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Opinion

The Warburg Effect: How Does it Benefit Cancer Cells?

Maria V. Liberti[1,2] and Jason W. Locasale[2,*]

Cancer cells rewire their metabolism to promote growth, survival, proliferation, and long-term maintenance. The common feature of this altered metabolism is the increased glucose uptake and fermentation of glucose to lactate. This phenomenon is observed even in the presence of completely functioning mitochondria and, together, is known as the 'Warburg Effect'. The Warburg Effect has been documented for over 90 years and extensively studied over the past 10 years, with thousands of papers reporting to have established either its causes or its functions. Despite this intense interest, the function of the Warburg Effect remains unclear. Here, we analyze several proposed explanations for the function of Warburg Effect, emphasize their rationale, and discuss their controversies.

Trends

Both glycolytic and mitochondrial metabolism are essential for cell proliferation in both past and present conceptions of the Warburg Effect.

Numerous proposals for the function of the Warburg Effect have emerged over the years.

Each of the proposed functions of the Warburg Effect is attractive, but also raises questions.

Signal transduction functions for the Warburg Effect appear likely, but are difficult to test experimentally.

Glucose Metabolism and the Warburg Effect

The metabolism of glucose, the central macronutrient, allows for energy to be harnessed in the form of **ATP** (see Glossary) through the oxidation of its carbon bonds. This process is essential for sustaining all mammalian life. In mammals, the end product can be lactate or, upon full oxidation of glucose via respiration in the mitochondria, CO_2. In tumors and other proliferating or developing cells, the rate of glucose uptake dramatically increases and lactate is produced, even in the presence of oxygen and fully functioning mitochondria. This process, known as the **Warburg Effect**, has been studied extensively (Figure 1). However, after careful inspection, it becomes apparent that its benefits for cell growth and survival are not yet resolved. In this analysis, we focus on several proposals for its function and, in each case, we discuss their appeal as well as the questions that are raised. Before our discussion of each proposal, we first introduce the Warburg Effect in a historical context with an emphasis on the lesser-appreciated aspects of its conceptual development. It is our hope that this retrospective and subsequent analysis bring additional context to current ideas in cancer metabolism.

Historical Perspectives of the Warburg Effect

During the 1920s, Otto Warburg and colleagues made the observation that tumors were taking up enormous amounts of glucose compared with what was seen in the surrounding tissue. Additionally, glucose was fermented to produce lactate even in the presence of oxygen, hence the term '**aerobic glycolysis**' [1,2]. However, it was also noted that respiration alone could maintain tumor viability. Therefore, it was concluded that, to kill tumor cells by depriving them of energy, both glucose and oxygen had to be eliminated [3]. Subsequently, in 1929, an English biochemist, Herbert Crabtree, extended Warburg's work and studied the heterogeneity of glycolysis in tumor types. He confirmed Warburg's findings, but further discovered that the magnitude of respiration in tumors was variable, with many tumors exhibiting a substantial amount of respiration [4]. Therefore, Crabtree concluded that not only do tumor cells exhibit aerobic glycolysis, but that there is also variability in fermentation, presumably due to environmental or genetic influences.

[1]Department of Molecular Biology and Genetics, Graduate Field of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY, USA
[2]Department of Pharmacology and Cancer Biology, Duke University Medical Center, Duke Molecular Physiology Institute, Duke Cancer Institute, Durham, NC, USA

*Correspondence: jason.locasale@duke.edu (J.W. Locasale).

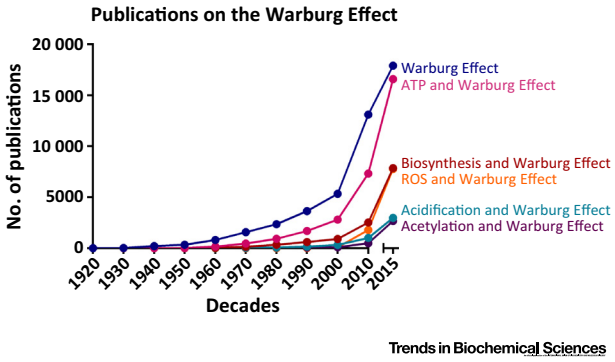

Figure 1. The Frequency of Publications on the Warburg Effect from the 1920s to the 2010s. The Warburg Effect has been studied extensively since the 1920s with a surge in the number of publications from the 2000s to today. Many of the proposed functions of the Warburg Effect have also gained renewed interest. Although energy (ATP), biosynthesis, and reactive oxygen species (ROS) have been intricately studied in the context of the Warburg Effect, acidification and acetylation have only recently gained attention. Publication numbers were obtained from Google Scholar.

Glossary

Aerobic glycolysis: enhanced rate of glycolysis and fermentation to lactate that occurs in the presence of functioning mitochondria.
ATP: adenosine triphosphate, cellular energy currency.
Flux: the rate of the overall chemical reaction resulting from the conversion of one metabolite to another through a defined metabolic pathway.
NADH: reduced nicotinamide adenine dinucleotide (NAD^+); a reducing agent involved in redox reactions that is responsible for the transfer of electrons. NADH is a key reducing equivalent in glycolysis and mitochondria.
NADPH: reduced nicotinamide adenine dinucleotide phosphate ($NADP^+$); most well known for its use in reductive biosynthesis and regenerating reduced glutathione.
Reactive oxygen species (ROS): reduced forms of oxygen that are chemically reactive.
Warburg Effect: another name for aerobic glycolysis; coined by Efraim Racker during the early 1970s.

Contrary to the findings of these previous works and for reasons unclear to these authors, Warburg later proposed that dysfunctional mitochondria are the root of aerobic glycolysis [5]. Warburg further hypothesized that this event is the primary cause of cancer. This phenomenon was then termed the Warburg Effect during the early 1970s by Efraim Racker, who also pointed out that previous data showed respiratory capability of tumors. Racker developed his own theories about the origins of the Warburg Effect, ranging from imbalances in intracellular pH to defects in ATPase activity [6]. It was later observed by Racker, Jeffrey Flier, and Morris Birnbaum that aerobic glycolysis was a controllable process that can be directly regulated by growth factor signaling. By that time, the discovery of oncogenes led to the conclusion that aberrant regulation of growth factor signaling is an initiating event in oncogenesis. Thus, their observations brought newfound significance to Warburg's hypothesis in cancer biology [7–10]. Nevertheless, it remained unclear whether the Warburg Effect was a bystander in cancer pathogenesis until more recently, when genetic and pharmacological studies conclusively showed that the Warburg Effect was required for tumor growth [11,12]. Coming back to the original findings on tumor metabolism, it is now apparent that targeting both aerobic glycolysis and mitochondrial metabolism may be required [13–16]. Throughout this history, the function of the Warburg Effect has remained controversial. Here, we discuss several of the major proposals and argue that the functions of the Warburg Effect for tumor growth remain unknown even today.

Warburg Effect and Rapid ATP Synthesis

Per unit of glucose, aerobic glycolysis is an inefficient means of generating ATP compared with the amount obtained by mitochondrial respiration [17,18]. However, the rate of glucose metabolism through aerobic glycolysis is higher, such that the production of lactate from glucose occurs 10–100 times faster than the complete oxidation of glucose in the mitochondria. In fact, the amount of ATP synthesized over any given period of time is comparable when either form of glucose metabolism is utilized [19]. Thus, a reasonable hypothesis for why cells would employ aerobic glycolysis could account for this inherent difference in kinetics.

Theoretical calculations using evolutionary game theory support the hypothesis that cells with a higher rate, but lower yield, of ATP production may gain a selective advantage when competing for shared and limited energy resources (Figure 2, Key Figure) [20,21]. In fact, tumor microenvironments have limited availability of glucose and undergo competition for nutrients with stromal cells and the immune compartment [22,23]. Additional support comes from a recent study, which showed that when changes to the cellular environment were induced to greatly increase ATP demand by altering the demand of ATP-dependent membrane pumps, aerobic glycolysis increased rapidly and oxidative phosphorylation remained constant [24]. This finding provides additional rationale for the function of the Warburg Effect to be supporting the rapid production of ATP that can be rapidly tuned to support the demand for ATP synthesis.

Key Figure

Summary of the Proposed Functions of the Warburg Effect

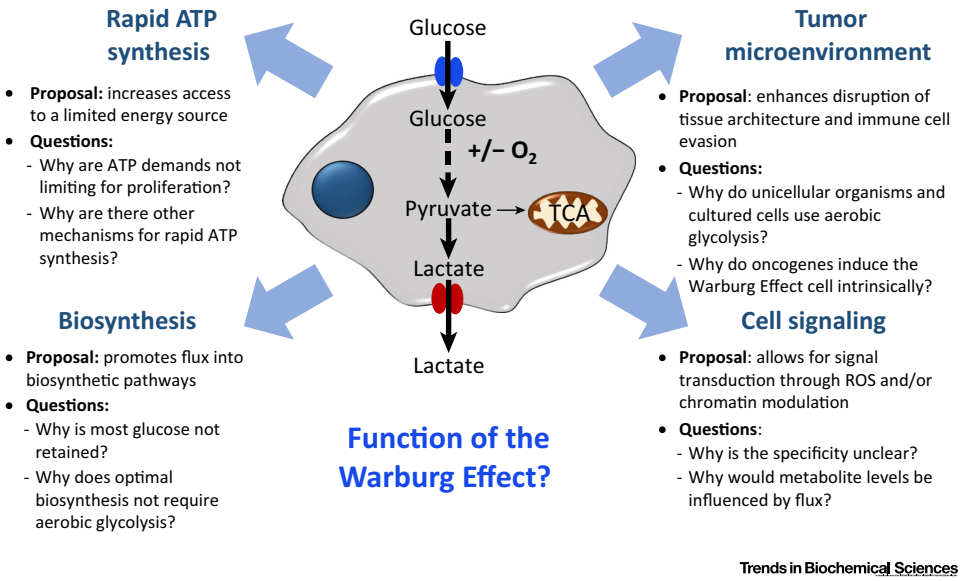

Figure 2. The Warburg Effect is defined as an increase in the rate of glucose uptake and preferential production of lactate, even in the presence of oxygen. Each of these functions has been hypothesized to be the function of the Warburg Effect. Abbreviations: ROS, reactive oxygen species; TCA, tricarboxylic acid cycle.

Despite this attractive proposal, there are difficulties. Simple empirical calculations indicate that the amount of ATP required for cell growth and division may be less than that required for normal cellular maintenance [18,25]. Thus, ATP demand may never reach limiting values during tumor cell growth. Furthermore, the mechanisms that are available to other cell types in cases of rapid ATP demand are also present in tumor cells. For example, rapid ATP synthesis from creatine kinases in exercised muscle or adenylate kinase under hormonal changes are present in most tumor cells and should be able to meet ATP demand. Thus, further studies are needed to show whether this mechanism can account for the role of aerobic glycolysis.

Warburg Effect and Biosynthesis

The Warburg Effect has been proposed to be an adaptation mechanism to support the biosynthetic requirements of uncontrolled proliferation (Figure 2). In this scenario, the increased glucose consumption is used as a carbon source for anabolic processes needed to support cell proliferation [17,26–32]. This excess carbon is diverted into the multiple branching pathways that emanate from glycolysis, and is used for the generation of nucleotides, lipids, and proteins. One example is the diversion of glycolytic **flux** into *de novo* serine biosynthesis through the enzyme phosphoglycerate dehydrogenase (PHGDH) [18]. In addition to the usage of additional carbon from enhanced glucose metabolism for cellular building blocks, a now famous argument is that, rather than having a rate-limiting demand for ATP, proliferating cells are in greater need of reducing equivalents in the form of **NADPH**. Increased glucose uptake allows for greater synthesis of these reducing equivalents in the oxidative branch of the pentose phosphate pathway, which are then used in reductive biosynthesis, most notably in *de novo* lipid synthesis [17,33].

Another proposed mechanism to account for the biosynthetic function of the Warburg Effect is the regeneration of NAD^+ from **NADH** in the pyruvate to lactate step that completes aerobic glycolysis. In this scenario, NADH that is produced by glyceraldehyde phosphate dehydrogenase (GAPDH) must be consumed to regenerate NAD^+ to keep glycolysis active. This high rate of glycolysis allows for supply lines to remain open that can, for example, siphon 3-phosphoglycerate (3PG) to serine for one-carbon metabolism-mediated production of NADPH and nucleotides [17,25]. These proposals together conclude that the Warburg Effect supports a metabolic environment that allows for rapid biosynthesis to support growth and proliferation.

Furthermore, others have proposed that aerobic glycolysis is a tradeoff to support biosynthesis [34,35,62]. In these scenarios, the inefficient way of making ATP occurs as a cost of maintaining high fluxes through anabolic pathways. These pathways require increased expression of biosynthesis genes, such as those involved in nucleotide and lipid metabolism, and the tradeoff occurs by limiting the use of mitochondria to preserve the high expression of biosynthetic enzymes in the face of the limited number of proteins that can be made. Another scenario of such a tradeoff comes from the idea that the physical volume available per cell may limit mitochondria number and, thus, any requirements for energy and biomass that exceed the limited mitochondrial capacity needs to be produced from aerobic glycolysis [36–38]. This concept has been termed the 'solvent capacity constraint'. In both these cases, the Warburg Effect is an adaptation to support biomass production in the face of limited options for ATP generation.

The attractiveness of this proposal comes in part from its ability to provide a simple explanation for the apparent correlation between aerobic glycolysis and cell growth and proliferation. Furthermore, it appears intuitive to some that the branching pathways from glycolysis would be used to a greater extent during the Warburg Effect, since the rate of glycolysis is larger and lactate production in this case would serve to regenerate NAD^+ to allow for glycolysis to continue. Also, the requirements of NADPH for lipid generation can be summarized in a very simple chemical equation showing that the demand for NADPH is higher than that of ATP for biosynthesis [17].

However, there are major limitations for this proposed function of the Warburg Effect. First, during aerobic glycolysis, most of the carbon is not retained and is instead excreted as lactate [25]. In fact, the overall equation of one glucose molecule being converted into two lactate molecules with no overall gain or loss of NAD^+ and NADH leaves no room for biomass. That is, due to the stoichiometry of glycolysis, biomass production is mutually exclusive with lactate generation and it is not possible for the regeneration of NAD^+ by lactate alone to account for biosynthesis. Thus, the avenues that lead to the biosynthesis from glucose occur in the complete absence of making lactate, which is a hallmark of the Warburg Effect. Also, it is now widely accepted that mitochondria are key components of the biosynthetic program whose substrates in the tricarboxylic acid (TCA) cycle are used for nucleotide, amino acid, and lipid biosynthesis [39,40]. In light of this evidence, it remains difficult to fathom how the Warburg Effect can directly promote biosynthesis.

Regarding proposals that define the Warburg Effect as a tradeoff to promote biosynthesis, recent estimates from quantitative proteomics show that the cost of protein production for conducting aerobic glycolysis is enormous. In fact, cells devote as much as 10% of their entire proteome and half of all of their metabolic genes to produce proteins involved in glycolysis [41]. By contrast, biosynthetic programs in cells require lower amounts of protein. Thus, the cost of producing proteins for aerobic glycolysis is as large, if not larger, than the cost of producing proteins for biosynthesis. These proposals are further challenged by the evidence showing that mitochondrial functions occur concomitantly with the Warburg Effect and, thus, limiting mitochondrial activity appears not to occur during the Warburg Effect. Ultimately, further

research is needed to elucidate whether the Warburg Effect functions to support biosynthetic programs.

Warburg Effect and the Tumor Microenvironment

Separate from the cell-intrinsic functions described in the previous sections, the Warburg Effect may present an advantage for cell growth in a multicellular environment. Acidification of the microenvironment and other metabolic crosstalk are intriguing possibilities. Elevated glucose metabolism decreases the pH in the microenvironment due to lactate secretion (Figure 2) [42]. The potential benefits of acidosis to cancer cells are multifold. An acid-mediated invasion hypothesis suggests that H^+ ions secreted from cancer cells diffuse into the surrounding environment and alter the tumor–stroma interface, allowing for enhanced invasiveness [42,43]. A recent study showed that tumor-derived lactate is a contributor to M2 tissue-associated macrophage (TAM) polarization [44]. Also, as briefly mentioned the availability of glucose appears to be a result of direct competition between the tumor and tumor-infiltrating lymphocytes (TIL) [22,23]. The high rates of glycolysis limit the availability of glucose to TILs, which require sufficient glucose for their effector functions. Supporting this proposal is direct evidence indicating that targeting aerobic glycolysis in the tumor has the added benefit of increasing the supply of glucose to TILs, thus boosting their main function, which is to eradicate the tumor cells. Together, this body of evidence indicates that tumor cells can communicate with cells in the immune system to support protumor immunity.

It is likely that the Warburg Effect provides an overall benefit that supports a tumor microenvironment conducive to cancer cell proliferation. However, the Warburg Effect is thought to be an early event in oncogenesis that is an immediate consequence of an initial oncogenic mutation, such as that of *KRAS* in pancreatic cancer or *BRAF* in melanoma; thus, it occurs not only before cell invasion, but also in benign and early-stage lesions [45,46]. Another issue is that, in conditions completely isolated from the environment, such as in the growth phase of unicellular yeast, the Warburg Effect remains the choice of energy metabolism from glucose [38]. Altogether, these data suggest that non-cell-intrinsic functions of the Warburg Effect are insufficient to entirely explain its functions.

The Warburg Effect and Cell Signaling

We and others have proposed that the Warburg Effect confers direct signaling functions to tumor cells [18,39,47–49]. This proposal is particularly attractive since it identifies a direct causal role of altered glucose metabolism in promoting tumorigenesis as a result of this signal transduction affecting other cellular processes. Two areas of signaling function are the generation and modulation of **reactive oxygen species** (ROS) and the mediation of chromatin state. Other studies have identified additional possible signaling mechanisms [23,50].

Maintaining the appropriate balance of ROS is essential [51]. Excessive ROS damages cell membranes and nucleic acids, and has other deleterious effects. Insufficient ROS disturbs signaling processes that are beneficial for cell proliferation, such as by inactivating phosphatase and tensin homolog (PTEN) and tyrosine phosphatases. The Warburg Effect causes alterations in mitochondrial redox potential, ultimately changing ROS generation [18].

An important determinant of redox potential in cells is the NADH that is available in the mitochondria for electron transport. Cellular mechanisms to maintain redox homeostasis are in place when glycolysis rates fluctuate. Up to a certain extent of glycolysis, the malate–aspartate shuttle through the mitochondria is able to restore the NADH imbalance [18]. However, when glycolysis rates are faster than can be accommodated by the malate–aspartate shuttle, the conversion of pyruvate into lactate via lactate dehydrogenase (LDH) is able to regenerate NAD^+. This process may also affect the homeostasis of ROS generation by affecting the concentration

of reducing equivalents in the mitochondria (Figure 2) [18,52]. This consequence of the Warburg Effect may be directly involved in oncogene-induced senescence (OIS) [53]. OIS has a tumor-suppressive cellular function and a recent study reported that increased glucose oxidation through pyruvate dehydrogenase (PDH) can regulate OIS. This finding shows that the redox balance of NADH may contribute to direct signaling roles for the Warburg Effect.

In addition, metabolic pathways that stimulate redox homeostasis are upregulated alongside the Warburg Effect. For example, the pentose phosphate pathway coming from glycolysis generates NADPH. *De novo* serine metabolism, which feeds into the one-carbon metabolism, produces NADPH and glutathione, which modulate ROS levels [54,55]. Together, these findings provide direct biochemical links between aerobic glycolysis and ROS availability that could in turn affect myriad signaling processes.

In addition to cell signaling through ROS, a signaling link between glucose metabolism and histone acetylation has been well documented [56–59]. The status of chromatin structure is responsible for regulating different cellular functions, including DNA repair and gene transcription. It has been established that acetyl-CoA, the substrate for histone acetylation, can be regulated by glucose flux [59]. Studies have shown that there is a direct link between cellular metabolism and regulation of growth genes, and that intracellular acetyl-CoA levels may represent a widely conserved mechanism that promotes this important link [60]. The activity of ATP-citrate lyase, the enzyme responsible for converting citrate into acetyl-coA, can influence histone acetylation levels [47]. Elevated levels of acetyl-CoA may be enough to drive cells into the growth phase via histone acetylation [56]. Removal of glucose or reduction of ATP-citrate lyase results in loss of acetylation on several histones and causes decreased transcription of genes involved in glucose metabolism. This indicates that there is some interplay between glucose metabolism and histone acetylation. Supporting this idea, glycolytic metabolism has been found to impact chromatin structure [58].

In addition to histone acetylation responding to glucose availability in cells, deacetylation can also be influenced by nutrient availability [39]. Deacetylation has an important role in nutrient sensing and signaling since the activity of multiple deacetylases is modulated by NAD^+ levels. Also, the NAD^+:NADH ratio increases in nutrient-deprived conditions [39,56,57]. Therefore, both acetylation and deacetylation may be influenced by nutrient availability, indicating that their statuses may be consequences of the Warburg Effect. These multiple lines of evidence point to glycolysis having cell signaling functions.

However, difficulties also limit this proposal from being the general mechanism that benefits cancer cells by undergoing aerobic glycolysis. One such limitation is that it is hard to imagine how molecular specificity arises through such a gross global signaling mechanism. In contrast to, for example, growth factor signaling in which ligand binding to a substrate induces conformational and enzymatic activity changes that affect specific cellular processes, a mechanism whereby the state of glycolysis signals to other cellular processes lacks obvious sources of specificity. Another limitation is that such proposals typically lack falsifiability. This means it is difficult to design experiments to conclusively show that a specific signaling mechanism, such as chromatin structure modulation, directly comes from the status of glucose metabolism as the key benefit for aerobic glycolysis. One reason for this is that the biochemical interaction occurs rapidly but the cellular phenotypic alterations evolve over longer timescales, resulting in many confounding factors that occur along the way. Genetic models that could test these hypotheses are difficult to conceive, and other experiments lack the ability to test whether specific cellular outcomes occur through such signaling mechanisms and not through indirect means. The extent to which these general features, such as ROS signaling homeostasis and chromatin structure organization, are key events in tumorigenesis also remains unclear [61]. In the future, such specificity and ability to experimentally test these hypotheses may come from observing quantitative aspects of the mechanism, as

has been shown in other studies of signal transduction. Experiments that can precisely control the levels of acetyl-CoA and ROS could allow researchers to decouple many of the downstream effects of the Warburg Effect.

Concluding Remarks

Extensive research on the Warburg Effect and its functions in cancer cells has advanced our understanding of its causes and requirements for tumor cell proliferation [29,52]. However, we argue that it has left us with a surprising lack of clarity regarding its ontology. These uncertainties should challenge us to better understand its function in promoting tumor growth. It is likely that we will require a better understanding of the biology of Warburg Effect if therapeutic advances are to be made in treating and preventing cancer using dietary and pharmacological intervention in metabolism, and in using glucose metabolism to manipulate the immune system, which are currently subjects of intense interest (see Outstanding Questions).

Outstanding Questions

How does the Warburg Effect promote the development of Cancer?

How does the Warburg Effect impose dependencies on tumor growth?

How can experimental systems be devised that can conclusively test the proposals for the function of the Warburg Effect?

Does resolution of any given function of the Warburg Effect have immediate therapeutic consequences?

Does the function of the Warburg Effect provide insights into its role in tumor evolution?

Do the requirements of the Warburg Effect provide clues for its function?

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