

# A minimal model for explaining the higher ATP production in the Warburg effect

Stefan Schuster<sup>1</sup>, Daniel Boley<sup>2</sup>, Philip Möller<sup>1</sup>, and Christoph Kaleta<sup>3</sup>

<sup>1</sup>Dept. of Bioinformatics, Friedrich Schiller University, Ernst-Abbe-Platz 2, 07743 Jena, Germany

<sup>2</sup>Computer Science & Engineering, University of Minnesota, Minneapolis 55455, MN, USA

<sup>3</sup>Research Group Medical Systems Biology, Christian-Albrechts-University Kiel, Brunswiker Straße 10, Kiel 24105, Germany

## ABSTRACT

For producing ATP, tumor cells rely on glycolysis leading to lactate to about the same extent as on respiration. Thus, they use a higher fraction of glycolysis than the corresponding healthy cells. This is known as the Warburg effect (named after German biochemist Otto Warburg) and also applies to striated muscle cells, activated lymphocytes and microglia, endothelial cells and several other cell types. This effect is paradoxical at first sight because the ATP yield of glycolysis is much lower than that of respiration. Although a straightforward explanation is that glycolysis allows a higher ATP production rate, the question arises why the cell does not re-allocate protein to the high-yield pathway of respiration. We tackle this question by a minimal model only including three combined reactions. We consider the case where the cell can allocate protein on several enzymes in a varying distribution and model this by a linear programming problem in which not only the rates but also the maximal velocities are variable. Depending on side conditions and on protein costs, this leads to pure respiration, pure glycolysis, and respirofermentation as a mixed flux distribution.

Keywords: ATP-producing pathway, Elementary-modes analysis, Flux-Balance Analysis, Molar yield, Respiratory pathway, Warburg effect

## INTRODUCTION

For producing ATP, tumor cells in mammalian tissues rely much more on glycolysis leading to lactate (in comparison to respiration) than the healthy cells from which the tumor cells originated. This is known as the Warburg effect, named after German biochemist Otto Warburg (cf. Schulz et al., 2006; Vazquez et al., 2010; Shlomi et al., 2011). He published these observations in several German papers in the 1920s (e.g. Warburg, 1924) and in 1956 in English (Warburg, 1956). Warburg himself explained the effect by impaired function of mitochondria in tumor cells (Warburg, 1924, 1956). A similar phenomenon is observed in striated muscle cells under heavy exercise (Schmitz et al., 2013), activated lymphocytes (cf. Pearce et al., 2013), astrocytes (cf. Pellerin et al., 2007), microglia (Voloboueva et al., 2013), endothelial cells (cf. Ghesquière et al., 2014) and several other cell types. In the case of lymphocytes, the term Warburg effect is explicitly used as well (Pearce et al., 2013).

Metabolic pathways are characterized both by their rate and their molar yield. While the rate quantifies the moles of product built per time, the yield quantifies the moles of product per mole of substrate. The Warburg effect is paradoxical at first sight because the ATP-versus-glucose yield of glycolysis equals two and is, thus, much lower than that of respiration. The ATP yield of respiration depends on the biological species and partly on conditions. Typical values are near 30 (Rich, 2003). On the other hand, glycolysis can reach much higher rates than respiration. Warburg (1956) wrote that cancer cells can obtain about the same amount of energy from fermentation as from respiration. In striated muscle cells, glycolysis is up to 100 times faster than respiration (Voet and Voet, 2004). A mechanistic explanation is that the respiratory pathway is much longer and that the enzymes of the respiratory chain are located in the membrane and, thus, operate in a two-dimensional environment in contrast to the three-dimensional cytoplasm, which harbours the glycolytic enzymes. In line with these sterically based considerations, the problem of macromolecular crowding has been suggested as a further explanation for the low rate of oxidative phosphorylation (Vazquez et al., 2010).

Various explanations have been given for the Warburg effect:

1. compromised mitochondrial function (Warburg, 1956)
2. anaerobic conditions (Larbi et al., 2010)
3. poisoning of competitors by end products (Gatenby, 1995)
4. increased ATP production rate (Pfeiffer et al., 2001)
5. supply of precursors (Ghesquière et al., 2014)
6. regulatory effects by glycolytic enzymes (Pearce et al., 2013)
7. avoiding harmful effects by, for example, reactive oxygen species (Vander Heiden et al., 2001).

The contribution of these factors has been intensely and controversially discussed in the literature (e.g. Vander Heiden et al., 2009; Schuster et al., 2011; Pearce et al., 2013). For example, lack of oxygen inside of tumors is a natural explanation of the Warburg effect, as long as they are not yet vascularised. However, tumors usually show the Warburg effect even in the presence of oxygen (Shlomi et al., 2011).

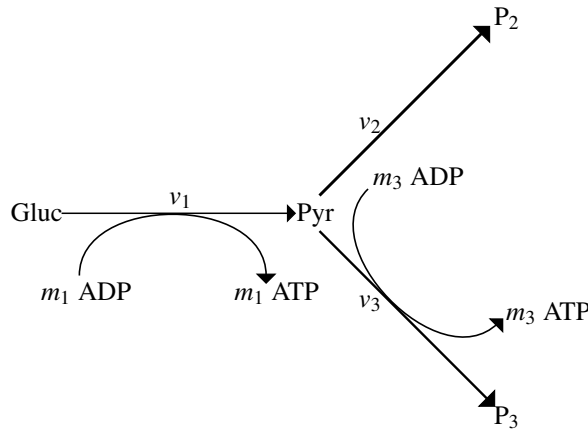
Here, we focus on the explanation in terms of higher ATP production rate, acknowledging that the others may be relevant as well. That is, we start from the assumption that a preferential use of glycolysis leads to an increase in that rate (Pfeiffer et al., 2001; Pfeiffer and Schuster, 2005). Thus, we analyze the trade-off between rate and yield.

The above reasoning leads to the optimization problem of maximizing ATP production rate. The enzyme concentrations in the pathways of glycolysis and respiration are regarded to be variable because they can change both during evolution and during development of a given organism. Thus, we allow for re-allocation of protein between enzymes and pathways. A plausible side constraint in the optimization problem is that the cell can provide a fixed amount of protein to energy metabolism. This constraint goes back to the work by Waley (1964) and Heinrich et al. (1987) in metabolic optimization. The question arises why the cell does not re-allocate protein to the high-yield pathway (respiration) (cf. Müller et al., 2014). One may assume that investing it into respiration would always imply a higher ATP formation than investing it into glycolysis. Here, we tackle this question by mathematical modeling. We establish a simple, paradigmatic example network as a minimal model, thus combining and streamlining several useful features of earlier models (Vazquez et al., 2010; Shlomi et al., 2011; Schuster et al., 2011). We discuss the applicability of the model to several cell types besides tumor cells. We use linear programming and consider both the rates and the maximal velocities as variables. Our approach can be considered to be part of a methodological framework called constraint-based modeling (CBM) (Price et al., 2004; Ruppin et al., 2010).

## METHODS

The term glycolysis is used in the literature with slightly different meanings: it may refer to the conversion of glucose into pyruvate, being a prerequisite of respiration. Alternatively, it may denote the conversion of glucose into lactate (or, in micro-organisms, ethanol, acetate etc.), which in microbiology is often called fermentation. Here, we use the term glycolysis in the former sense.

Our minimal model only includes three combined reactions: glycolysis up to pyruvate, conversion of pyruvate into a fermentation product such as lactate or into biomass, and the tricarboxylic acid (TCA) cycle together with oxidative phosphorylation (Fig. 1). Glucose is consumed by reaction 1 to produce 2 moles of pyruvate and  $m_1$  moles of ATP per mole of glucose. The stoichiometric coefficient of pyruvate (2) is not relevant here as long as we count the rates  $v_2$  and  $v_3$  in terms of glucose consumption. Only pyruvate is considered as an internal metabolite, while all other substances are considered as external metabolites, that is, their concentrations are fixed. Fermentation is modeled by the pathway leading from glucose to  $P_2$ . Respiration is modeled by the pathway leading to  $P_3$  and produces  $m_1 + m_3$  moles of ATP per mole of glucose. For our calculation we use the typical values  $m_1 = 2$  and  $m_3 = 30$  (see Introduction). In case that also reaction 2 produces ATP, also  $m_2$  is positive, such as in acetate fermentation by *Escherichia coli*, where  $m_2 = 1$ . The conversion of  $NAD^+$  into NADH in the TCA cycle is included implicitly because we consider the ATP produced on the basis of that portion of NADH. In contrast, we here neglect the NADH consumed in the second reaction.



**Figure 1.** Minimal reaction scheme for analyzing the Warburg effect. Gluc, glucose; Pyr, pyruvate, P<sub>2</sub>, fermentation product such as lactate or ethanol; P<sub>3</sub>, respiration products such as CO<sub>2</sub> and H<sub>2</sub>O; v<sub>i</sub>, reaction rates, m<sub>i</sub>, stoichiometric coefficients of ATP.

To describe the Warburg effect, we phrase the following linear optimization problem:

Maximize

$$J_{ATP}(\mathbf{v}) = \mathbf{m}^T \mathbf{v} \tag{1a}$$

s.t.

$$\mathbf{N}\mathbf{v} = 0 \tag{1b}$$

$$\mathbf{v} \leq \mathbf{v}_{cap} \tag{1c}$$

$$\boldsymbol{\alpha}^T \mathbf{v} \leq C \tag{1d}$$

$$\mathbf{v} \geq \mathbf{0} \tag{1e}$$

where lower case bold letters refer to column vectors. The equation system (1) can be explained as follows. The ATP production rate is given by a linear combination of rates with the stoichiometric coefficients of ATP as weighting factors. In particular, for glycolysis, we have  $m_1 = 2$ . Eq. (1b) is the steady-state condition with  $\mathbf{N}$  denoting the stoichiometry matrix. This condition is commonly used in metabolic modeling (cf. Heinrich and Schuster, 1996). Relation (1c) can be used to include upper limits on the rates, as arising, for example, from capacity constraints for the glucose uptake or the enzymes of oxidative phosphorylation due to limited space in membranes. The capacities should not be confused with the maximal velocities of the reaction rates, which are variable due to re-allocation of protein. If these constraints are irrelevant for some or all rates, the corresponding maximal capacities can be set equal to infinity.

The constraint (1d) reflects the organism's resource limits in creating enzymes for the reactions (cf. Müller et al., 2014). The coefficients  $\alpha_i$  express the different turnover numbers as well as the different synthesis costs of enzymes, which are largely determined by the molar masses of the enzymes and the different synthesis costs of amino acids (Akashi and Gojobori, 2002; Sajitz-Hermstein and Nikoloski, 2010; Müller et al., 2014). Written in terms of enzyme concentrations (to which the maximal velocities are proportional), such a side constraint has been used in metabolic modeling earlier (Waley, 1964; Heinrich et al., 1987). Furthermore, relation (1d) can reflect macromolecular crowding (Vazquez et al., 2010), that is, volume limitations in the cell.

Moreover, we can assume that the first and third overall reactions are irreversible because they include at least one irreversible partial reaction. For example, the hexokinase and phosphofructokinase reactions involved in glycolysis are irreversible. The second reaction may be reversible (e.g. lactate dehydrogenase or alanine aminotransferase). However, for simplicity's sake, we here consider it to be irreversible as well. In future studies, the model could be extended so that the second reaction is reversible, so that cells such as neurons can be described that take up lactate and respire it.

The irreversibility constraint implies that the rates of all overall reactions are non-negative, as expressed by inequality (1e). System (1) is a linear program (LP) and, thus, can be solved easily. For the system under consideration (Fig. 1), it only involves three variables. By the steady-state condition

(1b), we can eliminate  $v_1$  and write the system in terms of  $v_2$  and  $v_3$ .

Maximize

$$J_{ATP}(v_2, v_3) = m_1 v_2 + (m_1 + m_3) v_3 \quad (2a)$$

s. t.

$$v_2 + v_3 \leq v_{1,cap} \quad (2b)$$

$$v_2 \leq v_{2,cap}$$

$$v_3 \leq v_{3,cap}$$

$$(\alpha_1 + \alpha_2) v_2 + (\alpha_1 + \alpha_3) v_3 \leq C \quad (2c)$$

$$v_2 \geq 0, v_3 \geq 0 \quad (2d)$$

For solving the LP, we use MATLAB with the CVX package.

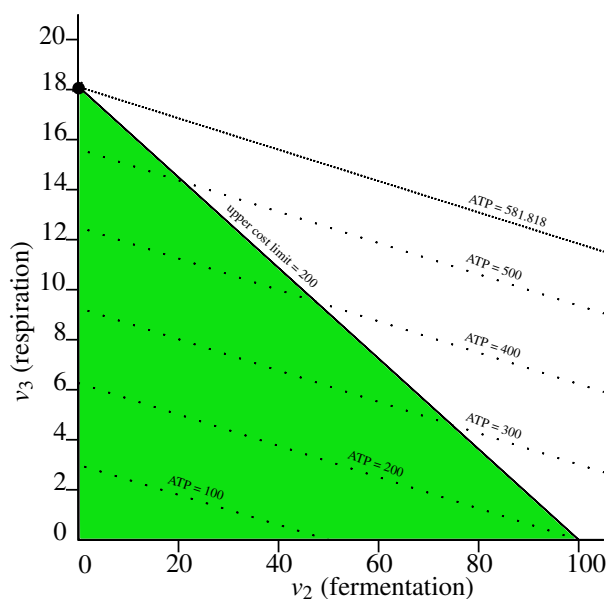
## RESULTS AND DISCUSSION

By eliminating  $v_1$ , we are left with an LP in two variables (see above), for which it is easy to analyze the feasible region graphically. Figs. 2 and 3 show the feasible region for (2) if the individual capacity constraints (2b) are absent. The level contours for the objective function are shown in the figures.

We now distinguish two cases depending on whether the costs for the high-yield pathway (quantified by the coefficient  $\alpha_3$ ) are low or high:

### Case (i): "Cheap" High-yield Pathway.

Fig. 2 shows the situation when respiration costs are low, with suitably chosen parameter values. Solving the LP leads to a flux distribution corresponding to pure respiration. Note that the solution must always be situated at a vertex of the admissible region because the optimization problem is linear.

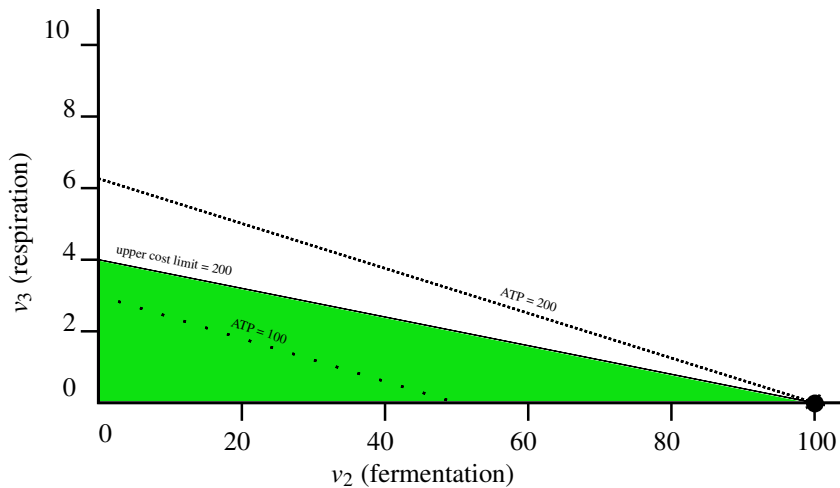


**Figure 2.** Feasible region for (2) with low cost respiration:  $\alpha_1=1$ ,  $\alpha_2=1$ ,  $\alpha_3 = 10$ ,  $C = 200$  and  $v_{i,cap} = \infty$ ,  $i = 1, 2, 3$  (colored region). Also shown are the level contours for the objective function  $J_{ATP}$  with stoichiometric coefficients  $m_1 = 2$ ,  $m_3 = 30$ . The optimal solution ( $\bullet$ ) is  $(v_2, v_3) = (0, 18.18)$ , corresponding to  $v_1 = 18.18$ ,  $J_{ATP} = 581.8$  and a yield ratio of  $J_{ATP}/v_1 = 32$ . This situation corresponds to pure respiration if respiratory enzymes are cheap.

### Case (ii): "Costly" High-yield Pathway.

Fig. 3 shows the situation when respiration is costly. The maximum feasible value for ATP production occurs using pure fermentation. This explains why fermentation can be advantageous even though its yield is lower. It does not pay for the cell to re-allocate protein to the high-yield pathway whenever the investment into the latter pathway is very costly.

We have seen above that, depending on the costs for the pathways, either pure respiration or pure fermentation results from the linear program. However, as observed already by Warburg (1924),



**Figure 3.** Feasible region for (2) with costly respiration:  $\alpha_1=1$ ,  $\alpha_2=1$ ,  $\alpha_3 = 50$ ,  $C = 200$  and  $v_{i,cap} = \infty$ ,  $i = 1, 2, 3$  (colored region). Also shown are the level contours for the objective function  $J_{ATP}$  with stoichiometric coefficients  $m_1 = 2$ ,  $m_3 = 30$ . The optimal solution ( $\bullet$ ) is  $(v_2, v_3) = (100, 0)$ , corresponding to  $v_1 = 100$ ,  $J_{ATP} = 200$  and a yield ratio of  $J_{ATP}/v_1 = 2$ . This situation corresponds to pure fermentation if respiratory enzymes are costly.

usually a mixture of the two pathways is used, the so-called respirofermentation. This metabolic mode can be obtained by imposing an additional constraint, notably on the substrate uptake or availability. Due to the low yield of fermentation, this pathway consumes glucose very fast. Thus, the limitation of glucose uptake due to its availability or the maximal capacity of glucose transporters becomes relevant. This can be written as relation (1c) for  $i=1$ .

By using appropriate values for  $v_{1,cap}$ , the feasible region can be restricted as shown in Fig. 4. It is now a quadrangle. Solving the linear program shows that the solution leads to positive rate values for both  $v_2$  and  $v_3$  ( $\bullet$  in Fig. 4). This corresponds to respirofermentation. Due to the limited substrate uptake, the cell has left-over enzyme resources that can be used in respiration. If the parameter  $v_{1,cap}$  is lowered even more, the solution finally turns into pure respiration (Fig. 5).

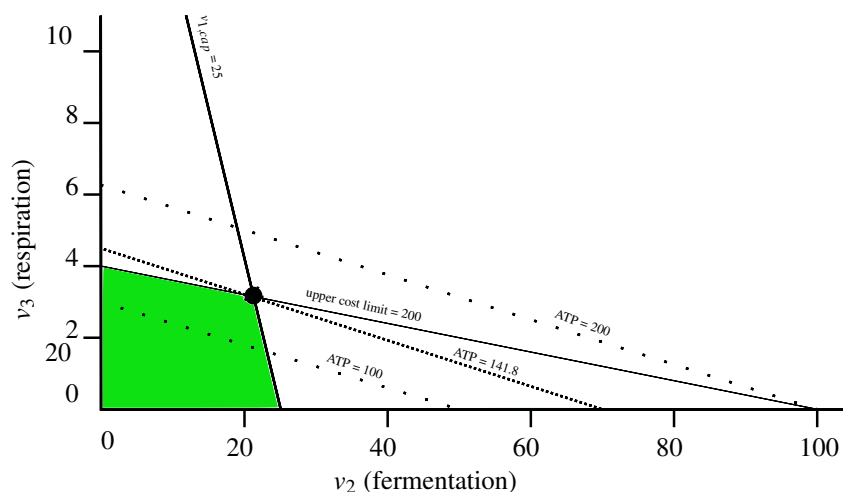
Here, we have presented and analyzed a minimal model for explaining the higher ATP production in the Warburg effect. The model is surprisingly powerful. It shows that depending on protein costs and substrate availability, pure respiration, pure fermentation or respirofermentation are predicted by the model. A reallocation of protein to the high-yield pathway only pays if the synthesis costs for that pathway are low enough. If these costs are above a certain threshold given in Schuster et al. (2011), it is better to concentrate protein on high-rate/low-efficiency pathways such as glycolysis. As the respiratory chain is quite costly for the cell, this leads to a straightforward explanation of the Warburg effect. The model applies not only to tumor cells but also to many other human cells as well as cells of microbes and other organisms. The qualitative results do not depend on the exact values of the stoichiometric coefficients  $m_1$  and  $m_3$ .

Respirofermentation is relevant in tumor cells, activated lymphocytes and many other cells (see Introduction). Limited substrate availability is especially relevant for the region inside of tumors. In contrast, pure respiration is obtained either when the respiratory pathway is "cheap", which is rarely the case, or when it is costly but substrate is very scarce. The latter case applies to baker's yeast and many other yeasts at low glucose levels. These cells indeed use pure respiration under this condition.

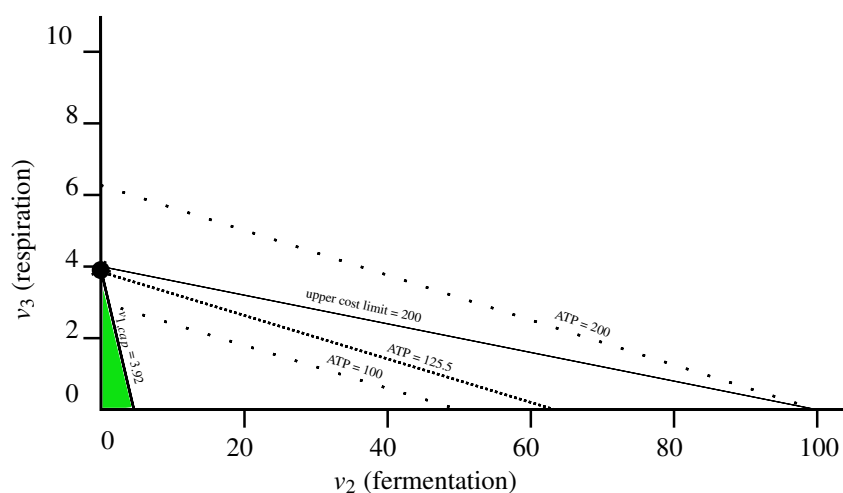
Pure fermentation (in higher organisms rather called pure glycolysis) is observed, for example, in lactobacilli and mammalian erythrocytes. The latter are very small to pass thin capillaries and are packed with hemoglobin. Thus, space constraints have led to expulsion of mitochondria, in agreement with the predictions of our model.

Pure respiration is predicted to occur if the respiratory enzymes are cheap. However, this is not likely to be the case in many cell types. Another possibility is that the upper bound on glucose availability is even lower than shown in Fig. 4. If that bound is low enough, the admissible region is a triangle delimited by the abscissa, ordinate and the line corresponding to that bound (Fig. 5). This is in agreement with the observation that baker's yeast, *E. coli* and many other cells use pure respiration at very low glucose levels.

It is more complicated to explain the usage of pure respiration in many cell types of multicellular organisms (animals, plants, multicellular fungi). Non-proliferating cells show a low nutrient con-



**Figure 4.** Feasible region for (2) with same value as in Fig. 3, but with a moderate limit on the uptake of Gluc:  $v_1 = v_2 + v_3 \leq v_{1, cap} = 25$ . Also shown are the level contours for the objective function  $J_{ATP}$ . The optimal solution ( $\bullet$ ) is  $(v_2, v_3) = (21.94, 3.06)$ , corresponding to  $v_1 = 25$ ,  $J_{ATP} = 141.8$  and a yield ratio of  $J_{ATP}/v_1 \approx 5.67$ . This situation corresponds to respirofermentation.



**Figure 5.** Feasible region for (2) with same value as in 3, but with a stricter limit on the uptake of Gluc:  $v_1 = v_2 + v_3 \leq v_{1, cap} = 3.92$ . The optimal solution ( $\bullet$ ) is  $(v_2, v_3) = (0, 3.92)$ , corresponding to  $v_1 = 3.92$ ,  $J_{ATP} = 125.5$  and a yield ratio of  $J_{ATP}/v_1 \approx 32.02$ . This situation corresponds to pure respiration at low glucose levels even if respiration is costly.

sumption and, thus, pure or predominant respiration (Vander Heiden et al., 2009). It can be assumed that they have evolved towards a more cooperative, economical use of glucose, that is, towards using high-yield pathways. Moreover, optimization criteria other than maximizing ATP production rate are likely to be relevant for them, since they do not proliferate at all or not as fast as microbial or tumor cells. The "selfish" usage of glucose by glycolysis is avoided in many cells of multicellular organisms by regulatory mechanisms, which are not considered in our model. These may include the action of the immune system, which serves, among other purposes, for cleaning the body from tumor cells.

## CONCLUSION

To verify the model predictions by experiment, it would obviously be useful to measure ATP production rate in cancer cells of a particular type and in the corresponding healthy cells. An obstacle would be that tumorigenesis alters many parameters in cells besides energy metabolism so that a comparison is difficult.

Besides a limitation of substrate availability, a constraint on oxygen is often relevant in living organisms. This is related to explanation 2 (anaerobic conditions) mentioned in the Introduction. By including this in our model, a solution corresponding to pure respiration can be changed into

one corresponding to respirofermentation or (under completely anaerobic conditions) even to pure fermentation (not shown). Again, this may be relevant for the region inside of tumors, where oxygen is scarce. Moreover, some yeasts, for example *Kluyveromyces marxianus* and *Pichia fermentans*, cells use respiration under aerobic conditions and fermentation when no oxygen is available (Veiga et al., 2000). Such a constraint has been used earlier in Flux Balance Analysis (Famili et al., 2003). As mentioned in the Introduction, a further promising extension is to define the second reaction to be reversible. Preliminary calculations (not shown) point to a regime at very low upper limit values  $v_{1, cap}$ , in which the fermentation product (e.g. lactate or ethanol) is taken up and degraded by respiration. This corresponds, for example, to the respiration of ethanol in baker's yeast after the diauxic shift.

The approach of maximizing the ATP synthesis flux is based on the assumption that cells producing ATP (and/or biomass) as fast as possible should have a selective advantage (Kacser and Beeby, 1984; Waddell et al., 1999; Werner et al., 2010). This is likely to be particularly relevant for micro-organisms, which can outcompete other species or strains when growing fast (Pfeiffer et al., 2001; Schuster et al., 2008; Teusink et al., 2009). It is of interest to compare this optimization criterion with that of maximizing molar yield. While production rate corresponds to moles of product per time, yield corresponds to moles of product per mole of substrate. Maximizing yield is a plausible optimization criterion as well and is, in fact, often used in Flux Balance Analysis (cf. Price et al., 2004). It may be especially relevant for multicellular organisms.

The present analysis is based on an earlier study (Schuster et al., 2011) where the constraint (1c) was not yet considered. There, we considered the maximal velocities of enzymes as variables, but they are somewhat redundant as variables in the optimal solution, since they are mathematically free to float. In an optimal solution, they can be set to the optimal values of  $v$  to obtain a feasible solution.

When no additional constraint on single reaction rates is included, the solutions to the optimization problem under study always correspond to pure elementary flux modes, as has been shown earlier by Wortel et al. (2014) and Müller et al. (2014) for more general networks. As shown above, a mixed mode such as respirofermentation can be obtained by an additional constraint.

Some of our results have been obtained earlier by Shlomi et al. (2011), who used a genome-scale model for explaining the Warburg effect. In our opinion, it is unnecessary, for that purpose, to study such a large network. Molenaar et al. (2009) proposed and analysed a medium-scale model, where the Warburg effect is explained by the balance of the protein costs between glucose uptake and catabolism. When glucose is expensive (low concentration), it is completely utilized, otherwise (high concentration) it can be "squandered".

A useful feature of our model is that it can be treated analytically. It is linear in the reaction rates, while the underlying rate laws can be nonlinear. The linear program can be solved easily, all the more as it includes only two independent variables. A complementary approach is based on evolutionary game theory (Pfeiffer et al., 2001).

Here, we focused on the explanation of the Warburg effect in terms of ATP production rate. It is worth noting that this explanation is consistent with the argument in terms of precursor supply. Whenever the glycolytic rate is high while respiration is limited, much pyruvate can be used for building biomass. The rate  $v_2$  then could be interpreted as the transamination of pyruvate into alanine, which is then incorporated into proteins. This optimization criterion would always lead to pure fermentation.

## ACKNOWLEDGMENTS

We thank Jean-Pierre Mazat (Bordeaux), Stefan Müller (Vienna), Sabine Peres (Orsay) and several members of the Bioinformatics Dept. in Jena for stimulating discussions.

## REFERENCES

- Akashi, H. and Gojobori, T. (2002). Metabolic efficiency and amino acid composition in the proteomes of *Escherichia coli* and *Bacillus subtilis*. *Proc. Natl. Acad. Sci. U S A.*, 99(6):3695–3700.
- Famili, I., Forster, J., Nielsen, J., and Palsson, B. O. (2003). *Saccharomyces cerevisiae* phenotypes can be predicted by using constraint-based analysis of a genome-scale reconstructed metabolic network. *Proc. Natl. Acad. Sci. U S A.*, 100(23):13134–13139.
- Gatenby, R. A. (1995). The potential role of transformation-induced metabolic changes in tumor-host interaction. *Cancer Res.*, 55(18):4151–4656.
- Ghesquière, B., Wong, B., Kuchnio, A., and Carmeliet, P. (2014). Metabolism of stromal and immune cells in health and disease. *Nature*, 511(7508):167–176.

- Heinrich, R., Holzhütter, H., and Schuster, S. (1987). A theoretical approach to the evolution and structural design of enzymatic networks; Linear enzymatic chains, branched pathways and glycolysis of erythrocytes. *Bull. Math. Biol.*, 49(5):539–595.
- Heinrich, R. and Schuster, S. (1996). *The Regulation of Cellular Systems*. Chapman & Hall, New York.
- Kacser, H. and Beeby, R. (1984). Evolution of catalytic proteins. On the origin of enzyme species by means of natural selection. *J. Mol. Evol.*, 20(1):38–51.
- Larbi, A., Zelba, H., Goldeck, D., and Pawelec, G. (2010). Induction of hif-1alpha and the glycolytic pathway alters apoptotic and differentiation profiles of activated human t cells. *J. Leukoc. Biol.*, 87(2):265–273.
- Müller, S., Regensburger, G., and Steuer, R. (2014). Enzyme allocation problems in kinetic metabolic networks: optimal solutions are elementary flux modes. *J. Theor. Biol.*, 347:182–190.
- Molenaar, D., van Berlo, R., de Ridder, D., and Teusink, B. (2009). Shifts in growth strategies reflect tradeoffs in cellular economics. *Mol. Syst. Biol.*, 5:323.
- Pearce, E., Poffenberger, M., Chang, C.-H., and Jones, R. (2013). Fueling immunity: insights into metabolism and lymphocyte function. *Science*, 342(6155). 1242454.
- Pellerin, L., Bouzier-Sore, A., Aubert, A., Serres, S., Merle, M., Costalat, R., and Magistretti, P. (2007). Activity-dependent regulation of energy metabolism by astrocytes: an update. *Glia*, 55(12):1251–1262.
- Pfeiffer, T. and Schuster, S. (2005). Game-theoretical approaches to studying the evolution of biochemical systems. *Trends Biochem. Sci.*, 30(1):20–25.
- Pfeiffer, T., Schuster, S., and Bonhoeffer, S. (2001). Cooperation and competition in the evolution of ATP producing pathways. *Science*, 292(5516):504–507.
- Price, N., Reed, J., and Palsson, B. (2004). Genome-scale models of microbial cells: evaluating the consequences of constraints. *Nat. Rev. Microbiol.*, 2(11):886–897.
- Rich, P. (2003). The molecular machinery of Keilin's respiratory chain. *Biochem. Soc. Trans.*, 31:1095–1105.
- Ruppin, E., Papin, J., de Figueiredo, L., and Schuster, S. (2010). Metabolic reconstruction, constraint-based analysis and game theory to probe genome-scale metabolic networks. *Curr. Opin. Biotechnol.*, 21(4):502–510.
- Sajitz-Hermstein, M. and Nikoloski, Z. (2010). A novel approach for determining environment-specific protein costs: the case of Arabidopsis thaliana. *Bioinformatics*, 26(18):i582–i588. doi:10.1093/bioinformatics/btq390.
- Schmitz, J., Groenendaal, W., Wessels, B., Wiseman, R., Hilbers, P., Nicolay, K., Prompers, J., Jeneson, J., and van Riel, N. (2013). Combined in vivo and in silico investigations of activation of glycolysis in contracting skeletal muscle. *Am. J. Physiol. Cell Physiol.*, 304(2):C180–C193.
- Schulz, T., Thierbach, R., Voigt, A., Drewes, G., Mietzner, B., Steinberg, P., Pfeiffer, A., and Ristow, M. (2006). Induction of oxidative metabolism by mitochondrial frataxin inhibits cancer growth: Otto Warburg revisited. *J. Biol. Chem.*, 281(2):977–981.
- Schuster, S., de Figueiredo, L. F., Schroeter, A., and Kaleta, C. (2011). Combining Metabolic Pathway Analysis with Evolutionary Game Theory. Explaining the occurrence of low-yield pathways by an analytic optimization approach. *Biosystems*, 105(2):147–153.
- Schuster, S., Pfeiffer, T., and Fell, D. A. (2008). Is maximization of molar yield in metabolic networks favoured by evolution? *J. Theor. Biol.*, 252(3):497–504.
- Shlomi, T., Benyamini, T., Gottlieb, E., Sharan, R., and Ruppin, E. (2011). Genome-scale metabolic modeling elucidates the role of proliferative adaptation in causing the Warburg effect. *PLoS Comput. Biol.*, 7(3):e1002018.
- Teusink, B., Wiersma, A., Jacobs, L., Notebaart, R., and Smid, E. (2009). Understanding the adaptive growth strategy of Lactobacillus plantarum by in silico optimisation. *PLoS Comput. Biol.*, 5(6):e1000410.
- Vander Heiden, M., Cantley, L., and Thompson, C. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*, 324(5930):1029–1033.
- Vander Heiden, M. G., Plas, D., Rathmell, J. C., Fox, C., Harris, M. H., and Thompson, C. (2001). Growth factors can influence cell growth and survival through effects on glucose metabolism. *Mol. Cell. Biol.*, 21(17):5899–5912.
- Vazquez, A., Liu, J., Zhou, Y., and Oltvai, Z. (2010). Catabolic efficiency of aerobic glycolysis: the Warburg effect revisited. *BMC Syst. Biol.*, 4(58).
- Veiga, A., Arrabaça, J. D., and Loureiro-Dias, M. C. (2000). Cyanide-resistant respiration is frequent, but confined to yeasts incapable of aerobic fermentation. *FEMS Microbiol. Lett.*, 190(1):93–97.



- Voet, D. and Voet, J. (2004). *Biochemistry*. John Wiley & Sons, 3rd edition.
- Voloboueva, L., Emery, J., Sun, X., and Giffard, R. (2013). Inflammatory response of microglial BV-2 cells includes a glycolytic shift and is modulated by mitochondrial glucose-regulated protein 75/mortalin. *FEBS Lett.*, 587(6):756–762.
- Waddell, T., Repovic, P., Meléndez-Hevia, E., Heinrich, R., and Montero, F. (1999). Optimization of glycolysis: new discussion. *Biochem. Educ.*, 27(1):12–13.
- Waley, S. (1964). A note on the kinetics of multi-enzyme systems. *Biochem. J.*, 91(3):514–517.
- Warburg, O. (1924). Über den Stoffwechsel der Carcinomzelle. *Die Naturwissenschaften*, 12:1131–1137.
- Warburg, O. (1956). On the origin of cancer cells. *Science*, 123(3191):309–314.
- Werner, S., Diekert, G., and Schuster, S. (2010). Revisiting the thermodynamic theory of optimal ATP stoichiometries by analysis of various ATP-producing metabolic pathways. *J. Mol. Evol.*, 71(5-6):346–355.
- Wortel, M., Peters, H., Hulshof, J., Teusink, B., and Bruggeman, F. (2014). Metabolic states with maximal specific rate carry flux through an elementary flux mode. *FEBS J.*, 281(6):1547–1555.