

The Warburg effect and mitochondrial oxidative phosphorylation: Friends or foes?

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ABSTRACT

Cancer cells display an altered energy metabolism, which was proposed to be the root of cancer. This early discovery was done by O. Warburg who conducted one of the first studies of tumor cell energy metabolism. Taking advantage of cancer cells that exhibited various growth rates, he showed that cancer cells display a decreased respiration and an increased glycolysis proportional to the increase in their growth rate, suggesting that they mainly depend on fermentative metabolism for ATP generation.

Warburg's results and hypothesis generated controversies that are persistent to this day. It is thus of great importance to understand the mechanisms by which cancer cells can reversibly regulate the two pathways of their energy metabolism as well as the functioning of this metabolism in cell proliferation. In this review, we discuss the origin of the decrease in cell respiratory rate, whether the Warburg effect is mandatory for an increased cell proliferation rate, the consequences of this effect on two major players of cell energy metabolism that are ATP and NADH, and the role of the microenvironment in the regulation of cellular respiration and metabolism both in cancer cell and in yeast.

Two well-known deviations of cell energy metabolism were described almost a century ago: the Crabtree effect and the Warburg effect. Whereas both lead to a decrease in mitochondrial ATP synthesis rate, they are mechanistically very different. The Crabtree effect is defined as the glucose-induced repression of respiratory flux [1]. The addition of external glucose to Crabtree-sensitive cells triggers in a few seconds the partial inhibition of O₂ consumption, which excludes the involvement of gene expression and de novo protein synthesis. The early discoveries from O. Warburg showed that cancer cells display a decreased respiration along with an enhanced lactate production, whose respective rates correlate with the increase in cellular proliferation. The Warburg effect in cancer cells may require several hours or even days to develop, and hence transcriptional/translational regulations are necessarily involved, which makes it very different from the Crabtree effect. This review will focus on the Warburg effect and H. Crabtree's contribution to studying this effect.

Otto Warburg seminal work on cancer cell energy metabolism has been extensively described. Basically, he studied cancer cells oxidative

phosphorylation flux (cellular respiration) and their glycolytic fluxes as a function of the cancer cells proliferation rate. Fig. 1 has been elaborated using the initial data in O. Warburg's paper [2]. From this figure, one can see a clear correlation between these three parameters: an increase in cancer cells proliferation rate (P1 to P4) is associated with a decrease in oxygen consumption and an increase in glycolytic flux (assessed here as the fermentation flux). This increase in the glycolytic fermentation was shown to occur even when oxygen was plentiful hence the "aerobic glycolysis" term. O. Warburg's interpretation of these data was that in cancer cells, mitochondrial oxidative phosphorylation is impaired, which leads to an increase in glycolytic flux to compensate for the decrease in ATP synthesis flux from mitochondria. This led to a dispute between O. Warburg and his colleagues, notably H. Crabtree, who pursued O. Warburg study assessing the carbohydrate metabolism of several strains of transplantable mouse tumors [1]. Because the dispute was relative to whether mitochondrial oxidative phosphorylation were impaired in cancer cells, great focus was put on cellular respiration and glycolytic flux. A key parameter was forgotten here: the

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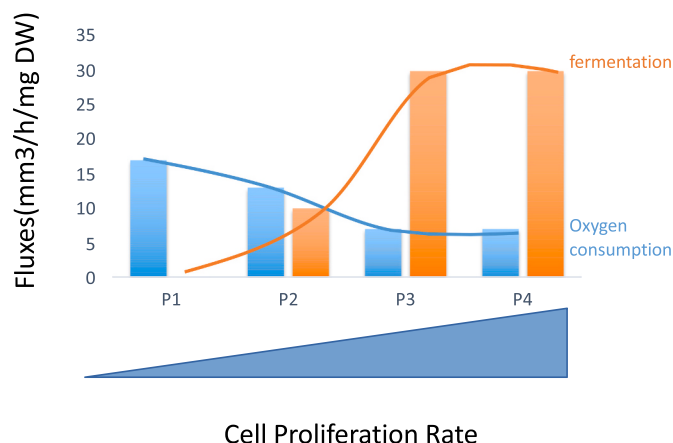


Fig. 1. Illustration of the Warburg Effect. Data are from Warburg's seminal paper [2]. P1 to 4 are normal or cancer cells with increasing proliferation rates. P1 being the lowest proliferation rate. P1: chorion of young embryos, P2: Earle's cancer cells (low malignancy), P3: Earle's cancer cells (high malignancy), P4: ascite cancer cells. Oxygen consumption flux is the amount of oxygen in cubic millimeters that 1 mg of tissue (dry weight) consumes per hour at 38 °C with oxygen saturation, fermentation is the amount of lactic acid in cubic millimeters that 1 mg of tissue (dry weight) develops per hour at 38 °C with oxygen saturation.

cancer cells proliferation rate. However, this key paper by H. Crabtree showed that in the numerous tumors he studied, an important number of tumors exhibited high respiration, “both in its absolute value and also in its relation to the aerobic and anaerobic glycolysis”. This respiration was qualified as being “high” both in comparison with other tumors and with the rate of glycolysis i.e. even if the tumor exhibited a high rate of glycolysis, a high rate of respiration could be measured. Further, the magnitude of the respiration of the tumors grafted subcutaneously was considerably higher than that of the tumors grafted intraperitoneally, showing that the carbohydrate metabolism of tumors is to some extent influenced by the environment in which they grow (see below). The controversy ceased in the 1960' when Warburg admitted that the respiration of cancer cells might be “insufficient” rather than damaged [3].

In this review, we will try to highlight the difficulties encountered when trying to define the energy metabolic deviation of cancer cells. The relevance of the assessed parameters will also be discussed.

1. Mitochondrial oxidative phosphorylation and the Warburg effect

Mitochondrial oxidative phosphorylation is a highly regulated process, both thermodynamically and kinetically [4]. Within a cell, two nonmutually-exclusive ways regulate mitochondrial respiratory rate, a kinetic regulation of oxidative phosphorylation [5] and/or a regulation of the amount of mitochondrial respiratory chain units [6,7]. This allows for ATP synthesis to be adjusted according to cellular energy demand. In order to distinguish between an impairment of mitochondrial oxidative phosphorylation and a regulation of this process, one has to characterize the origin of the modulation in cellular respiratory rate. To determine the amount of mitochondrial respiratory chain units, quantifying mitochondrial cytochromes aa3, b and cc1 has proven quite reliable [6,7]. However, the inconvenient of that measurement is that it requires a significant number of cells. Mitochondrial citrate synthase activity as well as mitochondrial cytochrome oxidase activity require less material and have been used for decades now in the field of mitochondrial pathologies [8]. A kinetic regulation of oxidative phosphorylation can be first investigated using well-known modulators of their function such as oligomycin that inhibits mitochondrial ATP synthase (basal respiratory rate, controlled by the mitochondrial inner membrane permeability to

protons) and an uncoupler such as CCCP or DNP, which will allow to determine the maximal respiratory rate that can be achieved by the cells, given that there is no kinetic control upstream the respiratory chain. To provide an example, a correlated increase (or decrease) in basal cellular respiration, maximal respiration rate and mitochondrial cytochromes content, will point to an increase (or decrease) in mitochondrial respiratory chain units. In contrast, the same observations without any modification of the mitochondrial cytochrome content, will point to a kinetic regulation of oxidative phosphorylation. It should be stressed here that a mitochondrial defect impairing the activity of one of the respiratory chain complexes, will exhibit similar features as a kinetic regulation. This points out the complexity of both the system and the difficulties relatives to the interpretation of the results.

This highlights the fact that a decrease in cellular respiration does not necessarily originate in a defect in mitochondrial function (Warburg's hypothesis). Further, subsequent studies have clearly shown that oxidative phosphorylation are functional in numerous cancer cells lines where cellular respiration is coupled to ATP synthesis and can be uncoupled [9,10]. In some cancer cells, the decrease in respiratory rate was shown to be highly dependent on the carbon substrate provided to the cells, and reversible when glucose was replaced with galactose (a carbon substrate that leads to restriction of the glycolytic flux due to an important kinetic control on the first steps of its metabolism) [11].

The mechanisms leading to a decrease in cellular respiration in cancer cells are multiple and may vary from one tumor to another. Only a few will be reviewed here that illustrate our point.

Pyruvate dehydrogenase (PDH) is a convergence point between glucose and fatty acids oxidation. PDH converts pyruvate to acetyl-coA that feeds the TCA cycle and thus mitochondrial oxidative phosphorylation with NADH and FADH₂. This enzyme was shown to be highly down-regulated when phosphorylated by pyruvate dehydrogenase kinase (PDK) [12]. This leads to an increase in kinetic control upstream of the respiratory chain and decreases cellular respiration. Decreased mitochondrial metabolism caused by inhibition of PDH by PDK was found to be involved in many diseases, including cancers and therefore in the Warburg effect [13,14]. PDK has four isoforms that have different phosphorylating site specificities and binding affinities for PDH [15]. These isoforms have all been linked to cancer and contribute to the Warburg phenotype [16]. The expression of PDK1 and PDK3 is up-regulated in low levels of oxygen [17,18] and PDK2 and PDK4 expression is upregulated in low nutrient conditions [19], both conditions being well-known characteristics of tumor environment. Since PDH is inhibited by PDK isoforms over-expression in cancer cells, therapeutic strategies have been developed to specifically inhibit the activity of PDKs in order to restore PDH activity and therefore enhance cellular respiration [16]. For example, the restoration of the PDH activity by the knock-down of PDK1 reverts the Warburg phenotype and decreases tumor growth [20]. However, decrease in proliferation rate upon PDH activation also occurs in non-cancerous cells [20–22]. This suggests that even though PDH inhibition might play a role in the Warburg's phenotype of cancer cells, it is not the primary cause of this effect.

HIF1 α is a well-known transcription factor involved in the cellular response to hypoxia [23]. Long-standing evidence suggests it is involved in the pathogenesis of various solid tumors [24]. Hypoxia-induced stabilization of this transcription factor activates a subset of genes involved in the regulation of cellular energy metabolism and this reprogramming eventually leads to an increase in glycolytic flux and a decrease in mitochondrial respiration. Consequently, this transcription factor was long thought to be at the origin of the observed Warburg effect. However, two major questions remained unanswered: 1) if HIF1 α induced transcriptional reprogramming leads to a Warburg effect, how was this effect induced in conditions where oxygen is plentiful and thus where HIF1 α cannot be stabilized? 2) does this induction lead to an increase in glycolysis and a decrease in oxidative phosphorylation associated with an increased proliferation rate? The first question was resolved by the discovery of oncometabolites such as fumarate, succinate and 2-

hydroxyglutarate, that accumulate when succinate dehydrogenase is mutated [25], which were shown to be able to stabilize HIF1 α even when oxygen is plentiful. It should be stressed here that this is a situation where mitochondria are, as a consequence of succinate dehydrogenase mutation, indeed defective (Warburg's hypothesis) and where a consequent rewiring of Krebs cycle intermediary metabolites fluxes occurs. Further, Rasola and coworkers showed that succinate dehydrogenase can be inhibited by upregulation of mitochondrial TRAP1, as seen in a variety of tumors [26–28]. Thus, oncometabolites can accumulate even with a perfectly functional succinate dehydrogenase. The second interrogation is more difficult to address with data from the literature where the three parameters involved in the Warburg effect are rarely assessed at the same time and in the same experimental conditions.

Our group investigated the induction of the Warburg effect in yeast. The rationales behind that study were multiple. First, in vitro studies conducted on cancer cells are most of the time done in both hyperoxia and hyperglycemia, affecting two crucial intermediates of cell energy metabolism (oxygen and glucose). Second, when one studies energy metabolism on a cancer cell, the Warburg effect is already established and the processes leading to this profound remodeling of energy metabolism cannot be analyzed. Last, this model allowed us to simultaneously assess the three key parameters of the Warburg effect in identical experimental conditions. We showed that the Warburg effect can be reconstituted in yeast upon glucose addition to cells growing on non-fermentable carbon substrates [29]. The full effect is induced in about 4 h. A decrease in oxidative phosphorylation associated to an increase in glycolysis and proliferation rate were evidenced. To analyze the origin of the decrease in oxidative phosphorylation rate, mitochondrial cytochromes and cellular respiratory rate were assessed. We were able to show a linear relationship between these two parameters which points to a decrease in respiratory chain units being at the origin of the decrease in cellular respiration (Fig. 2). This was further confirmed by evidencing a decrease in mitochondrial biogenesis upon glucose addition to cells [29].

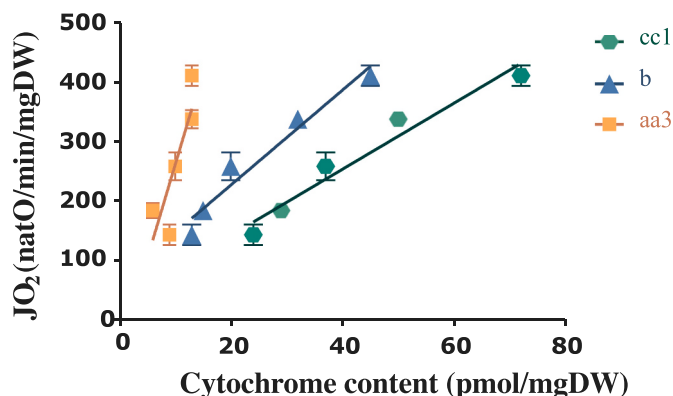


Fig. 2. Relationship between respiratory rate and mitochondrial cytochromes. Data are from Bouchez et al. 2020 [29]. The growth medium of *S. cerevisiae* was supplemented with 60 mM of glucose at T0. Cytochrome content was quantified every 2 h in cells in the presence of glucose in the medium. The cellular content of mitochondrial cytochromes $c + c_1$, b and $a + a_3$ was calculated as described in Dejean et al. [49] considering the respective molar extinction coefficient values and the reduced-minus-oxidized spectra recorded using a dual beam spectrophotometer (Varian, Cary 4000). Oxygen consumption was measured polarographically at 28 °C using a Clark oxygen electrode in a 1 mL thermostatically controlled chamber. 1 mL of culture was transferred to the chamber and respiratory rates (JO_2) were determined from the slope of a plot of O_2 concentration vs. time. The measured activities are normalized per mg dry weight. Respiration assays of growing cells were performed in the growth medium. A linear regression between respiratory rate and cytochrome content is presented for each cytochrome. Results shown represent means of at least four separate experiments $\pm$ SD.

2. Is the Warburg effect dispensable for cell proliferation?

As stated above, the Warburg effect is the result of the simultaneous changes of three parameters: growth, respiration and glycolysis. If one of these parameters does not evolve accordingly to what is expected then the dysregulation of energy metabolism can no longer be referred to as a Warburg effect. In order to determine whether the Warburg effect was dispensable for tumor cell growth, both glycolysis flux and mitochondrial oxidative phosphorylation have been targeted. The potential of oxidative phosphorylation upregulation in decreasing cell proliferation rate has been investigated both in tumor cells and in yeast.

One of the main functions of PGC-1 α is the control of energy metabolism, which is achieved by acting both on mitochondrial biogenesis and oxidative phosphorylation. This is confirmed by numerous in vitro and in vivo studies that have demonstrated that PGC-1 α is involved in mitochondrial biogenesis. This has been very well reviewed by Frederic Bost and Lisa Kaminski [30] and only key points will be addressed here. Overexpression of PGC-1 α in adipocytes, muscle cells, cardiac myocytes and osteoblasts leads to an increase in the amount of mitochondrial DNA [30] for review [31–34]. PGC-1 α initiates mitochondrial biogenesis by activating transcription factors that regulate the expression of mitochondrial proteins that are encoded by nuclear DNA [35]. Since a decrease in cellular respiration was evidenced in tumor cells, investigators overexpressed or upregulated PGC1 α in these cells to restore mitochondrial activity and assess its consequences on tumor cell growth. Unfortunately, the consequences of such an increase in PGC1 α activity depend on the type of cancer. In melanoma and breast cancer cells for example, PGC1 α overexpressing cells exhibit a high rate of mitochondrial oxidative metabolism and a significant proliferative and survival potential associated to a decrease in the invasive properties of these cells [36,37]. In other cancers, including hepatocarcinoma [38], colon cancer [39–41], renal cell carcinoma [42] and ovarian cancer [43], overexpression of PGC1 α in cell lines inhibits proliferation.

In our yeast model, as stated above, we were able to show that the decrease in cellular respiration upon establishment of the Warburg effect was due to a decrease in mitochondrial biogenesis. Ectopic overexpression of HAP4p-the yeast analog of PGC1 α - associated to the deletion of the *hxx2* isoform of hexokinase enabled us to generate a strain where glucose addition did not significantly alter cellular respiratory rate [29]. However, both proliferation rate and glycolysis flux were increased similarly to the wild type. Since the Warburg effect requires the concurrent modulation of cellular respiration, glycolysis flux and proliferation rate, this strain no longer exhibited a Warburg effect. However, upon glucose addition, this strain was able to grow as fast as a wild type strain with a significant glycolytic flux. This shows that repression of mitochondrial oxidative phosphorylation is not a prerequisite to promote cell growth. This was further confirmed in a yeast strain (*Candida utilis*) known to not exhibit glucose induced oxidative phosphorylation repression [29]. The possibility of a decorrelation between an increase in cell growth and the activity of mitochondrial oxidative phosphorylation is in agreement with the above-mentioned results in cancer cells where overexpression of PGC1 α led to opposite results depending on the cancer origin. Further, results obtained on cancer cells/cell lines seem to highly depend on the kind of cancer. However, as was first evidenced by Crabtree and is now well accepted, the tumor environment plays a crucial role on its metabolism, and it might very well be that conflicting results originate in distinct experimental conditions, with modulation of key parameters that rewire metabolism (see below 4- the microenvironment).

3. Are the Warburg's effect induced modifications in ATP synthesis and NADH reoxidation fluxes necessary for cell proliferation?

Both ATP and NADH are major players of cell energy metabolism. The free energy of the ATP molecule is used for most energy conversion

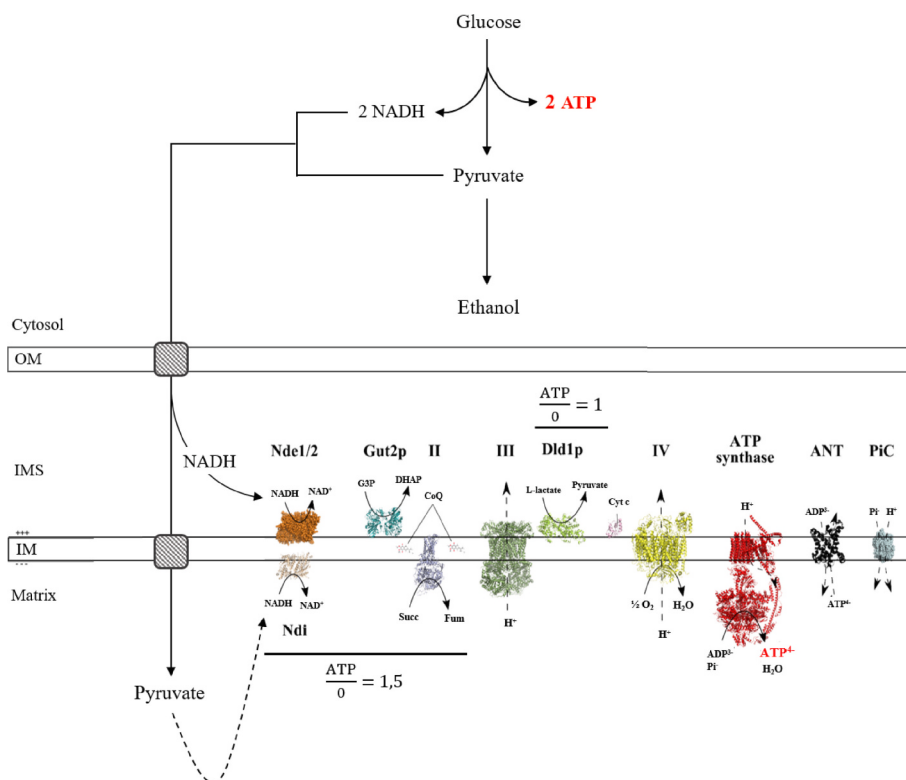
processes and is required for cell growth, maintenance etc. NADH is generated by catabolic pathways and is required for anabolism. Upon establishment of the Warburg effect, ATP synthesis is no longer mostly produced by the high efficiency pathway oxidative phosphorylation but in the cytosol through the low efficiency pathway glycolysis. Whether this metabolic switch has consequences in terms of ATP synthesis flux and/or kinetics remains debated to this day. In terms of NADH re-oxidation flux, the Warburg effect has a major impact since glycolysis will produce NADH and decreasing cellular respiration will decrease oxidative phosphorylation ability to reoxidize this NADH. This is when fermentation of pyruvate to lactic acid (ethanol in yeast) takes place, allowing glycolysis to be redox neutral, when the fermentation flux is two times the glucose consumption flux.

Surprisingly, calculations suggest that the amount of ATP required for mammalian cell growth and division may be far less than that required for basal cellular maintenance [44–46], suggesting that the possible decrease in ATP synthesis rate might not impact cell proliferation.

In our yeast model, we made use of both the glycolytic flux and the cellular respiratory rate that were assessed in the presence or absence of glucose in wild type and mutant strains to calculate the rate of ATP synthesis during cell growth (Scheme 1). Yeast cells were grown on non-fermentable substrate namely lactate before glucose addition. Because yeast mitochondria exhibit a lactate dehydrogenase (LDH) that belongs to the respiratory chain, this allows mitochondrial ATP synthesis from this carbon source. Since this LDH gives its electrons to cytochrome c, there is only one proton pumping coupling site associated to lactate oxidation. Consequently, only one ATP per oxygen consumed will be synthesized in this condition (ATP/O = 1). Assessing the cellular oxygen

consumption flux in the presence or absence of antimycin A allowed us to determine that lactate oxidation accounts for 30 % of the total flux both in the presence and absence of glucose [47]. The 70 % remaining of the flux came from the mitochondrial dehydrogenases. Because yeast has no proton pumping complex I, the ATP/O ratio from these dehydrogenases is constant and was shown to be around 1.5 [48]. The mitochondrial ATP synthesis flux was thus calculated accordingly: 70 % ATP/O = 1.5 and 30 % ATP/O = 1. Regarding the glycolytic ATP synthesis flux, we considered that fermentation of glucose into EtOH leads to the synthesis of 2ATP. This flux might hence be slightly underestimated since we do not consider the pyruvate that will be reoxidized by the mitochondria. To assert that our conclusions were robust, we compared the glycolytic ATP synthesis flux that was determined through fermentation and the one determined from glucose consumption rate. Fig. 3A & B show that there is no clear relationship between the cellular proliferation rate and the ATP synthesis flux. In fact, the ATP synthesis flux under our experimental conditions can sustain almost a doubling of the cell growth rate.

In conclusion, in our model, there is no clear relationship between the growth rate and ATP synthesis flux. More precisely, when mitochondrial oxidative phosphorylation are not repressed upon glucose addition to cells (see above the $\Delta h x k 2$ -HAP4 overexpressing strain), it seems that the increase in ATP synthesis in these cells is not allocated to cell proliferation but rather gets consumed in some “futile cycle”. An explanation to this result might lie in previous studies from our laboratory [49]. This study aimed at increasing mitochondrial content within yeast cell by activating the cAMP/PKA signaling pathway, which is known to be involved in the regulation of mitochondrial biogenesis [50]. Under conditions where the growth rate was already optimal, i.e.



Scheme 1. Determination of the ATP synthesis flux in Yeast. Yeast cells were grown on non-fermentable substrate namely lactate before glucose addition. There is a single site for proton pumping associated to lactate oxidation. Consequently, only one ATP per oxygen consumed will be synthesized in this condition (ATP/O = 1). Assessing the cellular oxygen consumption flux in the presence or absence of antimycin A allowed us to determine that lactate oxidation accounts for 30 % of the total flux both in the presence and absence of glucose. The 70 % remaining of the flux came from the mitochondrial dehydrogenases. Because yeast has no proton pumping complex I, the ATP/O ratio from these dehydrogenases is constant and was shown to be around 1.5. The mitochondrial ATP synthesis flux was thus calculated accordingly: 70 % ATP/O = 1.5 and 30 % ATP/O = 1. Regarding the glycolytic ATP synthesis flux, we considered that fermentation of glucose into EtOH leads to the synthesis of 2 ATP OR glucose consumption flux leads to the synthesis of 2 ATP.

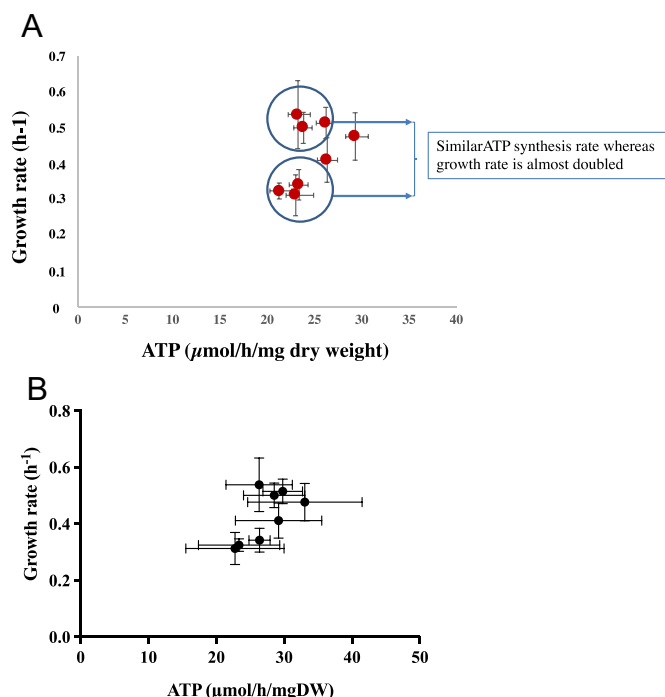


Fig. 3. Relationship between growth and ATP synthesis flux. (A) ATP synthesis flux was calculated from respiratory flux and EtOH synthesis flux. (B) ATP synthesis flux was calculated from respiratory flux and glucose consumption flux. Because yeast has no proton pumping complex I in the mitochondrial respiratory chain, the ATP/O ratio from these dehydrogenases is constant and was shown to be around 1.5 [48]. The mitochondrial ATP synthesis flux was thus calculated accordingly: 70 % ATP/O = 1.5 and 30 % ATP/O = 1. Regarding the glycolytic ATP synthesis flux, we considered that fermentation of glucose into EtOH leads to the synthesis of 2 ATP. Data are from Bouchez et al. 2020 [29]. Results shown represent means of at least three separate experiments $\pm$ SD.

high lactate concentration, exogenous cAMP led to a proliferation of well-coupled mitochondria within cells. This phenomenon was associated with a rise in the respiratory activity. The enthalpy balance of these cells showed an increase in the heat dissipation yield (41 % vs. 33 %) and a large decrease in the biomass yield (21 % vs. 40 %). This shows that the maintenance of the mitochondrial compartment comes at high energetic cost and leads to a decrease in the energy conversion processes efficiency within the cell. Consequently, even though our mutant Δ hxx2-HAP4 overexpressing strain cells exhibit a high respiratory rate due to a high mitochondrial content [29], this is associated to a decrease in its growth yield. This shows that energy conversion efficiency can vary depending on cellular mitochondrial content and excludes any univocal relationship between ATP synthesis flux and cellular proliferation.

NADH reoxidation flux calculation is less prone to errors since it requires fermentation (1 NADH is reoxidized per EtOH produced) and consumption of $\frac{1}{2}$ O₂ is associated with two electrons transferred, hence 1 NADH. Consequently, the NADH reoxidation flux equals the EtOH production flux plus the oxygen consumption flux. Fig. 4 shows that in our yeast model, there is no relationship between NADH reoxidation flux and cell proliferation rate, which shows that this parameter does not control cell proliferation rate. This seems counter-intuitive since upon the Warburg effect induction, only half of the glucose is fermented into EtOH [29]. Notably, this is comparable to what has been assessed in over 60 cancer cell lines [51], the other half of the NADH generated will thus have to be reoxidized by the mitochondria. However, it has been shown that there is quite a reorientation of oxidizing fluxes upon glucose addition to yeast cells. First, studies have shown that in this condition

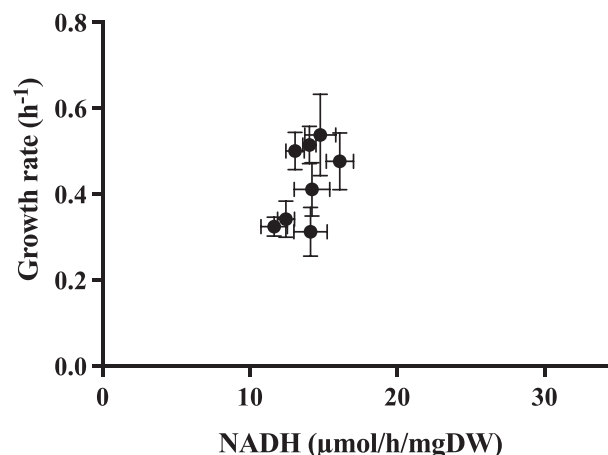


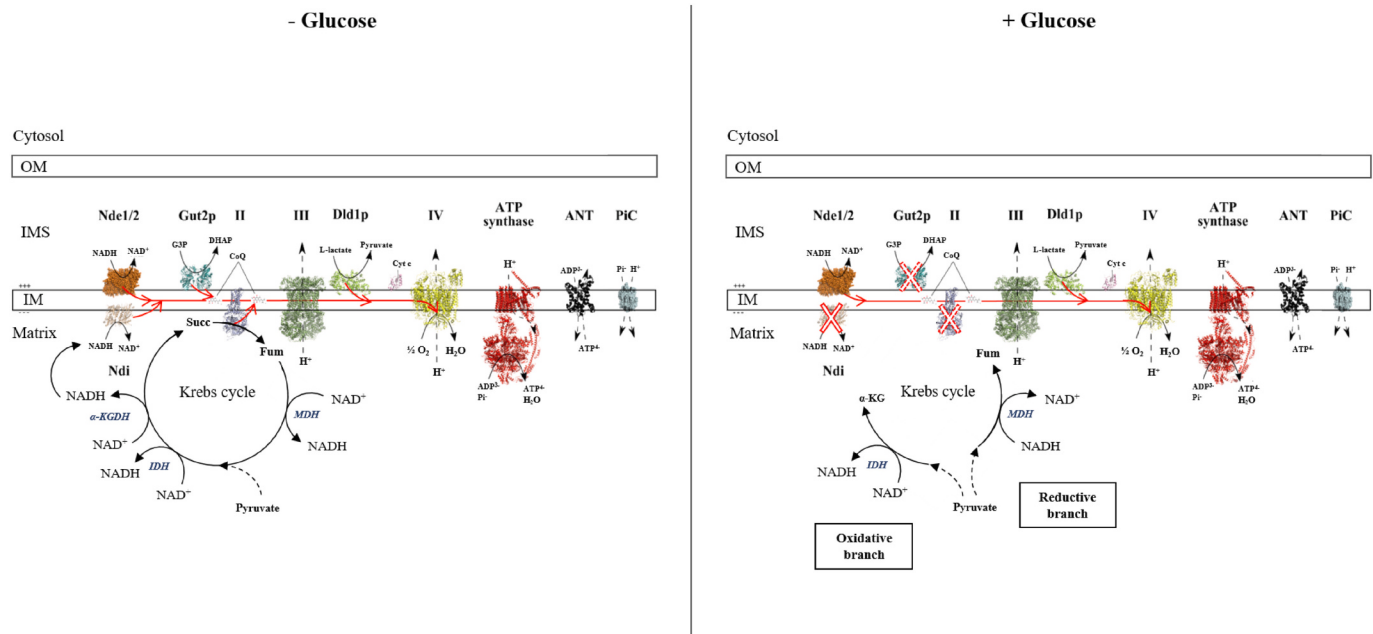
Fig. 4. Relationship between growth and NADH reoxidation flux. (A) NADH reoxidation flux was calculated from respiratory flux and EtOH synthesis flux. One NADH is reoxidized per EtOH produced and consumption of $\frac{1}{2}$ O₂ is associated with two electrons transferred hence 1 NADH. Consequently, the NADH reoxidation flux equals the EtOH production flux plus the oxygen consumption flux. Data are from Bouchez et al. 2020 [29]. Results shown represent means of at least three separate experiments $\pm$ SD.

[52] the Krebs cycle does not act as a cycle but rather as two half cycles, making it redox neutral. Consequently, there is no more NADH generated in the mitochondrial matrix. Second, work from our laboratory has shown that the external NADH dehydrogenase of the yeast respiratory chain has the right of way on other dehydrogenases [53] making the reoxidation of cytosolic NADH a priority for the cell. Scheme 2 illustrates this flux reorientation process that allows for glycolytic NADH to be reoxidized in priority under conditions where mitochondrial activity is decreased and reoxidation flux requirement increases due to glycolytic activity. To our knowledge, it remains to be shown whether such remodeling of NADH reoxidation fluxes occur in cancer cells where pyruvate oxidation could have the right of way on succinate and/or glycerol-3-phosphate for example.

4. The microenvironment

As stated in the introduction, in his 1929 paper, Crabtree investigated the carbohydrate metabolism of several strains of transplantable mouse tumors. These strains were propagated in his laboratory and he observed a great variability in the absolute and relative extent of energy metabolism. He showed that this variability was not to be attributed to the different strains but rather among tumors of the same strain. Tumor metabolism was measured on two series of tumors transplanted either subcutaneously or intraperitoneally. Crabtree's work showed that the respiration of the subcutaneous tumors was a great deal higher than the respiration of the intraperitoneal tumors. Whereas the tumor respirations varied greatly, the anaerobic glycolysis was comparable in both cases. This very early work by Crabtree has been unfortunately poorly followed up. However, it is seminal in the sense that later scientific disagreements regarding mitochondrial oxidative phosphorylation in tumor cells could have been avoided, had this result been common knowledge. This would have allowed scientists to take great care of their experimental conditions/tumor environment when comparing their results. Also, it should be stressed here that our results in our yeast model are in close agreement with these results since whereas the link between the glycolytic flux and growth rate seems pretty tight, mitochondrial oxidative phosphorylation can vary greatly without any major impact on cell proliferation [29].

Another very interesting study highlighting the importance of the tumor cells growing conditions on their metabolism was done recently in lung cancer cells [54]. In this study, the authors infused mice with



Scheme 2. Reorientation of NADH reoxidizing flux in yeast upon glucose addition. There is a reorientation of oxidizing fluxes upon glucose addition to yeast cells. Studies have shown that in this condition [52] the Krebs cycle does not act as a cycle but rather two half cycles, making it redox neutral. Consequently, there is no more NADH generated in the mitochondrial matrix. Moreover, work from our laboratory has shown that the external NADH dehydrogenase of the yeast respiratory chain has the right of way on other dehydrogenases [53] making the reoxidation of cytosolic NADH a priority for the cell.

lung cancers with isotope-labeled glucose or glutamine and compared the fate of these nutrients in tumor and normal tissue. The main result was that glutamine utilization by both lung tumors and normal lung was minimal, with lung tumors showing increased glucose contribution to the TCA cycle relative to normal lung tissue. However, when these very same cells were cultured *in vitro*, glutamine utilization was restored, showing that the *in vivo* environment is an important determinant of the metabolic phenotype of cancer cells.

5. Conclusion

From these studies, two parameters seem intrinsically linked, namely cell growth and glycolysis/fermentation. Mitochondrial oxidative phosphorylation activity can vary considerably without much impact on cell growth in some models which might be due to the high energetic cost of mitochondrial maintenance. Further, some tumors have been shown to exhibit mitochondrial dysfunction (for instance succinate dehydrogenase mutation) whereas others have functional oxidative phosphorylation. This highlights the great variability of oxidative phosphorylation phenotype in tumors. Further, early studies from Crabtree pointed to a major role of the *in vivo* environment in the modulation of oxidative phosphorylation. Altogether, these data show that there cannot be a univocal relationship between cell proliferation, fermentation and oxidative phosphorylation as proposed by O. Warburg.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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