HHS Public Access

Author manuscript

Mol Cell. Author manuscript; available in PMC 2023 January 31.

Published in final edited form as:

Mol Cell. 2022 September 01; 82(17): 3119–3121. doi:10.1016/j.molcel.2022.08.004.

The Warburg effect: saturation of mitochondrial NADH shuttles triggers aerobic lactate fermentation

Hyllana C. D. Medeiros[1], **Sophia Y. Lunt**[1,2,*]

[1]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, 48824, USA

[2]Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, 48824, USA

Abstract

In this issue of *Molecular Cell,* Wang *et al.* investigate the Warburg effect in proliferating cells and demonstrate that lactate fermentation is a secondary mechanism activated after mitochondrial shuttles exceed their capacity to oxidize cytosolic NADH.

The Warburg effect, also known as aerobic glycolysis and aerobic fermentation, was first described in the 1920s by Otto Warburg when he observed that cancer cells consume more glucose and ferment it to lactate even in the presence of oxygen (Warburg, 1925). This is puzzling from an energy perspective, as generating adenosine 5′-triphosphate (ATP) via glucose fermentation is less efficient than using mitochondrial oxidative phosphorylation (OXPHOS). Warburg concluded that cancers cells shift ATP production from OXPHOS to aerobic glycolysis due to mitochondrial dysfunction. However, many cancer cells have functional and active mitochondria (Liberti and Locasale, 2016; Zhang et al., 2018). Moreover, the Warburg effect has also been observed in proliferating normal cells. Several hypotheses have been proposed to explain the Warburg effect: rapid ATP synthesis, generation of biosynthetic building blocks, acidification of the microenvironment, metabolic crosstalk, and cell signaling by modulating reactive oxygen species and histone acetylation (Liberti and Locasale, 2016; Lunt and Vander Heiden, 2011). A century after Warburg's initial observation, the scientific community has yet to reach a consensus on the purpose of aerobic glycolysis. In this issue of *Molecular Cell*, Wang and colleagues (Wang, 2022) unravel the question of aerobic lactate fermentation.

The authors first surveyed lactate excretion, metabolic fluxes, and proliferation rates in NCI-60 cells, a collection of 60 different human tumor cell lines. They found no correlation between lactate excretion and proliferation. Interestingly, lactate excretion rates were strongly correlated with all components of the malate-aspartate shuttle (MAS), a main shuttle for translocating electrons produced in the cytoplasm into the mitochondria.

* Corresponding Author contact information: Sophia Y. Lunt, Ph.D., Biochemistry Building, 603 Wilson Rd Room 522A, Michigan State University, East Lansing, MI 48824, USA, sophia@msu.edu.

Declaration of Interests

Sophia Y. Lunt is a member of the Van Andel Institute Metabolism & Nutrition Program Internal Advisory Board.

Based on these results, the authors hypothesized that lactate production is driven by the need to regenerate reducing equivalents when NADH shuttles are saturated. In the sixth step of glycolysis, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) reduces cytosolic NAD^+ to NADH while oxidizing glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate. For glycolysis to continue, NAD^+ must be regenerated as a substrate for GAPDH. There are three major mechanisms to oxidize NADH to NAD^+ in the cytosol: 1) malate dehydrogenase 1 (MDH1), a component of the MAS, 2) glycerol 3-phosphate dehydrogenase 1 (GPD1), a component of the glycerol 3-phosphate shuttle (G3PS), and 3) lactate dehydrogenase (LDH), which converts pyruvate to lactate. Since NAD^+ and NADH cannot directly cross the inner mitochondrial membrane, the MAS and the G3PS transfer electrons from NADH in the cytosol to the mitochondria.

To further investigate the correlation between lactate excretion and NADH shuttles, Wang *et al.* increased the capacities of the MAS and the G3PS through overexpression of MDH1 and GPD1, respectively. They found that increasing the activity of the MAS and the G3PS decreased lactate production without decreasing the rate of cellular proliferation. Consistently, MDH1 or GPD1 knockdown increased lactate excretion, suggesting the activities of the MAS and the G3PS regulate lactate production. Treating the cells with an external electron acceptor, α-ketobutyrate (AKB), provides cells with an additional pathway for oxidizing NADH to NAD^+ by reducing AKB to α-hydroxybutyrate (AHB). AKB addition led to decreased lactate excretion and increased cellular proliferation, demonstrating that lactate production is not essential for cellular proliferation as long as there are sufficient alternative sources for regenerating cytosolic NAD^+. Moreover, *in vivo* studies in nude mice showed that overexpression of MDH1 increased tumor growth rates. Intratumoral infusion studies with [U-^{13}C] glucose in mice revealed that in tumors overexpressing MDH1, the relative amount of ^{13}C-labeled lactate was lower while ^{13}C-labeled citrate was higher compared to the amount of ^{13}C-labeled pyruvate. These results indicate that increased MDH1 expression in tumors diverts glucose away from lactate fermentation and towards oxidation by the tricarboxylic acid cycle and OXPHOS.

To determine the preferred mechanism of NAD^+ regeneration, the authors used low doses of the glycolytic inhibitor 2-deoxygluose to reduce glycolytic flux and found that most of the NADH produced by GAPDH in proliferating cells was oxidized by the MAS and the G3PS. LDH only became activated after the rate of NAD^+ regeneration by the MAS and the G3PS were saturated, although total NADH levels oxidized by LDH versus MDH1 or GPD1 vary between cell lines. Overall, the experiments support that the MAS and the G3PS are the preferred mechanisms for regenerating cytosolic NAD^+ until glycolysis outpaces the maximum flux of MDH1 and GPD1, at which point LDH becomes the primary mechanism of NAD^+ regeneration.

Normal proliferating cells also exhibit aerobic glycolysis (Lunt and Vander Heiden, 2011). Therefore, the authors examined non-transformed 3T3 cells during proliferation and quiescence. In proliferating cells, saturation of the MAS and the G3PS were also found to drive lactate production. During quiescence, the MAS is not saturated, and the G3PD does not play a significant role. Increased LDHA expression was observed upon saturation of MDH1 and GPD1 in both transformed and non-transformed proliferating cells. Indeed,

glucose fermentation regenerates cytosolic NAD^+ when NADH shuttles are insufficient in skeletal muscle, heart, and the brain (Kane, 2014). It remains to be uncovered whether NADH shuttle saturation triggering lactate production is common in all cells that display aerobic glycolysis, including immune cells as well as pathogens (Chou et al., 2022).

Wang *et al.* demonstrate that aerobic fermentation is a mechanism to support high glycolytic flux when capacities of the MAS and the G3PD are saturated (Figure 1) in both transformed and non-transformed proliferating cells. Their findings are consistent with previous work showing that aerobic glycolysis is driven by an increased demand for NAD^+ relative to ATP (Fernandez-de-Cossio-Diaz and Vazquez, 2017; Luengo et al., 2021) and support that mitochondrial metabolism is in fact elevated, not impaired, during proliferation (Ahn and Metallo, 2015). Building on the authors' discovery that saturation of the MAS and the G3PD triggers aerobic fermentation, future work will reveal why proliferating cells increase expression of LDH but do not increase expression of MDH1 or GPD1 when NAD^+ regeneration becomes limited. Further studies may also clarify the exact purpose of increased glucose consumption during cellular proliferation to improve our understanding of the Warburg effect in tumor progression and guide advanced cancer therapy strategies.

Funding

H.C.D.M. and S.Y.L. acknowledge support from the National Cancer Institute of the National Institutes of Health under Award Number R01CA270136 and the National Institute of Environmental Health Sciences of the National Institutes of Health under Award Number R01ES030695.

References

Ahn CS, and Metallo CM (2015). Mitochondria as biosynthetic factories for cancer proliferation. Cancer Metab. 3, 1. 10.1186/s40170-015-0128-2. [PubMed: 25621173]

Chou W-C, Rampanelli E, Li X, and Ting JP-Y (2022). Impact of intracellular innate immune receptors on immunometabolism. Cell. Mol. Immunol. 19, 337–351. 10.1038/s41423-021-00780-y. [PubMed: 34697412]

Fernandez-de-Cossio-Diaz J, and Vazquez A (2017). Limits of aerobic metabolism in cancer cells. Sci. Rep. 7, 13488. 10.1038/s41598-017-14071-y. [PubMed: 29044214]

Kane DA (2014). Lactate oxidation at the mitochondria: a lactate-malate-aspartate shuttle at work. Front. Neurosci. 8. 10.3389/fnins.2014.00366. [PubMed: 24550770]

Liberti MV, and Locasale JW (2016). The Warburg Effect: How Does it Benefit Cancer Cells? Trends Biochem. Sci. 41, 211–218. 10.1016/j.tibs.2015.12.001. [PubMed: 26778478]

Luengo A, Li Z, Gui DY, Sullivan LB, Zagorulya M, Do BT, Ferreira R, Naamati A, Ali A, Lewis CA, et al. (2021). Increased demand for NAD+ relative to ATP drives aerobic glycolysis. Mol. Cell 81, 691–707.e6. 10.1016/j.molcel.2020.12.012. [PubMed: 33382985]

Lunt SY, and Vander Heiden MG (2011). Aerobic Glycolysis: Meeting the Metabolic Requirements of Cell Proliferation. Annu. Rev. Cell Dev. Biol. 27, 441–464. 10.1146/annurev-cellbio-092910-154237. [PubMed: 21985671]

Warburg O (1925). The Metabolism of Carcinoma Cells. J. Cancer Res. 9, 148–163. 10.1158/jcr.1925.148.

Wang Y, Stancliffe E, Gowle-Grider R, Wang R, Wang C, Schwaiger-Haber M, Shriver LP, and Patti GJ (2022). Saturation of the mitochondrial NADH shuttles drives aerobic glycolysis in proliferating cells. Mol Cell.

Zhang G, Darshi M, and Sharma K (2018). The Warburg Effect in Diabetic Kidney Disease. Semin. Nephrol. 38, 111–120. 10.1016/j.semnephrol.2018.01.002. [PubMed: 29602394]

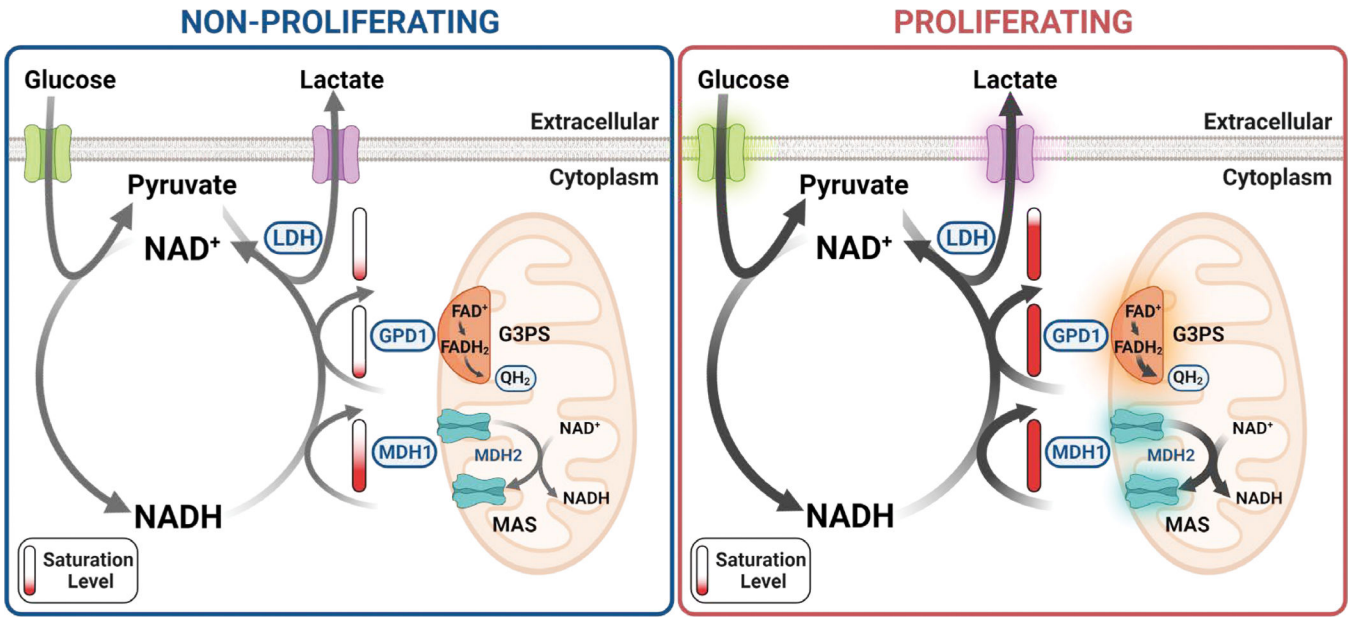

Figure 1. Aerobic fermentation is driven by saturation of the NADH shuttles MAS and G3PS in proliferating cells.

Wang *et al.* analyzed proliferating cancer cells as well as non-transformed cells to investigate the Warburg effect. To continue glycolysis, cytosolic NAD^+ levels must be maintained to enable the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase. Non-proliferating cells (left) oxidize NADH to NAD^+ mainly by malate dehydrogenase 1 (MDH1), a component of the malate-aspartate shuttle (MAS) in the inner mitochondrial membrane. MDH1 activity is not saturated in non-proliferating cells. Depending on the cell line and availability of oxygen, non-proliferating cells also use lactate dehydrogenase (LDH) to oxidize NADH. They further oxidize small quantities of NADH using glycerol 3-phosphate dehydrogenase 1 (GPD1), a component of the glycerol 3-phosphate shuttle (G3PS) in the inner mitochondrial membrane. In proliferating cells that exhibit the Warburg effect (right), high rates of glycolysis increase NADH levels, amplifying demand for NADH oxidation. When NAD^+ regeneration by the MAS and the G3PS reach maximum capacity due to MDH1 and GPD1 saturation, LDH expression and activity rise to oxidize excess NADH, elevating lactate production. Created with BioRender.com.