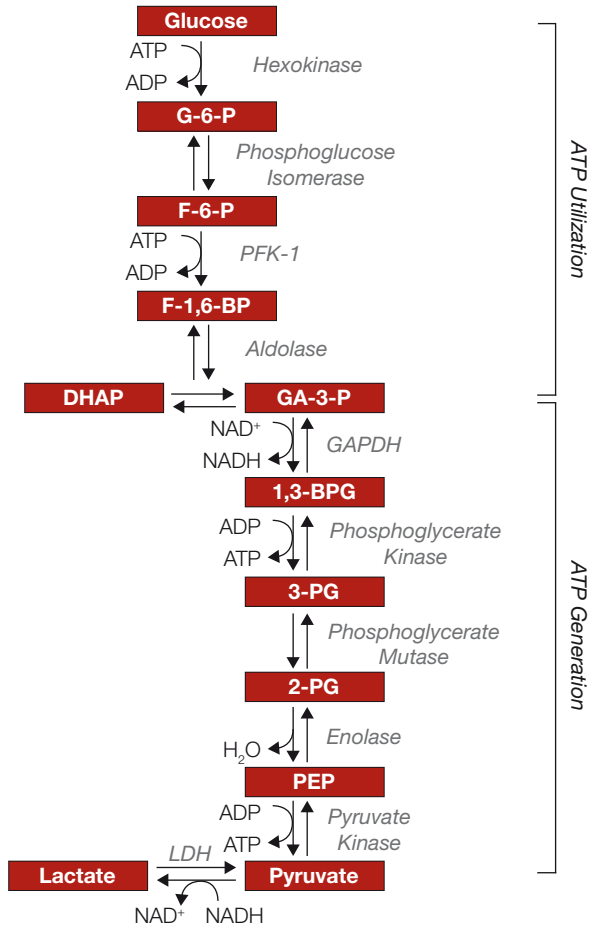

Fig. 1 *Catabolism of glucose via glycolysis. Following facilitated transport across the plasma membrane, glucose is degraded by the pathway of glycolysis. Glycolysis itself can be separated into an ATP utilization stage and an ATP generating stage. The first consumes 2 ATP molecules in course of phosphorylating glucose and cleaving it to yield 2 molecules of GA-3-P. The ATP generating stage converts 2 molecules of GA-3-P to either pyruvate of lactate, generating 4 molecules of ATP. Glycolysis therefore generates a net 2 moles of ATP per mole of glucose. Several of the enzymes directly involved in glycolysis are up-regulated in order to support the Warburg effect in proliferating cells.* 1,3-BPG *1,3-bisphosphoglycerate,* DHAP *dihydroxyacetone phosphate,* F-6-P *fructose-6-phosphate,* F-1,6-BP *fructose-1,6-bisphosphate,* G-6-P *glucose-6-phosphate,* GA-3-P *glyceraldehyde-3-phosphate,* GAPDH *glyceraldehyde-3-phosphate,* LDH *lactate dehydrogenase,* PEP *phosphoenolpyruvate,* 2-PG *2-phosphoglycerate,* 3-PG *3-phosphoglycerate*

[4]. The first reaction of the ATP generating stage of glycolysis, catalyzed by glyceraldehyde-3-phosphate dehydrogenase (GAPDH), is coupled to the reduction of NAD^+ to NADH. ATP itself is generated by reactions catalyzed by phosphoglycerate kinase and pyruvate kinase. The continual regeneration of NAD^+ is required to ensure that glycolysis is not limited. In differentiated cells and tissues, under aerobic conditions, NAD^+ is re-generated from NADH via the malate-aspartate shuttle and the mitochondrial electron transport chain; whereas, under anaerobic conditions lactate dehydrogenase (LDH) couples the reduction of pyruvate to lactate with the regeneration of NAD^+.

The oxidation of glucose-derived carbon requires the transport of pyruvate into the mitochondrial matrix via a monocarboxylate (MCT) transporter [5], and its subsequent oxidative decarboxylation, catalyzed by the pyruvate dehydrogenase (PDH) complex yielding acetyl-CoA [6]. The PDH complex consists of PDH, PDH kinase (PDK), and PDH phosphatase (PDHP). The flux of pyruvate through PDH is subjected to regulation by both substrate/product ratios and covalent mechanisms. The increased generation of pyruvate, decreased ratios of NADH/NAD^+ and acetyl-CoA/CoA, as well as PDHP-mediated dephosphorylation increase flux through PDH. Conversely, increased ratios of NADH/NAD^+ and acetyl-CoA/CoA, as well as PDK-mediated phosphorylation decreases flux through PDH, thereby restricting the oxidation of glucose-derived carbon units [6].

Underlying molecular mechanisms

The Warburg effect is a predominant metabolic phenotype observed in proliferating cells. Detailed studies in a variety of cancer cell types have revealed that this metabolic phenotype may arise from a number of cellular alterations and occur downstream of growth promoting signal transduction pathways (PI3K-Akt-mTOR), mutations in proto-oncogenes (*Myc*), in response to loss of function mutations of tumor suppressor genes (p53), and transcriptional responses to hypoxia-inducible factor-1α (HIF1α) [3]. These alterations in cellular signaling elicit concerted effects on the intermediary metabolism of glucose in both the ATP utilizing and ATP generating stages of glycolysis, and facilitate enhanced glycolysis characteristic of the Warburg effect.

Growth factor-mediated activation of the PI3K-Akt-mTOR pathway via transcriptional and post-transcriptional effects increases the expression and cell surface localization of GLUT1 glucose transporters [7]. Similarly, loss of function mutations of the p53 tumor suppressor de-represses the transcription of GLUT1 and GLUT4 [8]. These alterations can increase cellular glucose uptake. Glucose entering the cytosolic compartment is efficiently trapped as glucose-6-phosphate (G6P) by hexokinase II, a HIF1α- and Myc-target gene up-regulated in a variety of cancer cells [9]. Loss of p53 function also enhances flux through the ATP utilizing stage of glycolysis, secondary to the loss of *T*P53-induced glycolysis and apoptosis regulator (TIGAR) [10]. TIGAR is functionally similar to the fructose-2,6-bisphospahatase (FBPase) domain of the bifunctional enzyme, 6-phosphofructo-2-kinase (PFK-2), which converts fructose-6-phosphate to fructose-2,6-bisphosphate, a potent allosteric stimulator of PFK-1. Thus, p53 can restrain flux through PFK-1 via TIGAR-mediated degradation of fructose-2,6-bisphosphate [10], whereas loss of p53 function via loss of TIGAR can promote flux through PFK-1 [11].

ATP is generated by the phosphoglycerate kinase and pyruvate kinase reactions of glycolysis and both are of central importance in mediating the Warburg effect. HIF1α and Myc increase the expression of phosphoglycerate kinase in a cooperative manner [12], which contributes to increasing glycolytic flux. Pyruvate kinase (PK) catalyzes the final reaction of glycolysis, coupling the production of pyruvate with the production of ATP. Proliferating cells, including cancer cells predominantly express the PKM2 isoform [13]. Interestingly, the affinity of dimeric PKM2 for its substrate PEP is low, and PKM2 is therefore nearly inactive at physiological/pathophysiological intracellular PEP concentrations [14]. These effects are associated with increased cellular ADP/ATP ratios, which enhance flux through the PFK-1 reaction of glycolysis. These effects may appear paradoxical, as the generation of the glycolytically derived pyruvate via the PK reaction is limited, which would be expected to decrease lactate release and hence decrease an important component of the Warburg effect. However, a recent report identifies a novel/alternative pathway of glycolysis in proliferating cells that circumvents this reaction and generates pyruvate from PEP by transferring the phosphate from PEP to the catalytic histidine residue of the glycolytic enzyme phosphoglycerate mutase [15]. The generated pyruvate can subsequently be reduced to lactate and hence maintain the Warburg effect.

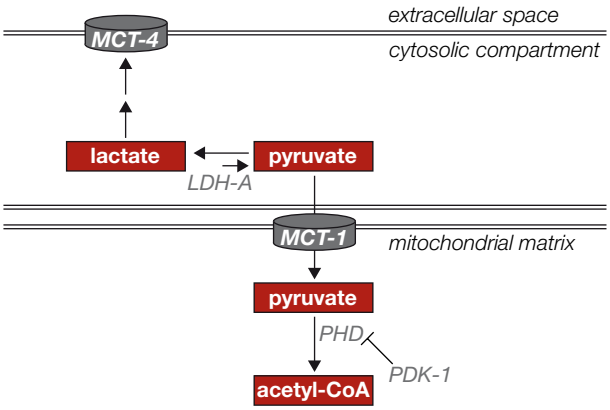

Fig. 2 *Pyruvate/lactate metabolism and the Warburg effect. Pyruvate is the usual end product of glycolysis in the presence of oxygen; however, the Warburg effect is characterized by the conversion of glucose to lactate, and the subsequent release of lactate to the extracellular space. Several alterations in the enzymes involved in the metabolism of pyruvate and lactate facilitate this aspect of the Warburg effect. Increased expression of pyruvate dehydrogenase kinase-1 (PDK-1) restricts the mitochondrial oxidation of glucose-derived pyruvate. The increased expression of lactate dehydrogenase-A (LDH-A) preferentially converts pyruvate to lactate, while increased expression of monocarboxylate transporter 4 (MCT-4) increases the efflux of lactate from the cytosol to the extracellular space.*

Mechanisms affecting pyruvate/lactate metabolism and lactate release are also required to support the Warburg effect. The expression of the LDH-A isoform is under the transcriptional control of both HIF1α and Myc [16]. LDH-A effectively converts glycolytically derived pyruvate to lactate. HIF1α also upregulates the expression of the plasma membrane MCT4 isoform [17], as well as PDK1 in proliferating cells [9]. Taken together, these metabolic alterations provide a means to regenerate NAD^+ (LDH-A) required for glycolysis, facilitate lactate release (MCT4), and restrict glucose oxidation (PDK1) (Fig. 2).

The Warburg effect and anabolic metabolism

The Warburg effect is of central importance in supporting the requirements of cell division which require a doubling of total biomass (including cellular nucleic acid, lipid, and protein contents) [3]. The non-oxidative arm of the pentose phosphate pathway (PPP) utilizes the glycolytic intermediate, fructose-6-phosphate for the synthesis of ribose-5-phosphate required for nucleotide biosynthesis [18]. Proliferating cancer cells

in particular also have high requirements for *de novo* lipid synthesis, evidenced from the elevated expression of lipogenic enzymes including ATP citrate lyase (ACL), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS) [19]. ACL can contribute to maintaining the Warburg effect as it cleaves cytosolic citrate (originating from the mitochondrial matrix), yielding oxaloacetate and acetyl-CoA. To ensure that the requirements for *de novo* lipid synthesis do not deplete the TCA cycle, the cycle is replenished via the anaplerotic flux of glutamine [20]. These effects of ACL decrease the content of cytosolic citrate, enhancing PFK-1 activity, as well as providing substrate (i.e., acetyl-CoA) for *de novo* lipid synthesis via ACC and FAS. The metabolism of cytosolic oxaloacetate can also contribute to supporting the Warburg effect as malate dehydrogenase couples the conversion of oxaloacetate to malate with the formation of NAD^+. Malic enzyme subsequently couples the conversion of malate to pyruvate (which can subsequently be converted to lactate and exported) with the production of nicotinamide adenine dinucleotide phosphate (NADPH), which serves as a cofactor in both the PPP and *de novo* lipid synthesis [18]. These complimentary activities of the Warburg effect and several pathways of anabolic cellular metabolism (Fig. 3) meet the requirements of cellular proliferation, and in cancer cells support tumor development and progression.

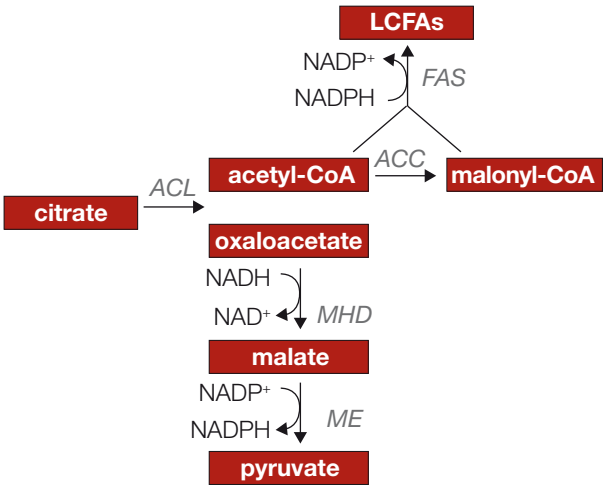

Fig. 3 *Cytosolic metabolism of citrate for de novo lipid synthesis. Citrate originating in the mitochondrial matrix can gain access to the cytosolic compartment. In the cytosol, citrate can be cleaved by the lipogenic enzyme ATP citrate lyase, generating acetyl-CoA which itself can contribute to de novo lipid synthesis. Oxaloacetate generated from the ATP citrate lyase (ACL) reaction can be converted to malate and subsequently pyruvate by the activities of malate dehydrogenase (MDH) and malic enzyme (ME) respectively. Pyruvate itself can be converted to lactate and released into the extracellular space.* ACC *acetyl-CoA carboxylase,* FAS *fatty acid synthase,* LCFAs *long chain fatty acids.*

Conclusions

Alterations in the intermediary metabolism of glucose, exemplified by the Warburg effect are critical in supporting the biosynthetic requirements of cellular proliferation. The Warburg effect adapts cellular metabolic phenotype, such that enhanced flux through glycolysis provides a number of critically important substrates for the macromolecular synthesis of cellular biomass. As such a greater understanding of molecular mechanisms regulating the intermediary metabolism of glucose is relevant for the identification novel pharmacological targets that can influence cellular proliferation. With regards to cancer cells, this may lead to the development of promising therapeutic agents to limit tumor development and progression. •

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