MYC overrides HIF-1α to regulate proliferating primary cell metabolism in hypoxia

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# Abstract

Hypoxia requires metabolic adaptations to sustain energetically demanding cellular activities. While the metabolic consequences of hypoxia have been studied extensively in cancer cell models, comparatively little is known about the metabolic response of primary cells to hypoxia. We performed metabolic flux analyses of human lung fibroblasts and pulmonary artery smooth muscle cells proliferating in hypoxia. Unexpectedly, hypoxia decreased glycolysis despite activation of hypoxia-inducible factor 1α (HIF-1α) and increased glycolytic enzyme expression. While HIF-1α activation by prolyl hydroxylase (PHD) inhibition did increase glycolysis in normoxia, this effect was abrogated by hypoxia. Multi-omic profiling revealed distinct molecular responses to hypoxia and PHD inhibition, suggesting a critical role for MYC in modulating HIF-1α responses to hypoxia. Consistent with this hypothesis, MYC knockdown in hypoxia increased glycolysis and MYC overexpression in normoxia decreased glycolysis stimulated by PHD inhibition. These data suggest that MYC signaling in hypoxia uncouples an increase in HIF-dependent glycolytic gene transcription from glycolytic flux.

# Keywords

hypoxia / metabolic flux analysis / hypoxia-inducible factor / prolyl hydroxylase / MYC

# Introduction

Cellular responses to hypoxia propel many physiologic and pathologic processes from wound healing and angiogenesis to vascular remodeling and fibrosis (W. D. Lee et al., 2019; Semenza, 2012). These activities require cells to continue energetically demanding tasks, such as macromolecular biosynthesis and proliferation, despite limited oxygen availability. Since respiration is the most efficient way for cells to produce energy, cell metabolism must adapt to meet energy demands when oxygen supply is limiting. Understanding how these metabolic adaptations sustain critical cellular processes in hypoxia is fundamentally important to our understanding of human health and disease.

Cells typically respond to hypoxia by shifting energy production away from respiration and toward glycolysis. This response is mediated primarily by stabilization of the hypoxia-inducible transcription factor 1α (HIF-1α). HIF-1α activates the transcription of glucose transporters, glycolytic enzymes, lactate dehydrogenase, and pyruvate dehydrogenase kinase, while decreasing the expression of tricarboxylic acid (TCA) cycle and electron transport chain enzymes (Lee et al., 2020; Semenza, 2012). Although HIF-1α is constitutively expressed, it is hydroxylated by prolyl hydroxylase enzymes (PHDs) in normoxia and targeted for proteasomal degradation. PHDs are the principal oxygen sensors in metazoan cells (Kaelin and Ratcliffe, 2008). PHDs are α-ketoglutarate-dependent dioxygenase enzymes that require molecular oxygen for their enzymatic activity. When oxygen tension falls, PHD activity decreases, leading to HIF-1α stabilization and activation of its downstream transcriptional program. Overall, this transcriptional program should increase glycolytic capacity and divert glucose-derived pyruvate from oxidative phosphorylation toward lactate fermentation to maintain glycolytic ATP production.

In addition to metabolic changes designed to maintain energy supply, hypoxic cells also reduce energy demand through down-regulation of Na+/K+-ATPase, slowing protein translation, and decreasing cell proliferation (Hubbi and Semenza, 2015; Wheaton and Chandel, 2011). In particular, HIF-1α decreases cell proliferation by activating cyclin-dependent kinase inhibitor expression, inhibiting cell-cycle checkpoint progression (Gardner et al., 2001), and antagonizing pro-proliferative MYC signaling (Koshiji et al., 2004). Despite these canonical effects of HIF-1α activation, there are many examples where cells continue to proliferate despite hypoxic stress, including cancer cells, stem cells, and lung vascular cells (Hubbi and Semenza, 2015). How these cells meet the metabolic needs of sustained proliferation in hypoxia and how these adaptations are regulated are active areas of investigation (Jain et al., 2020; Lee et al., 2020; Oldham et al., 2015). Since hypoxia is a prominent feature of cancer biology as tumor growth outstrips blood supply, most detailed metabolic studies of hypoxic cell metabolism have used tumor cell models, yielding important insights into the metabolic pathobiology of cancer (Garcia-Bermudez et al., 2018; Jiang et al., 2016; J. W. Lee et al., 2019; Meléndez-Rodríguez et al., 2019; Metallo et al., 2011; Wise et al., 2011). For example, stable isotope tracing and metabolic flux analyses identified a critical role for the reductive carboxylation of glutamine-derived α-ketoglutarate for lipid biosynthesis in supporting tumor growth (Gameiro et al., 2013; Metallo et al., 2011; Scott et al., 2011; Wise et al., 2011), and metabolomic studies identified aspartate as a limiting metabolite for cancer cell proliferation under hypoxia (Garcia-Bermudez et al., 2018). By contrast, comparatively little is known about metabolic adaptations of primary cells to hypoxia. Indeed, the importance of reductive carboxylation or aspartate biosynthesis remains to be elucidated in primary cells. A more complete understanding of primary cell metabolic adaptations to hypoxia would provide an important context for understanding how metabolic reprogramming supports normal cellular responses to hypoxia, how these responses may be (mal)adaptive in a variety of disease contexts, and how the hypoxia metabolic program in primary cells differs from that observed in cancer cells.

To address these questions, we have developed models of bioenergetic carbon flux in human lung fibroblasts (LFs) and pulmonary artery smooth muscle cells (PASMCs) cultured in 21% or 0.5% oxygen. These cells may be exposed to a wide range of oxygen concentrations *in vivo*, continue to proliferate despite hypoxic culture conditions *in vitro*, and play important roles in the pathology of non-cancerous diseases in which tissue hypoxia is a prominent feature, including pulmonary hypertension and pulmonary fibrosis. We found that hypoxia fails to increase glycolysis in these primary cells despite robust up-regulation of the HIF-1α transcriptional program. In normoxia, HIF-1α stabilization by the PHD inhibitor molidustat (BAY-85-3934, “BAY”) (Flamme et al., 2014) did increase glycolysis and lactate efflux; however, hypoxia blocked this response. These findings suggested that important hypoxia-dependent regulatory mechanisms override the metabolic consequences of HIF-1α-dependent glycolytic gene expression. Transcriptomic profiling identified a critical role for the transcription factor MYC in the adaptive response to hypoxia. Using knockdown and overexpression approaches, we demonstrated that MYC attenuates HIF-driven glycolysis in hypoxia and following HIF stabilization in normoxia.

# Results

## Hypoxia uncouples HIF-dependent glycolytic gene expression from glycolytic metabolic flux

The goal of this study was to characterize hypoxia-induced metabolic changes in proliferating primary LFs and PASMCs. To accomplish this goal, we used metabolic flux analysis to model how cell metabolism supports cell proliferation. Metabolic flux analysis fits cell proliferation rate, extracellular flux measurements, and 13C isotope labeling patterns to a computational model of cell metabolism (Antoniewicz, 2018). This analysis reconstructs comprehensive flux maps that depict the flow of carbon from extracellular substrates, through intracellular metabolic pathways, and into cell biomass and metabolic by-products (Young et al., 2014). These models assume that cells are at a metabolic pseudo-steady state over the experimental time course (Buescher et al., 2015). Exponential growth phase is thought to reflect metabolic pseudo-steady state as cells in culture steadily divide at their maximal condition-specific rate, provided nutrient supply does not become limiting (Ahn and Antoniewicz, 2011; Buescher et al., 2015). Thus, we first set out to define experimental conditions to capture exponential growth phase in normoxic and hypoxic cultures.

Cells were seeded and placed into hypoxia for 24 h prior to sample collection to provide adequate time for activation of the hypoxia-dependent transcriptional program (**Figure 1A**). We selected 0.5% oxygen for hypoxia as this level yielded the most reproducible phenotypic differences compared to 21% oxygen, while being physiologically relevant and above the KM of cytochrome *c* oxidase (electron transport chain complex IV) for oxygen (Lee et al., 2020; Wenger et al., 2015). From this starting point, we identified the optimal cell seeding density and time course to capture exponential cell growth (**Figure 1B**). Hypoxia decreased cell proliferation rates (**Figure 1C**), but slower growth was not associated with decreased cell viability (**Figure 1 - figure supplement 1A**). As anticipated, hypoxic cells demonstrated robust stabilization of HIF-1α associated with up-regulation of its downstream targets, glucose transporter 1 (GLUT1) and lactate dehydrogenase A (LDHA) (**Figure 1D-H**). These changes persisted for the duration of the experimental time course.

We next determined the extracellular fluxes of glucose (GLC), lactate (LAC), pyruvate (PYR), and amino acids (**Figure 1I-J**). Flux calculations incorporated cell growth rate, extracellular metabolite concentrations, metabolite degradation rates, and medium evaporation rate (Murphy and Young, 2013) (**Figure 1 - figure supplement 1B-C**). Interestingly, while we observed a modest increase in glucose uptake, we found that hypoxia actually decreased lactate efflux (**Figure 1I**). This finding was confirmed by measuring the rate of [U-13C3]-lactate produced from LFs cultured with [U-13C6]-glucose (**Figure 1 - figure supplement 2**). Hypoxia decreased lactate efflux despite activating HIF-1α and increasing glycolytic enzymes expression (**Figure 1D-H**).

To test if more severe hypoxia would augment glycolysis, we cultured cells in 0.2% ambient oxygen (**Figure 1 - figure supplement 3**). Under these conditions, we observed no change in glucose or lactate fluxes, similar to 0.5% oxygen culture. To test if this unexpected response was unique to LFs, we studied PASMCs under 0.5% oxygen conditions (**Figure 1 - figure supplement 4**). Similar to LFs, we observed no change in glucose uptake and reduced lactate efflux in PASMCs. Together, these data suggest that hypoxia uncouples HIF-1α target gene expression and glycolytic flux in proliferating primary cells.

Since hypoxia did not increase glycolysis in LFs, we wanted to determine how these cells responded to HIF-1α stabilization in normoxia. To activate HIF-1α, LFs were treated with the PHD inhibitor molidustat (BAY, 10 μM) using a similar time course as our hypoxia experiments (**Figure 2**). Like hypoxia, BAY decreased cell growth rate (**Figure 2B-C**) and activated the HIF-1α transcriptional program (**Figure 2D-H**). Unlike hypoxia, HIF-1α stabilization in normoxia markedly increased glucose uptake and lactate efflux (**Figure 2I**). Although hypoxia and BAY treatments increased in HIF-1α, GLUT1, and LDHA, to a similar degree, the glycolytic response differed markedly between these treatments.

## Extracellular fluxes are treatment and cell-type dependent

In addition to glucose and lactate, we also determined the extracellular fluxes of pyruvate and amino acids (**Figure 1J, Figure 1 - figure supplement 3J, Figure 1 - figure supplement 4J, Figure 2J**). To our knowledge, this is the first comprehensive extracellular flux profiling of key metabolic substrates in primary cells. In LFs, overall, changes in extracellular fluxes were modest, with hypoxia generally decreasing the fluxes of all measured metabolites. These findings were similar with 0.2% oxygen exposure (**Figure 1 - figure supplement 3J**).

Notably, we observed a significant decrease in glutamine consumption in hypoxic LFs. This finding contrasts with previous studies of cancer cell metabolism demonstrating increased glutamine uptake as a key feature of the metabolic response to hypoxia (Gameiro et al., 2013; Metallo et al., 2011; Wise et al., 2011). In these systems, glutamine-derived α-ketoglutarate was reductively carboxylated by isocitrate dehydrogenase enzymes to generate citrate for lipogenesis. In addition, glutamine has been shown to support TCA cycling in hypoxia in a Burkitt lymphoma model (Le et al., 2012). Unlike LFs, PASMCs did exhibit a trend toward increased glutamine uptake (**Figure 1 - figure supplement 4J**). To examine the relative importance of glucose and glutamine to the proliferation of these cells in hypoxia, we measured LF and PASMC growth rates in the absence of either substrate (**Figure 3**). In LFs, absence of either glucose or glutamine reduced cell proliferation to a similar extent (**Figure 3A**). In hypoxia, glucose deficiency decreased LF proliferation rate further, while glutamine deficiency had no additional impact. These findings are consistent with extracellular flux measurements demonstrating decreased glutamine consumption by LFs in hypoxia. Interestingly, neither glucose nor glutamine deficiency decreased PASMC proliferation (**Figure 3B**), suggesting a high degree of metabolic flexibility in these cells.

In LFs, among all of the measured amino acid fluxes, proline consumption uniquely increased (**Figure 1J**). Hypoxia increases collagen expression in these cells (Liu et al., 2013) and proline constitutes ~ 10% of the total amino acid content of collagens. Together, these data suggest an important contribution of extracellular proline to collagen production in hypoxic LFs as has been observed in other fibroblast cell lineages (Szoka et al., 2017).

In PASMCs, we observed increased consumption of the branched-chain amino acids (BCAAs) leucine and valine as well as arginine (**Figure 1 - figure supplement 4J**), which was not observed in LFs. BCAAs are transaminated by branch chain amino transferase enzymes to branched chain α-keto acids (BCKAs). BCKAs are further metabolized to yield acyl-CoA derivatives for lipogenesis or oxidation (Crown et al., 2015; Mann et al., 2021). Previous studies have shown that hypoxia up-regulates arginase expression in hypoxic PASMCs (Chen et al., 2009; Xue et al., 2017) to support polyamine and proline synthesis required for cell proliferation (Li et al., 2001). Interestingly, activation of these metabolic pathways in hypoxia was not observed in LFs and suggests distinct metabolic dependencies of these different cell types.

Compared to hypoxia treatment, BAY demonstrated more modest effects on amino acid fluxes generally (**Figure 2J**). In particular, glutamate efflux was not affected by BAY treatment, while it was reduced by hypoxia. Alanine efflux was increased by BAY treatment, but decreased by hypoxia. In addition to the glucose and lactate fluxes noted above, these findings further highlight fundamental differences in the metabolic consequences of HIF-1α activation in normoxia and hypoxia.

## Isotope tracing reveals altered substrate utilization in hypoxia

To investigate intracellular metabolic reprogramming in hypoxic cells, we performed 13C stable isotope tracing with [U-13C6]-glucose, [1,2-13C2]-glucose, and [U-13C5]-glutamine. Isotopic enrichment of downstream metabolites in glycolysis and the TCA cycle were determined by LC-MS (**Figure 4 - figure supplement 1**, **Figure 4 - figure supplement 2**). Overall, relatively small changes in the patterns of isotope incorporation were observed following hypoxia or BAY treatment. The most substantial differences were observed in pyruvate (PYR), the terminal product of glycolysis, and citrate (CIT), a central metabolic node in TCA and fatty acid metabolism (**Figure 4A-C**). Both hypoxia and BAY treatments decreased incorporation of glucose-derived carbon into pyruvate (**Figure 4A**) (*i.e.,* the unlabeled, or M0, fraction was greater). This suggests a greater contribution from an unlabeled carbon source, such as extracellular pyruvate, lactate, or alanine, than from glucose, to the intracellular pyruvate pool following PHD inhibition.

Total citrate labeling from [U-13C6]-glucose was unchanged across the treatment conditions (**Figure 4B**). As expected, we observed decreased M2 and M4 citrate isotopes, consistent with decreased pyruvate dehydrogenase activity in hypoxia. Interestingly, we observed increased M3 and M5 citrate isotopes. Pyruvate carboxylase catalyzes the carboxylation of pyruvate to oxaloacetate after which all three pyruvate carbons are incorporated into citrate by citrate synthase. Thus, this labeling pattern suggests a more prominent contribution of pyruvate carboxylase to sustain TCA cycle anaplerosis despite pyruvate dehydrogenase inhibition following HIF-1α activation. By contrast to glucose labeling, much less citrate was labeled by glutamine with hypoxia or BAY with a more pronounced effect of BAY treatment (**Figure 4C**), suggesting a less important contribution of glutamine to TCA anaplerosis under these conditions. In addition, the overall fraction of M5 citrate resulting from reductive carboxylation of glutamine-derived α-ketoglutarate was low (< 7%) (**Figure 4D**). Although a hypoxia-mediated increase in M5 citrate was observed, the overall fraction was much less than the 10-20% levels previously reported in cancer cells (Metallo et al., 2011; Wise et al., 2011).

The stable isotope labeling patterns in PASMCs were generally similar to LFs (**Figure 4 - figure supplement 2**). The most notable differences between LF and PASMC labeling were observed in citrate. Compared with LFs, a much lower fraction of total citrate was labeled by glucose in PASMCs. Less activity of pyruvate carboxylase in these cells was suggested by decreased M3 and M5 citrate isotopes after glucose labeling. Interestingly, the M5 citrate fraction in PASMCs was more consistent with previous reports from the cancer literature (**Figure 4D**), suggesting activation of glutamine anaplerosis for biomass synthesis in these cells.

## Glycolytic flux in hypoxia is closely coupled to cell growth rate

The mass isotopomer distribution for a given metabolite is determined by the complex relationship among the rate of isotope incorporation into the metabolic network, the contributions of unlabeled substrates, and fluxes through related pathways. To clarify how these labeling patterns reflect changes in intracellular metabolite fluxes, we next generated metabolic flux models incorporating the extracellular flux measurements and stable isotope tracing data described above. Preliminary labeling time courses indicated that, even after 72 h of labeling, intracellular metabolites did not reach isotopic steady state (**Figure 5 - figure supplement 1**). Thus, we performed isotopically non-stationary metabolic flux analysis as implemented by Isotopomer Network Compartment Analysis (INCA) (Jazmin and Young, 2013; Murphy and Young, 2013; Young, 2014).

Overall, LF and PASMC metabolic fluxes were dominated by high rates of glucose uptake and glycolysis (**Figure 5 - figure supplement 2**). Approximately 10% of cytoplasmic pyruvate enters the TCA cycle with the balance converted to lactate. Consistent with extracellular flux measurements and isotope labeling patterns described above, hypoxia significantly decreased glycolysis, the TCA cycle, and amino acid metabolism (**Figure 5A**). A significant increase in pentose phosphate pathway flux was also observed, although the absolute flux through this pathway is low. By contrast, HIF-1α activation by BAY in 21% oxygen increased glycolysis and lactate fermentation by nearly 50% (**Figure 5B**), but had a similar effect on decreasing serine and glutamine uptake as hypoxia. Metabolite fluxes in DMSO-treated cells were similar to 21% oxygen controls (**Table 1**, **Table 2**).

In normoxia, the magnitude of intracellular metabolite fluxes was generally similar in LFs and PASMCs (**Figure 5 - figure supplement 2**, **Table 1**, **Table 3**). Compared to LFs, PASMCs had slower rates of glycolysis and faster rates of TCA metabolism driven, in part, by increased glutamine uptake (**Figure 5 - figure supplement 3**). In hypoxia, PASMCs exhibited similar decreases in glycolytic flux as LFs but also a marked, and unexpected, increase in TCA flux (**Figure 5 - figure supplement 4**). The increased TCA flux in PASMCs was driven by increased glutamine consumption. This finding is similar to a prior report of glutamine-driven oxidative phosphorylation in hypoxic cancer cells (Fan et al., 2013), where oxidative phosphorylation continued to provide the majority of cellular ATP even at 1% oxygen.

Given the global decrease in bioenergetic metabolic flux in hypoxic LFs, we hypothesized that these differences may be a consequence of decreased growth rate. After normalizing metabolite fluxes in normoxia and hypoxia to the cell growth rate, a modest increase (~10%) in glycolytic flux was observed (**Figure 5 - figure supplement 5**). This finding suggests that, while glycolysis increases relative to growth rate in hypoxic cells, regulators of cell proliferation rate override the anti-proliferative effects of the HIF-1α transcriptional program. Indeed, even after adjusting for cell growth rate, the relative increase in glycolytic flux is modest compared to the marked up-regulation of glycolytic protein levels and the glycolytic potential of these cells demonstrated by BAY treatment in normoxia. BAY treatment decreased cell proliferation rate (**Figure 2B-C**), indicating that, unlike hypoxia, BAY treatment in normoxia uncouples cell proliferation and metabolic flux.

## Hypoxia and BAY treatment increase lactate oxidation

Although the metabolite exchange fluxes for bidirectional reactions tend to be poorly resolved by metabolic flux analysis (Wiechert, 2007), two observations are worth highlighting (**Table 1**, **Table 2**, **Table 3**). First, consistent with the stable isotope tracing results, the modeled rate of reductive carboxylation through reverse flux by isocitrate dehydrogenase in LFs is low (~4 fmol/cell/h), unchanged by hypoxia, and modestly increased by BAY treatment. By contrast, the rate of reductive carboxylation increases 6-fold in PASMCs in hypoxia, highlighting a potentially important role for this pathway in the metabolic response of PASMCs to decreased oxygen availability (**Figure 6**).

Second, PHD inhibition is associated with a marked increase in the lactate transport exchange flux in LFs from ~ 0 to 1,500 and 700 fmol/cell/h in 0.5% oxygen and BAY treatment conditions, respectively, with similar results in PASMCs (**Figure 7A**). Since the net lactate transport flux is secretion, this observation suggests increased lactate uptake with hypoxia or BAY treatment, a finding that may be consistent with the HIF-driven increased expression of the reversible lactate transporter MCT4 (Contreras-Baeza et al., 2019). To investigate this hypothesis, LFs and PASMCs were treated with [U-13C3] lactate (2 mM) and 13C incorporation into intracellular metabolites was analyzed by LC-MS (**Figure 7B**, **Figure 4 - figure supplement 1**, **Figure 4 - figure supplement 2**). Lactate labeled ~50% of citrate and ~20% of downstream TCA cycle metabolites in both LFs and PASMCs, indicating that lactate may be an important respiratory fuel source in these cells even though lactate efflux is high. Although lactate has been used less commonly than glucose and glutamine in stable isotope tracing studies, Faubert and colleagues (2017) demonstrated lactate incorporation in human lung adenocarcinoma *in vivo*. In this study, lactate incorporation corresponded to regions of high glucose uptake as determined by [¹⁸F]-fluorodeoxyglucose positron emission tomography, suggesting that lactate consumption can occur even in areas of high glucose utilization. Subsequently, investigators have demonstrated the importance of lactate as a metabolic fuel *in vivo* (Hui et al., 2020, 2017). As predicted from our metabolic flux analysis, with hypoxia or BAY treatment, we observed increased labeling of the TCA metabolites citrate (CIT), α-ketoglutarate (AKG), malate (MAL), and aspartate (ASP) in LFs. Interestingly, although increased labeling of pyruvate was observed in hypoxic PASMCs, the label was not incorporated into the TCA cycle as observed in LFs (**Figure 4 - figure supplement 2**).

In addition to downstream metabolites, we also observed hypoxia- and BAY-dependent increases in lactate incorporation in fructose bisphosphate (FBP) and 3-phosphoglycerate (3PG). This observation is consistent with prior reports describing hypoxia-mediated increases in gluconeogenesis and glycogen synthesis (Favaro et al., 2012; Owczarek et al., 2020; Pelletier et al., 2012). These data suggest that lactate also makes a small (~5% carbon) contribution to glycogen precursors. Together, these findings from stable isotope tracing of lactate reveal its important contribution to primary cell metabolism under standard culture conditions, but also reveal increased utilization of this substrate in hypoxia.

## Hypoxia prevents BAY from increasing glycolysis

To reconcile the differential effects of prolyl hydroxylase inhibition by hypoxia and BAY, we next addressed whether hypoxia could suppress the effects of BAY on glucose and lactate fluxes (**Figure 8**). LFs cultured in standard growth medium were treated with BAY and placed in either 21% or 0.5% oxygen. Similar to previous experiments, BAY treatment decreased cell growth rate, increased glucose uptake, and increased lactate efflux in 21% oxygen. However, when combined with 0.5% oxygen, BAY treatment was unable to enhance lactate efflux. These data demonstrate that hypoxia antagonizes the effects of HIF-1α activation on glycolytic flux in these cells.

We observed a similar effect of hypoxia on glycolysis when using RNA interference to silence PHD2 expression and activate HIF-1α gene transcription (**Figure 8 - figure supplement 1**).

These data indicate that the metabolic effects that we observe are not a consequence of off-target effects of BAY treatment.

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# Author contributions

W.M.O. conceived and designed the analysis. C.A.C., B.A.O., D.R.Z., S.M., K.L., and W.M.O. collected the data. J.D.Y. and W.M.O. contributed data or analysis tools. W.M.O. performed the analysis. W.M.O. drafted the manuscript. All authors participated in interpreting the results and revising the manuscript. All authors approve the final submission.

# Conflict of interest

The authors declare that they have no conflicts of interest.

# References

Ahn WS, Antoniewicz MR. 2011. Metabolic flux analysis of CHO cells at growth and non-growth phases using isotopic tracers and mass spectrometry. *Metab Eng* **13**:598–609. doi:[10.1016/j.ymben.2011.07.002](https://doi.org/10.1016/j.ymben.2011.07.002)

Antoniewicz MR. 2018. A guide to 13C metabolic flux analysis for the cancer biologist. *Exp Mol Med* **50**:1–13. doi:[10.1038/s12276-018-0060-y](https://doi.org/10.1038/s12276-018-0060-y)

Buescher JM, Antoniewicz MR, Boros LG, Burgess SC, Brunengraber H, Clish CB, DeBerardinis RJ, Feron O, Frezza C, Ghesquiere B, Gottlieb E, Hiller K, Jones RG, Kamphorst JJ, Kibbey RG, Kimmelman AC, Locasale JW, Lunt SY, Maddocks ODK, Malloy C, Metallo CM, Meuillet EJ, Munger J, Nöh K, Rabinowitz JD, Ralser M, Sauer U, Stephanopoulos G, St-Pierre J, Tennant DA, Wittmann C, Vander Heiden MG, Vazquez A, Vousden K, Young JD, Zamboni N, Fendt S-M. 2015. A roadmap for interpreting (13)C metabolite labeling patterns from cells. *Curr Opin Biotechnol* **34**:189–201. doi:[10.1016/j.copbio.2015.02.003](https://doi.org/10.1016/j.copbio.2015.02.003)

Chen B, Calvert AE, Cui H, Nelin LD. 2009. Hypoxia promotes human pulmonary artery smooth muscle cell proliferation through induction of arginase. *Am J Physiol Lung Cell Mol Physiol* **297**:L1151–1159. doi:[10.1152/ajplung.00183.2009](https://doi.org/10.1152/ajplung.00183.2009)

Contreras-Baeza Y, Sandoval PY, Alarcón R, Galaz A, Cortés-Molina F, Alegría K, Baeza-Lehnert F, Arce-Molina R, Guequén A, Flores CA, San Martín A, Barros LF. 2019. Monocarboxylate transporter 4 (MCT4) is a high affinity transporter capable of exporting lactate in high-lactate microenvironments. *J Biol Chem* **294**:20135–20147. doi:[10.1074/jbc.RA119.009093](https://doi.org/10.1074/jbc.RA119.009093)

Crown SB, Marze N, Antoniewicz MR. 2015. Catabolism of Branched Chain Amino Acids Contributes Significantly to Synthesis of Odd-Chain and Even-Chain Fatty Acids in 3T3-L1 Adipocytes. *PLoS One* **10**:e0145850. doi:[10.1371/journal.pone.0145850](https://doi.org/10.1371/journal.pone.0145850)

Fan J, Kamphorst JJ, Mathew R, Chung MK, White E, Shlomi T, Rabinowitz JD. 2013. Glutamine-driven oxidative phosphorylation is a major ATP source in transformed mammalian cells in both normoxia and hypoxia. *Mol Syst Biol* **9**:712. doi:[10.1038/msb.2013.65](https://doi.org/10.1038/msb.2013.65)

Faubert B, Li KY, Cai L, Hensley CT, Kim J, Zacharias LG, Yang C, Do QN, Doucette S, Burguete D, Li H, Huet G, Yuan Q, Wigal T, Butt Y, Ni M, Torrealba J, Oliver D, Lenkinski RE, Malloy CR, Wachsmann JW, Young JD, Kernstine K, DeBerardinis RJ. 2017. Lactate Metabolism in Human Lung Tumors. *Cell* **171**:358–371.e9. doi:[10.1016/j.cell.2017.09.019](https://doi.org/10.1016/j.cell.2017.09.019)

Favaro E, Bensaad K, Chong MG, Tennant DA, Ferguson DJP, Snell C, Steers G, Turley H, Li J-L, Günther UL, Buffa FM, McIntyre A, Harris AL. 2012. Glucose utilization via glycogen phosphorylase sustains proliferation and prevents premature senescence in cancer cells. *Cell Metab* **16**:751–764. doi:[10.1016/j.cmet.2012.10.017](https://doi.org/10.1016/j.cmet.2012.10.017)

Flamme I, Oehme F, Ellinghaus P, Jeske M, Keldenich J, Thuss U. 2014. Mimicking hypoxia to treat anemia: HIF-stabilizer BAY 85-3934 (Molidustat) stimulates erythropoietin production without hypertensive effects. *PLoS One* **9**:e111838. doi:[10.1371/journal.pone.0111838](https://doi.org/10.1371/journal.pone.0111838)

Gameiro PA, Yang J, Metelo AM, Pérez-Carro R, Baker R, Wang Z, Arreola A, Rathmell WK, Olumi A, López-Larrubia P, Stephanopoulos G, Iliopoulos O. 2013. In vivo HIF-mediated reductive carboxylation is regulated by citrate levels and sensitizes VHL-deficient cells to glutamine deprivation. *Cell Metab* **17**:372–385. doi:[10.1016/j.cmet.2013.02.002](https://doi.org/10.1016/j.cmet.2013.02.002)

Garcia-Bermudez J, Baudrier L, La K, Zhu XG, Fidelin J, Sviderskiy VO, Papagiannakopoulos T, Molina H, Snuderl M, Lewis CA, Possemato RL, Birsoy K. 2018. Aspartate is a limiting metabolite for cancer cell proliferation under hypoxia and in tumours. *Nat Cell Biol* **20**:775–781. doi:[10.1038/s41556-018-0118-z](https://doi.org/10.1038/s41556-018-0118-z)

Gardner LB, Li Q, Park MS, Flanagan WM, Semenza GL, Dang CV. 2001. Hypoxia inhibits G1/S transition through regulation of P27 expression. *J Biol Chem* **276**:7919–7926. doi:[10.1074/jbc.M010189200](https://doi.org/10.1074/jbc.M010189200)

Hubbi ME, Semenza GL. 2015. Regulation of cell proliferation by hypoxia-inducible factors. *Am J Physiol Cell Physiol* **309**:C775–782. doi:[10.1152/ajpcell.00279.2015](https://doi.org/10.1152/ajpcell.00279.2015)

Hui S, Cowan AJ, Zeng X, Yang L, TeSlaa T, Li X, Bartman C, Zhang Z, Jang C, Wang L, Lu W, Rojas J, Baur J, Rabinowitz JD. 2020. Quantitative Fluxomics of Circulating Metabolites. *Cell Metab* **32**:676–688.e4. doi:[10.1016/j.cmet.2020.07.013](https://doi.org/10.1016/j.cmet.2020.07.013)

Hui S, Ghergurovich JM, Morscher RJ, Jang C, Teng X, Lu W, Esparza LA, Reya T, Le Zhan null, Yanxiang Guo J, White E, Rabinowitz JD. 2017. Glucose feeds the TCA cycle via circulating lactate. *Nature* **551**:115–118. doi:[10.1038/nature24057](https://doi.org/10.1038/nature24057)

Jain IH, Calvo SE, Markhard AL, Skinner OS, To T-L, Ast T, Mootha VK. 2020. Genetic Screen for Cell Fitness in High or Low Oxygen Highlights Mitochondrial and Lipid Metabolism. *Cell* **181**:716–727.e11. doi:[10.1016/j.cell.2020.03.029](https://doi.org/10.1016/j.cell.2020.03.029)

Jazmin LJ, Young JD. 2013. Isotopically nonstationary 13C metabolic flux analysis. *Methods Mol Biol* **985**:367–390. doi:[10.1007/978-1-62703-299-5\_18](https://doi.org/10.1007/978-1-62703-299-5_18)

Jiang L, Shestov AA, Swain P, Yang C, Parker SJ, Wang QA, Terada LS, Adams ND, McCabe MT, Pietrak B, Schmidt S, Metallo CM, Dranka BP, Schwartz B, DeBerardinis RJ. 2016. Reductive carboxylation supports redox homeostasis during anchorage-independent growth. *Nature* **532**:255–258. doi:[10.1038/nature17393](https://doi.org/10.1038/nature17393)

Kaelin WG, Ratcliffe PJ. 2008. Oxygen sensing by metazoans: The central role of the HIF hydroxylase pathway. *Mol Cell* **30**:393–402. doi:[10.1016/j.molcel.2008.04.009](https://doi.org/10.1016/j.molcel.2008.04.009)

Koshiji M, Kageyama Y, Pete EA, Horikawa I, Barrett JC, Huang LE. 2004. HIF-1alpha induces cell cycle arrest by functionally counteracting Myc. *EMBO J* **23**:1949–1956. doi:[10.1038/sj.emboj.7600196](https://doi.org/10.1038/sj.emboj.7600196)

Le A, Lane AN, Hamaker M, Bose S, Gouw A, Barbi J, Tsukamoto T, Rojas CJ, Slusher BS, Zhang H, Zimmerman LJ, Liebler DC, Slebos RJC, Lorkiewicz PK, Higashi RM, Fan TWM, Dang CV. 2012. Glucose-independent glutamine metabolism via TCA cycling for proliferation and survival in B cells. *Cell Metab* **15**:110–121. doi:[10.1016/j.cmet.2011.12.009](https://doi.org/10.1016/j.cmet.2011.12.009)

Lee JW, Ko J, Ju C, Eltzschig HK. 2019. Hypoxia signaling in human diseases and therapeutic targets. *Exp Mol Med* **51**:1–13. doi:[10.1038/s12276-019-0235-1](https://doi.org/10.1038/s12276-019-0235-1)

Lee P, Chandel NS, Simon MC. 2020. Cellular adaptation to hypoxia through hypoxia inducible factors and beyond. *Nat Rev Mol Cell Biol* **21**:268–283. doi:[10.1038/s41580-020-0227-y](https://doi.org/10.1038/s41580-020-0227-y)

Lee WD, Mukha D, Aizenshtein E, Shlomi T. 2019. Spatial-fluxomics provides a subcellular-compartmentalized view of reductive glutamine metabolism in cancer cells. *Nat Commun* **10**:1351. doi:[10.1038/s41467-019-09352-1](https://doi.org/10.1038/s41467-019-09352-1)

Li H, Meininger CJ, Hawker JR, Haynes TE, Kepka-Lenhart D, Mistry SK, Morris SM, Wu G. 2001. Regulatory role of arginase I and II in nitric oxide, polyamine, and proline syntheses in endothelial cells. *Am J Physiol Endocrinol Metab* **280**:E75–82. doi:[10.1152/ajpendo.2001.280.1.E75](https://doi.org/10.1152/ajpendo.2001.280.1.E75)

Liu S-S, Wang H-Y, Tang J-M, Zhou X-M. 2013. Hypoxia-induced collagen synthesis of human lung fibroblasts by activating the angiotensin system. *Int J Mol Sci* **14**:24029–24045. doi:[10.3390/ijms141224029](https://doi.org/10.3390/ijms141224029)

Mann G, Mora S, Madu G, Adegoke OAJ. 2021. Branched-chain Amino Acids: Catabolism in Skeletal Muscle and Implications for Muscle and Whole-body Metabolism. *Front Physiol* **12**:702826. doi:[10.3389/fphys.2021.702826](https://doi.org/10.3389/fphys.2021.702826)

Meléndez-Rodríguez F, Urrutia AA, Lorendeau D, Rinaldi G, Roche O, Böğürcü-Seidel N, Ortega Muelas M, Mesa-Ciller C, Turiel G, Bouthelier A, Hernansanz-Agustín P, Elorza A, Escasany E, Li QOY, Torres-Capelli M, Tello D, Fuertes E, Fraga E, Martínez-Ruiz A, Pérez B, Giménez-Bachs JM, Salinas-Sánchez AS, Acker T, Sánchez Prieto R, Fendt S-M, De Bock K, Aragonés J. 2019. HIF1α Suppresses Tumor Cell Proliferation through Inhibition of Aspartate Biosynthesis. *Cell Rep* **26**:2257–2265.e4. doi:[10.1016/j.celrep.2019.01.106](https://doi.org/10.1016/j.celrep.2019.01.106)

Metallo CM, Gameiro PA, Bell EL, Mattaini KR, Yang J, Hiller K, Jewell CM, Johnson ZR, Irvine DJ, Guarente L, Kelleher JK, Vander Heiden MG, Iliopoulos O, Stephanopoulos G. 2011. Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. *Nature* **481**:380–384. doi:[10.1038/nature10602](https://doi.org/10.1038/nature10602)

Murphy TA, Young JD. 2013. ETA: Robust software for determination of cell specific rates from extracellular time courses. *Biotechnol Bioeng* **110**:1748–1758. doi:[10.1002/bit.24836](https://doi.org/10.1002/bit.24836)

Oldham WM, Clish CB, Yang Y, Loscalzo J. 2015. Hypoxia-Mediated Increases in L-2-hydroxyglutarate Coordinate the Metabolic Response to Reductive Stress. *Cell Metab* **22**:291–303. doi:[10.1016/j.cmet.2015.06.021](https://doi.org/10.1016/j.cmet.2015.06.021)

Owczarek A, Gieczewska K, Jarzyna R, Jagielski AK, Kiersztan A, Gruza A, Winiarska K. 2020. Hypoxia increases the rate of renal gluconeogenesis via hypoxia-inducible factor-1-dependent activation of phosphoenolpyruvate carboxykinase expression. *Biochimie* **171–172**:31–37. doi:[10.1016/j.biochi.2020.02.002](https://doi.org/10.1016/j.biochi.2020.02.002)

Pelletier J, Bellot G, Gounon P, Lacas-Gervais S, Pouysségur J, Mazure NM. 2012. Glycogen Synthesis is Induced in Hypoxia by the Hypoxia-Inducible Factor and Promotes Cancer Cell Survival. *Front Oncol* **2**:18. doi:[10.3389/fonc.2012.00018](https://doi.org/10.3389/fonc.2012.00018)

Scott DA, Richardson AD, Filipp FV, Knutzen CA, Chiang GG, Ronai ZA, Osterman AL, Smith JW. 2011. Comparative metabolic flux profiling of melanoma cell lines: Beyond the Warburg effect. *J Biol Chem* **286**:42626–42634. doi:[10.1074/jbc.M111.282046](https://doi.org/10.1074/jbc.M111.282046)

Semenza GL. 2012. Hypoxia-inducible factors in physiology and medicine. *Cell* **148**:399–408. doi:[10.1016/j.cell.2012.01.021](https://doi.org/10.1016/j.cell.2012.01.021)

Szoka L, Karna E, Hlebowicz-Sarat K, Karaszewski J, Palka JA. 2017. Exogenous proline stimulates type I collagen and HIF-1α expression and the process is attenuated by glutamine in human skin fibroblasts. *Mol Cell Biochem* **435**:197–206. doi:[10.1007/s11010-017-3069-y](https://doi.org/10.1007/s11010-017-3069-y)

Wenger RH, Kurtcuoglu V, Scholz CC, Marti HH, Hoogewijs D. 2015. Frequently asked questions in hypoxia research. *Hypoxia (Auckl)* **3**:35–43. doi:[10.2147/HP.S92198](https://doi.org/10.2147/HP.S92198)

Wheaton WW, Chandel NS. 2011. Hypoxia. 2. Hypoxia regulates cellular metabolism. *Am J Physiol Cell Physiol* **300**:C385–393. doi:[10.1152/ajpcell.00485.2010](https://doi.org/10.1152/ajpcell.00485.2010)

Wiechert W. 2007. The thermodynamic meaning of metabolic exchange fluxes. *Biophys J* **93**:2255–2264. doi:[10.1529/biophysj.106.099895](https://doi.org/10.1529/biophysj.106.099895)

Wise DR, Ward PS, Shay JES, Cross JR, Gruber JJ, Sachdeva UM, Platt JM, DeMatteo RG, Simon MC, Thompson CB. 2011. Hypoxia promotes isocitrate dehydrogenase-dependent carboxylation of α-ketoglutarate to citrate to support cell growth and viability. *Proc Natl Acad Sci U S A* **108**:19611–19616. doi:[10.1073/pnas.1117773108](https://doi.org/10.1073/pnas.1117773108)

Xue J, Nelin LD, Chen B. 2017. Hypoxia induces arginase II expression and increases viable human pulmonary artery smooth muscle cell numbers via AMPKα1 signaling. *Am J Physiol Lung Cell Mol Physiol* **312**:L568–L578. doi:[10.1152/ajplung.00117.2016](https://doi.org/10.1152/ajplung.00117.2016)

Young JD. 2014. INCA: A computational platform for isotopically non-stationary metabolic flux analysis. *Bioinformatics* **30**:1333–1335. doi:[10.1093/bioinformatics/btu015](https://doi.org/10.1093/bioinformatics/btu015)

Young JD, Allen DK, Morgan JA. 2014. Isotopomer measurement techniques in metabolic flux analysis II: Mass spectrometry. *Methods Mol Biol* **1083**:85–108. doi:[10.1007/978-1-62703-661-0\_7](https://doi.org/10.1007/978-1-62703-661-0_7)

# Figures

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| **Figure 1 - Effects of 0.5% oxygen on extracellular metabolite fluxes in lung fibroblasts.** (**A**) Lung fibroblasts (LFs) were cultured in 21% or 0.5% oxygen beginning 24 h prior to time 0. Samples were collected every 24 h for 72 h. (**B**) Growth curves of LFs in each experimental condition (n = 8). (**C**) Growth rates from (B) were determined by robust linear modeling of log-transformed growth curves. (**D**) Representative immunoblot of LF protein lysates cultured as in (A). (**E**) Relative change in HIF-1α protein levels from (D) normalized to 21% oxygen at time 0 (n = 4). (**F**) Relative change in GLUT1 mRNA levels normalized to 21% oxygen treatment at time 0 (n = 4). (**G**) Relative change in LDHA mRNA levels as in (F). (**H**) Relative change in LDHA protein levels as in (E). (**I**) Extracellular fluxes of glucose (GLC) and lactate (LAC) (n = 8). By convention, negative fluxes indicate metabolite consumption. (**J**) Extracellular fluxes of pyruvate (PYR) and amino acids. Data are mean ± SEM (\* p < 0.05). |

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| **Figure 1 - figure supplement 1 - Supporting data for extracellular flux calculations.** (**A**) Cell viability as assessed by live/dead cell staining with acridine orange plus propidium iodide did not differ between 21% and 0.5% oxygen culture conditions (n = 3 technical replicates). (**B**) Predicted well volumes were estimated from the change in culture plate mass over the experimental time course. Evaporation rates differed depending on the culture conditions and treatment. Although the mean evaporation rate is depicted, experiment-specific evaporation rates were used to calculate fluxes for each biological replicate (**C**) Metabolite accumulation (positive values) and degradation (negative values) rates. Data are mean ± SEM of 3-8 biological replicates. Rates significantly different from 0 (*) based on a probability value < 0.05 using Student’s one-sample* t\*-test were incorporated into flux calculations. |

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| **Figure 1 - figure supplement 2 - Quantifying lactate efflux generated by [U-13C6]-glucose.** Cells were cultured in MCDB131 supplemented with 8 mM [U-13C6]-glucose. Conditioned medium was collected, spiked with [D8]-ᴅʟ-valine internal standard, and analyzed by LC-MS. (**A**) A standard curve was generated from the peak area ratios of lactate and the internal standard prepared in unconditioned MCDB131 medium. (**B**) Lactate accumulates more slowly in the conditioned medium from hypoxic cells (n = 3 biological replicates). (**C**) Lactate efflux was decreased in hypoxia, similar to the data obtained from enzymatic lactate assay. Data are mean ± SEM (\* p < 0.05). |

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| **Figure 1 - figure supplement 3 - Effects of 0.2% oxygen on extracellular metabolite fluxes in lung fibroblasts.** (**A**) Lung fibroblasts (LFs) were cultured in 21% or 0.2% oxygen beginning 24 h prior to time 0. Samples were collected every 24 h for 72 h. (**B**) Growth curves of LFs in each experimental condition (n = 4). (**C**) Growth rates from (B) were determined by robust linear modeling of log-transformed growth curves. (**D**) Representative immunoblot of LF protein lysates cultured as in (A). (**E**) Relative change in HIF-1α protein levels from (D) normalized to 21% oxygen at time 0 (n = 4). (**F**) Relative change in GLUT1 mRNA levels normalized to 21% oxygen treatment at time 0 (n = 4). (**G**) Relative change in LDHA mRNA levels as in (F). (**H**) Relative change in LDHA protein levels as in (E). (**I**) Extracellular fluxes of glucose (GLC) and lactate (LAC) (n = 4). By convention, negative fluxes indicate metabolite consumption. (**J**) Extracellular fluxes of pyruvate (PYR) and amino acids. Data are mean ± SEM (\* p < 0.05). |

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| **Figure 1 - figure supplement 4 - Effects of 0.5% oxygen on extracellular metabolite fluxes in pulmonary artery smooth muscle cells.** (**A**) Pulmonary artery smooth muscle cells (PASMCs) were cultured in 21% or 0.5% oxygen beginning 24 h prior to time 0. Samples were collected every 24 h for 72 h. (**B**) Growth curves of LFs in each experimental condition (n = 4). (**C**) Growth rates from (B) were determined by robust linear modeling of log-transformed growth curves. (**D**) Representative immunoblot of LF protein lysates cultured as in (A). (**E**) Relative change in HIF-1α protein levels from (D) normalized to 21% oxygen at time 0 (n = 4). (**F**) Relative change in GLUT1 mRNA levels normalized to 21% oxygen treatment at time 0 (n = 4). (**G**) Relative change in LDHA mRNA levels as in (F). (**H**) Relative change in LDHA protein levels as in (E). (**I**) Extracellular fluxes of glucose (GLC) and lactate (LAC) (n = 4). By convention, negative fluxes indicate metabolite consumption. (**J**) Extracellular fluxes of pyruvate (PYR) and amino acids. Data are mean ± SEM (\* p < 0.05). |

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| **Figure 2 - Effects of pharmacologic prolyl hydroxylase inhibition on extracellular metabolite fluxes in lung fibroblasts.** (**A**) Lung fibroblasts (LFs) were treated with the prolyl hydroxlyase inhibitor molidustat (BAY, 10 μM) or DMSO beginning 24 h prior to time 0. Samples were collected every 24 h for 72 h. (**B**) Growth curves of LFs in each experimental condition (n = 8). (**C**) Growth rates from (B). (**D**) Representative immunoblot of LF protein lysates cultured as in (A). (**E**) Relative change in HIF-1α protein levels from (D) normalized to DMSO at time 0 (n = 4). (**F**) Relative change in GLUT1 mRNA levels normalized to DMSO at time 0 (n = 4). (**G**) Relative change in LDHA mRNA levels as in (F). (**H**) Relative change in LDHA protein levels as in (E). (**I**) Extracellular fluxes of glucose (GLC) and lactate (LAC) (n = 8). By convention, negative fluxes indicate metabolite consumption. (**J**) Extracellular fluxes of pyruvate (PYR) and amino acids. Data are mean ± SEM (\* p < 0.05). |

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| **Figure 3 - Cell growth rates following substrate deprivation.** (**A**, **B**) Lung fibroblasts (n = 8 biological replicates) (A) and pulmonary artery smooth muscle cells (n = 4 biological replicates) (B) were cultured in MCDB131 medium lacking either glucose (-GLC) or glutamine (-GLN) for 72 h. Growth rates were calculated from total DNA quantification. Data are mean ± SEM (\* p < 0.05; black compares 21% and 0.5% oxygen within a given treatment, red and blue compare substrate deficiency to replete medium in 21% and 0.5% oxygen, respectively. |

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| **Figure 4 - Stable isotope labeling of lung fibroblasts following hypoxic and pharmacologic PHD inhibition.** (**A**) Mass isotopomer distribution (MID) of pyruvate (PYR) following 72 h labeling with [U-13C6]-glucose (GLC). (**B**) MID of citrate after 72 h labeling with [U-13C6]-GLC (**C**) MID of citrate after 72 h labeling with [U-13C5]-glutamine (GLN). Data are mean ± SEM (n = 4, p < 0.05 indicated as \* 0.5% *v.* 21% oxygen, † BAY *v.* DMSO, ‡ Δoxygen *v.* ΔBAY). (**D**) Fraction of M5 citrate indicating reductive carboxylation after labeling with [U-13C5]-GLN in LFs and PASMCs (n = 3-4, \* p < 0.05). |

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| **Figure 4 - supplemental figure 1 - Mass isotopomer distributions after 72 h of labeling in lung fibroblasts.** Lung fibroblasts (LFs) were labeled with the indicated tracers and intracellular metabolites were analyzed by LC-MS after 72 h. Mass isotopomer distributions were adjusted for natural abundance. Data are the mean ± SEM of 4 biological replicates. Significant differences in labeling patterns between 21% and 0.5% oxygen (\*), DMSO and BAY treatment (†), and 0.5% oxygen and BAY treatment (‡) for each combination of metabolite and tracer are highlighted. |

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| **Figure 4 - supplemental figure 2 - Mass isotopomer distributions after 36 h of labeling in pulmonary artery smooth muscle cells.** Pulmonary artery smooth muscle cells (PASMCs) were labeled with the indicated tracers and intracellular metabolites were analyzed by LC-MS after 36 h. Mass isotopomer distributions were adjusted for natural abundance. Data are the mean ± SEM of 4 biological replicates. Significant differences in labeling patterns between 21% and 0.5% oxygen (\*) for each combination of metabolite and tracer are highlighted. |

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| **Figure 5 = Metabolic flux analysis of lung fibroblasts following hypoxic and pharmacologic PHD inhibition.** (**A**) Ratio of modeled metabolic fluxes in 0.5% oxygen compared to 21% oxygen. Fluxes with non-overlapping confidence intervals are highlighted with arrows colored according to the magnitude of the fold change. Arrow thickness corresponds to the absolute flux measured in hypoxia. (**B**) Ratio of metabolic fluxes in BAY-treated cells compared to DMSO-treated control. Arrows are colored as in (A) and arrow weights correspond to the absolute flux as measured in BAY-treated cells. |

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| **Figure 5 - figure supplement 1 - Isotope incorporation over the labeling time course.** LFs were cultured in 21% or 0.5% oxygen and labeled with the indicated tracers. Intracellular metabolites were analyzed by LC-MS (FBP, fructose-bisphosphate; PYR, pyruvate; CIT, citrate; MAL, malate). Mass isotopomer distributions were calculated and adjusted for natural abundance. Data show the total amount of metabolite labeling (*i.e.,* 1 - M0 fractional abundance). Data are the mean ± SEM of 4 biological replicates. |

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| **Figure 5 - figure supplement 2 - Isotopically non-stationary metabolic flux analysis of cell metabolism in 21% oxygen.** (**A**) Metabolic flux model of LF metabolism in 21% oxygen. Arrows are colored by log10(flux). (**B**) Metabolic flux model of PASMC metabolism in 21% oxygen as in (A). |

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| **Figure 5 - figure supplement 3 - Comparison of lung fibroblast and pulmonary artery smooth muscle cell metabolic fluxes.** Ratio of metabolic fluxes in PASMCs compared to LFs. Fluxes with non-overlapping confidence intervals are highlighted with arrows colored according to the magnitude of the change. Arrow thickness corresponds to the absolute flux measured in LFs. |

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| **Figure 5 - figure supplement 4 - Comparison of pulmonary artery smooth muscle cell metabolic fluxes in 21% and 0.5% oxygen.** Ratio of modeled metabolic fluxes in 0.5% oxygen compared to 21% oxygen. Fluxes with non-overlapping confidence intervals are highlighted with arrows colored according to the magnitude of the fold change. Arrow thickness corresponds to the absolute flux measured in hypoxia. |

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| **Figure 5 - figure supplement 5 - Growth rate adjusted changes in hypoxic lung fibroblast metabolism.** LF fluxes were normalized to cell growth rate. Graph depicts the ratio of normalized metabolic fluxes in LFs cultured in 0.5% oxygen compared to 21% oxygen control. Fluxes with non-overlapping confidence intervals are highlighted to indicate significant changes. |

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| **Figure 6 - Hypoxia increases reductive carboxylation in pulmonary artery smooth muscle cells.** (**A**) Reductive carboxylation describes the converstion of α-ketoglutarate (AKG) to isocitrate by reverse flux through isocitrate dehydrogenase (IDH). This yields M+5 citrate. (**B**) Exchange flux estimates for reductive carboxylation. Data show the model estimate with upper and lower bounds for LFs and PASMCs. |

# Tables

Table 1: LF fluxes in 21% and 0.5% oxygen

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| **Type** | **Pathway** | **ID** | **Reaction** | **Flux** | **LB** | **UB** | **Flux** | **LB** | **UB** | **Ratio** |
| NET | Transport | GLUT | GLC.x → GLC | 5.14e+02 | 5.11e+02 | 5.21e+02 | 4.41e+02 | 4.26e+02 | 4.58e+02 | 0.86 |
| PYRR | PYR.x → PYR.c | 7.56e+01 | 7.31e+01 | 7.96e+01 | 6.21e+01 | 5.83e+01 | 6.60e+01 | 0.82 |
| MCT | LAC ↔︎ LAC.x | 9.99e+02 | 9.98e+02 | 1.02e+03 | 8.91e+02 | 8.62e+02 | 9.25e+02 | 0.89 |
| ALAR | ALA → ALA.x | 2.25e+00 | 1.95e+00 | 2.49e+00 | 5.84e-01 | 1.10e-03 | 1.16e+00 | 0.26 |
| GLNR | GLN.x → GLN | 4.15e+01 | 4.06e+01 | 4.16e+01 | 1.43e+01 | 1.26e+01 | 1.94e+01 | 0.34 |
| GLUR | GLU ↔︎ GLU.x | 1.62e+01 | 1.58e+01 | 1.68e+01 | 7.55e+00 | 6.88e+00 | 8.15e+00 | 0.47 |
| ASPR | ASP → ASP.x | 2.57e+00 | 2.53e+00 | 2.68e+00 | 1.08e+00 | 4.17e-01 | 1.69e+00 | 0.42 |
| SERR | SER.x → SER | 1.42e+01 | 1.35e+01 | 1.49e+01 | 5.49e+00 | 4.99e+00 | 6.06e+00 | 0.39 |
| CYSR | CYX.x → CYS + CYS | 4.41e+00 | 4.23e+00 | 4.58e+00 | 1.65e+00 | 1.32e+00 | 2.08e+00 | 0.37 |
| GLYR | GLY → GLY.x | 2.05e+00 | 1.90e+00 | 2.15e+00 | 2.60e-01 | 2.00e-02 | 4.92e-01 | 0.13 |
| Glycolysis | HK | GLC → G6P | 5.14e+02 | 5.11e+02 | 5.21e+02 | 4.41e+02 | 4.26e+02 | 4.58e+02 | 0.86 |
| PGI | G6P ↔︎ F6P | 5.11e+02 | 4.99e+02 | 5.24e+02 | 4.23e+02 | 4.04e+02 | 4.40e+02 | 0.83 |
| PFK | F6P → FBP | 5.09e+02 | 5.00e+02 | 5.12e+02 | 4.32e+02 | 4.17e+02 | 4.49e+02 | 0.85 |
| ALDO | FBP ↔︎ DHAP + GAP | 5.09e+02 | 5.00e+02 | 5.12e+02 | 4.32e+02 | 4.17e+02 | 4.49e+02 | 0.85 |
| TPI | DHAP ↔︎ GAP | 5.08e+02 | 5.06e+02 | 5.08e+02 | 4.31e+02 | 4.15e+02 | 4.48e+02 | 0.85 |
| GAPDH | GAP ↔︎ 3PG | 1.02e+03 | 9.96e+02 | 1.04e+03 | 8.69e+02 | 8.35e+02 | 9.03e+02 | 0.85 |
| ENO | 3PG → PEP | 1.01e+03 | 9.99e+02 | 1.03e+03 | 8.68e+02 | 8.36e+02 | 9.00e+02 | 0.86 |
| PK | PEP → PYR.c | 1.04e+03 | 9.95e+02 | 1.04e+03 | 8.78e+02 | 8.36e+02 | 9.21e+02 | 0.84 |
| LDH | PYR.c ↔︎ LAC | 9.99e+02 | 9.98e+02 | 1.02e+03 | 8.91e+02 | 8.62e+02 | 9.25e+02 | 0.89 |
| GPT1 | PYR.c ↔︎ ALA | 1.19e+01 | 9.12e+00 | 1.19e+01 | 5.55e+00 | -9.08e+02 | 6.13e+00 | 0.47 |
| GPT2 | PYR.m ↔︎ ALA | -2.58e+00 | -4.56e+00 | 2.87e+00 | -2.40e-03 | -3.22e+01 | 9.11e+02 |  |
| Pentose phosphate pathway | G6PD | G6P → P5P + CO2 | 1.26e-07 | 0.00e+00 | 3.91e-01 | 1.62e+01 | 4.41e+00 | 2.89e+01 | 128571428.57 |
| TK1 | P5P + P5P ↔︎ S7P + GAP | -9.11e-01 | -9.29e-01 | -8.30e-01 | 4.76e+00 | -1.22e-01 | 9.62e+00 | -5.23 |
| TA | S7P + GAP ↔︎ F6P + E4P | -9.11e-01 | -9.29e-01 | -8.30e-01 | 4.76e+00 | -1.22e-01 | 9.62e+00 | -5.23 |
| TK2 | P5P + E4P ↔︎ F6P + GAP | -9.11e-01 | -9.29e-01 | -8.30e-01 | 4.76e+00 | -1.22e-01 | 9.62e+00 | -5.23 |
| Anaplerosis | PYRT | PYR.c → PYR.m | 1.16e+02 | 1.16e+02 | 1.19e+02 | 4.42e+01 | 3.82e+01 | 9.58e+02 |  |
| PC | PYR.m + CO2 → OAC | 1.88e+01 | 1.74e+01 | 1.91e+01 | 1.37e+01 | 9.82e+00 | 2.69e+01 |  |
| PEPCK | OAC → PEP + CO2 | 2.56e+01 | 1.58e+01 | 2.57e+01 | 9.66e+00 | 0.00e+00 | 2.60e+01 |  |
| ME2 | MAL → PYR.m + CO2 | 2.05e+00 | 9.51e-02 | 2.68e+00 | 1.00e-07 | 0.00e+00 | 2.25e+01 |  |
| ME1 | MAL → PYR.c + CO2 | 2.78e-02 | 0.00e+00 | 2.63e+01 | 8.71e-05 | 0.00e+00 | 2.52e+01 |  |
| FAO | FAO → AcCoA.m | 1.00e-07 | 0.00e+00 | 2.13e+00 | 6.58e-06 | 0.00e+00 | 7.73e-01 |  |
| GLDH | GLU ↔︎ AKG | 1.71e+01 | 1.56e+01 | 1.84e+01 | 9.11e-01 | -6.16e-01 | 7.27e+00 | 0.05 |
| GLS | GLN ↔︎ GLU | 3.78e+01 | 3.60e+01 | 3.86e+01 | 1.17e+01 | 1.01e+01 | 1.70e+01 | 0.31 |
| Tricarboxylic acid cycle | PDH | PYR.m → AcCoA.m + CO2 | 1.02e+02 | 8.76e+01 | 1.15e+02 | 3.05e+01 | 2.86e+01 | 5.24e+01 | 0.30 |
| CS | AcCoA.m + OAC → CIT | 1.02e+02 | 8.30e+01 | 1.11e+02 | 3.05e+01 | 2.88e+01 | 5.09e+01 | 0.30 |
| IDH | CIT ↔︎ AKG + CO2 | 2.49e+01 | 2.42e+01 | 2.53e+01 | 1.01e+01 | 8.75e+00 | 1.41e+01 | 0.41 |
| OGDH | AKG → SUC + CO2 | 4.19e+01 | 4.01e+01 | 4.25e+01 | 1.10e+01 | 7.87e+00 | 2.02e+01 | 0.26 |
| SDH | SUC ↔︎ FUM | 4.19e+01 | 4.01e+01 | 4.25e+01 | 1.10e+01 | 7.87e+00 | 2.02e+01 | 0.26 |
| FH | FUM ↔︎ MAL | 4.19e+01 | 4.01e+01 | 4.25e+01 | 1.10e+01 | 7.87e+00 | 2.02e+01 | 0.26 |
| MDH | MAL ↔︎ OAC | 1.17e+02 | 1.08e+02 | 1.24e+02 | 3.14e+01 | 2.62e+01 | 5.70e+01 | 0.27 |
| GOT | OAC ↔︎ ASP | 8.11e+00 | 8.06e+00 | 8.23e+00 | 4.98e+00 | 4.32e+00 | 5.64e+00 | 0.61 |
| Amino acid metabolism | PST | 3PG → SER | 1.95e+00 | 1.63e+00 | 2.00e+00 | 2.42e-01 | 1.34e-01 | 3.57e+01 |  |
| SHT | SER ↔︎ GLY + MEETHF | 6.38e+00 | 6.22e+00 | 6.43e+00 | 3.91e+00 | 3.71e+00 | 4.10e+00 | 0.61 |
| CYST | SER ↔︎ CYS | -7.12e+00 | -7.19e+00 | -6.81e+00 | -2.10e+00 | -2.97e+00 | -1.44e+00 | 0.30 |
| SD | SER → PYR.c | 1.17e+01 | 1.04e+01 | 1.20e+01 | 2.82e-01 | 0.00e+00 | 1.47e+00 | 0.02 |
| GLYS | CO2 + MEETHF → GLY | 3.39e+00 | 3.35e+00 | 3.49e+00 | 1.80e+00 | 1.66e+00 | 1.93e+00 | 0.53 |
| Biomass | BIOMASS | 1216\*AcCoA.c + 295.6\*ALA + 232.4\*ASP + 114.7\*CO2 + 71.43\*CYS + 57.14\*DHAP + 142.4\*G6P + 158.6\*GLN + 190.1\*GLU + 324.2\*GLY + 125.6\*MEETHF + 114.7\*P5P + 217.2\*SER → biomass | 2.38e-02 | 2.34e-02 | 2.39e-02 | 1.68e-02 | 1.61e-02 | 1.75e-02 | 0.71 |
| ACL | CIT → AcCoA.c + MAL | 7.74e+01 | 6.29e+01 | 1.04e+02 | 2.04e+01 | 1.95e+01 | 3.71e+01 | 0.26 |
| LIPS | AcCoA.c → lipid | 4.84e+01 | 4.55e+01 | 4.84e+01 | 1.00e-07 | 0.00e+00 | 1.68e+01 | 0.00 |
| Mixing | cPYR | 0\*PYR.c → PYR.ms | 1.00e+00 | 8.47e-01 | 1.00e+00 | 1.42e-01 | 0.00e+00 | 1.00e+00 |  |
| mPYR | 0\*PYR.m → PYR.ms | 1.00e-07 | 0.00e+00 | 1.53e-01 | 8.58e-01 | 0.00e+00 | 1.00e+00 |  |
| sPYR | PYR.ms → PYR.fix | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 |  |
| EXCH | Transport | MCT | LAC ↔︎ LAC.x | 1.00e-07 | 0.00e+00 | 1.05e-01 | 1.52e+03 | 1.35e+03 | 2.41e+03 | 15200000000.00 |
| GLUR | GLU ↔︎ GLU.x | 5.10e+00 | 4.77e+00 | 5.23e+00 | 1.54e+00 | 1.11e+00 | 2.54e+00 | 0.30 |
| Glycolysis | PGI | G6P ↔︎ F6P | 2.78e+05 | 1.77e+05 | Inf | 2.46e+05 | 0.00e+00 | Inf |  |
| ALDO | FBP ↔︎ DHAP + GAP | 1.43e+02 | 1.43e+02 | 1.43e+02 | 3.20e+02 | 2.79e+02 | 3.60e+02 | 2.24 |
| TPI | DHAP ↔︎ GAP | 4.33e+03 | 4.33e+03 | 1.09e+04 | 1.70e+03 | 1.06e+03 | 3.06e+03 | 0.39 |
| GAPDH | GAP ↔︎ 3PG | 4.42e+02 | 4.72e+00 | 4.50e+02 | 1.00e-07 | 0.00e+00 | 2.39e+02 |  |
| LDH | PYR.c ↔︎ LAC | 1.63e+03 | 1.62e+03 | 1.80e+03 | 4.80e+00 | 0.00e+00 | 3.51e+02 | 0.00 |
| GPT1 | PYR.c ↔︎ ALA | 1.00e-07 | 0.00e+00 | 2.61e-01 | 8.32e+02 | 0.00e+00 | 9.06e+02 |  |
| GPT2 | PYR.m ↔︎ ALA | 4.21e-04 | 0.00e+00 | 2.92e+00 | 1.28e-04 | 0.00e+00 |  |  |
| Pentose phosphate pathway | TK1 | P5P + P5P ↔︎ S7P + GAP | 9.97e+04 | 6.27e+03 | Inf | 1.47e+02 | 6.67e+01 | 2.60e+02 | 0.00 |
| TA | S7P + GAP ↔︎ F6P + E4P | 5.93e+00 | 5.79e+00 | 6.97e+00 | 2.35e-04 | 0.00e+00 | 7.54e+00 |  |
| TK2 | P5P + E4P ↔︎ F6P + GAP | 1.00e+07 | -Inf | Inf | 9.05e+00 | 4.10e+00 | 1.43e+01 |  |
| Anaplerosis | GLDH | GLU ↔︎ AKG | 1.52e+03 | 1.52e+03 | 7.13e+03 | 3.78e+02 | 1.93e+02 | 1.94e+03 |  |
| GLS | GLN ↔︎ GLU | 3.99e-01 | 0.00e+00 | 8.04e-01 | 1.00e-07 | 0.00e+00 | 3.84e-01 |  |
| Tricarboxylic acid cycle | IDH | CIT ↔︎ AKG + CO2 | 4.55e+00 | 4.03e+00 | 5.19e+00 | 2.52e+00 | 1.80e+00 | 4.50e+00 |  |
| SDH | SUC ↔︎ FUM | 1.22e+03 |  | Inf | 7.60e+01 | 2.57e+01 | Inf |  |
| FH | FUM ↔︎ MAL | 3.66e+05 | 1.95e+05 | Inf | 5.05e+05 | 3.06e+02 | Inf |  |
| MDH | MAL ↔︎ OAC | 1.11e+03 | 7.88e+02 | 2.38e+03 | 1.33e+02 | 7.22e+01 | 3.25e+02 | 0.12 |
| GOT | OAC ↔︎ ASP | 1.00e+07 | -Inf | Inf | 4.42e+01 | 0.00e+00 | Inf |  |
| Amino acid metabolism | SHT | SER ↔︎ GLY + MEETHF | 5.10e+00 | 8.92e-01 | 5.25e+00 | 6.07e-07 | 0.00e+00 | 3.32e+02 |  |
| CYST | SER ↔︎ CYS | 1.52e-05 | 0.00e+00 | 2.55e-04 | 1.46e-02 | 0.00e+00 | Inf |  |
| a SSR 391.7 [311.2-416.6] (95% CI, 362 DOF) | | | | | | | | | | |
| b SSR 334.3 [311.2-416.6] (95% CI, 362 DOF) | | | | | | | | | | |

Table 2: LF fluxes following DMSO and BAY treatment

|  | | | | **DMSOa** | | | **BAYb** | | |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Type** | **Pathway** | **ID** | **Reaction** | **Flux** | **LB** | **UB** | **Flux** | **LB** | **UB** | **Ratio** |
| NET | Transport | GLUT | GLC.x → GLC | 6.12e+02 | 6.12e+02 | 6.12e+02 | 8.80e+02 | 8.80e+02 | 8.80e+02 | 1.44 |
| PYRR | PYR.x → PYR.c | 9.98e+01 | 9.95e+01 | 1.01e+02 | 6.06e+01 | 6.06e+01 | 6.06e+01 | 0.61 |
| MCT | LAC ↔︎ LAC.x | 8.19e+02 | 8.17e+02 | 8.20e+02 | 1.33e+03 | 1.33e+03 | 1.33e+03 | 1.62 |
| ALAR | ALA → ALA.x | 2.67e+00 | 2.36e+00 | 3.29e+00 | 5.98e+00 | 5.88e+00 | 6.24e+00 | 2.24 |
| GLNR | GLN.x → GLN | 3.78e+01 | 3.77e+01 | 3.79e+01 | 2.06e+01 | 2.06e+01 | 2.06e+01 | 0.54 |
| GLUR | GLU ↔︎ GLU.x | 1.61e+01 | 1.56e+01 | 1.62e+01 | 1.68e+01 | 1.68e+01 | 1.68e+01 | 1.05 |
| ASPR | ASP → ASP.x | 2.36e+00 | 2.32e+00 | 2.49e+00 | 1.80e+00 | 1.80e+00 | 1.81e+00 | 0.76 |
| SERR | SER.x → SER | 1.03e+01 | 1.03e+01 | 1.06e+01 | 2.50e+00 | 2.50e+00 | 2.50e+00 | 0.24 |
| CYSR | CYX.x → CYS + CYS | 2.79e+00 | 2.79e+00 | 2.95e+00 | 3.07e-01 | 3.06e-01 | 3.07e-01 | 0.11 |
| GLYR | GLY → GLY.x | 2.52e+00 | 2.30e+00 | 2.73e+00 | 5.52e-01 | 4.30e-01 | 7.45e-01 | 0.22 |
| Glycolysis | HK | GLC → G6P | 6.12e+02 | 6.12e+02 | 6.12e+02 | 8.80e+02 | 8.80e+02 | 8.80e+02 | 1.44 |
| PGI | G6P ↔︎ F6P | 6.09e+02 | 6.08e+02 | 6.09e+02 | 8.42e+02 | 8.42e+02 | 8.42e+02 | 1.38 |
| PFK | F6P → FBP | 6.07e+02 | 6.07e+02 | 6.07e+02 | 8.65e+02 | 8.65e+02 | 8.65e+02 | 1.43 |
| ALDO | FBP ↔︎ DHAP + GAP | 6.07e+02 | 6.07e+02 | 6.07e+02 | 8.65e+02 | 8.65e+02 | 8.65e+02 | 1.43 |
| TPI | DHAP ↔︎ GAP | 6.06e+02 | 6.06e+02 | 6.06e+02 | 8.65e+02 | 8.65e+02 | 8.65e+02 | 1.43 |
| GAPDH | GAP ↔︎ 3PG | 1.21e+03 | 1.21e+03 | 1.21e+03 | 1.74e+03 | 1.74e+03 | 1.74e+03 | 1.44 |
| ENO | 3PG → PEP | 1.21e+03 | 1.21e+03 | 1.21e+03 | 1.57e+03 | 1.57e+03 | 1.57e+03 | 1.30 |
| PK | PEP → PYR.c | 1.23e+03 | 1.19e+03 | 1.23e+03 | 1.65e+03 | 1.65e+03 | 1.65e+03 | 1.34 |
| LDH | PYR.c ↔︎ LAC | 8.19e+02 | 8.17e+02 | 8.20e+02 | 1.33e+03 | 1.33e+03 | 1.33e+03 | 1.62 |
| GPT1 | PYR.c ↔︎ ALA | 9.62e+00 | 9.44e+00 | 9.62e+00 | 9.36e+00 | 9.32e+00 | 9.42e+00 | 0.97 |
| GPT2 | PYR.m ↔︎ ALA | 1.14e-01 |  |  | 2.28e-07 | -1.22e-05 | 6.41e-04 |  |
| Pentose phosphate pathway | G6PD | G6P → P5P + CO2 | 2.02e-02 | 0.00e+00 | 1.08e+00 | 3.64e+01 | 3.64e+01 | 3.64e+01 | 1801.98 |
| TK1 | P5P + P5P ↔︎ S7P + GAP | -9.06e-01 | -9.28e-01 | -9.06e-01 | 1.17e+01 | 1.17e+01 | 1.17e+01 | -12.89 |
| TA | S7P + GAP ↔︎ F6P + E4P | -9.06e-01 | -9.28e-01 | -9.06e-01 | 1.17e+01 | 1.17e+01 | 1.17e+01 | -12.89 |
| TK2 | P5P + E4P ↔︎ F6P + GAP | -9.06e-01 | -9.28e-01 | -9.06e-01 | 1.17e+01 | 1.17e+01 | 1.17e+01 | -12.89 |
| Anaplerosis | PYRT | PYR.c → PYR.m | 4.99e+02 | 4.97e+02 | 4.99e+02 | 5.50e+02 | 5.50e+02 | 5.50e+02 | 1.10 |
| PC | PYR.m + CO2 → OAC | 2.11e+01 | 2.07e+01 | 2.17e+01 | 9.05e+01 | 9.05e+01 | 9.05e+01 | 4.28 |
| PEPCK | OAC → PEP + CO2 | 1.36e+01 | 1.36e+01 | 1.37e+01 | 8.58e+01 | 8.58e+01 | 8.58e+01 | 6.31 |
| ME2 | MAL → PYR.m + CO2 | 1.30e+01 | 1.28e+01 | 1.37e+01 | 1.00e-07 | 0.00e+00 | 9.49e-06 | 0.00 |
| ME1 | MAL → PYR.c + CO2 | 3.20e-03 | 0.00e+00 | 1.73e+00 | 1.00e-07 | 0.00e+00 | 2.15e-05 |  |
| FAO | FAO → AcCoA.m | 1.00e-07 | 0.00e+00 | 3.48e+00 | 1.09e-04 | 8.34e-06 | 4.14e-02 |  |
| GLDH | GLU ↔︎ AKG | 1.33e+01 | 1.31e+01 | 1.35e+01 | -2.46e-01 | -2.47e-01 | -2.46e-01 | -0.02 |
| GLS | GLN ↔︎ GLU | 3.40e+01 | 3.35e+01 | 3.42e+01 | 1.88e+01 | 1.88e+01 | 1.88e+01 | 0.55 |
| Tricarboxylic acid cycle | PDH | PYR.m → AcCoA.m + CO2 | 4.90e+02 | 4.90e+02 | 4.92e+02 | 4.60e+02 | 4.60e+02 | 4.60e+02 | 0.94 |
| CS | AcCoA.m + OAC → CIT | 4.90e+02 | 4.84e+02 | 4.91e+02 | 4.60e+02 | 4.60e+02 | 4.60e+02 | 0.94 |
| IDH | CIT ↔︎ AKG + CO2 | 2.70e+01 | 2.70e+01 | 2.76e+01 | 1.45e+01 | 1.45e+01 | 1.45e+01 | 0.54 |
| OGDH | AKG → SUC + CO2 | 4.03e+01 | 3.99e+01 | 4.04e+01 | 1.43e+01 | 1.43e+01 | 1.43e+01 | 0.35 |
| SDH | SUC ↔︎ FUM | 4.03e+01 | 3.99e+01 | 4.04e+01 | 1.43e+01 | 1.43e+01 | 1.43e+01 | 0.35 |
| FH | FUM ↔︎ MAL | 4.03e+01 | 3.99e+01 | 4.04e+01 | 1.43e+01 | 1.43e+01 | 1.43e+01 | 0.35 |
| MDH | MAL ↔︎ OAC | 4.91e+02 | 4.91e+02 | 4.92e+02 | 4.60e+02 | 4.60e+02 | 4.60e+02 | 0.94 |
| GOT | OAC ↔︎ ASP | 7.91e+00 | 7.76e+00 | 7.98e+00 | 4.46e+00 | 4.46e+00 | 4.46e+00 | 0.56 |
| Amino acid metabolism | PST | 3PG → SER | 4.03e-01 | 3.74e-01 | 5.04e-01 | 1.73e+02 | 1.73e+02 | 1.73e+02 | 429.83 |
| SHT | SER ↔︎ GLY + MEETHF | 6.63e+00 | 6.59e+00 | 6.65e+00 | 2.85e+00 | 2.79e+00 | 2.93e+00 | 0.43 |
| CYST | SER ↔︎ CYS | -3.88e+00 | -3.91e+00 | -3.87e+00 | 2.03e-01 | 2.02e-01 | 2.03e-01 | -0.05 |
| SD | SER → PYR.c | 2.80e+00 | 2.80e+00 | 2.80e+00 | 1.70e+02 | 1.70e+02 | 1.70e+02 | 60.81 |
| GLYS | CO2 + MEETHF → GLY | 3.63e+00 | 3.50e+00 | 3.65e+00 | 1.41e+00 | 1.30e+00 | 1.46e+00 | 0.39 |
| Biomass | BIOMASS | 1216\*AcCoA.c + 295.6\*ALA + 232.4\*ASP + 114.7\*CO2 + 71.43\*CYS + 57.14\*DHAP + 142.4\*G6P + 158.6\*GLN + 190.1\*GLU + 324.2\*GLY + 125.6\*MEETHF + 114.7\*P5P + 217.2\*SER → biomass | 2.39e-02 | 2.39e-02 | 2.50e-02 | 1.14e-02 | 1.14e-02 | 1.14e-02 | 0.48 |
| ACL | CIT → AcCoA.c + MAL | 4.63e+02 | 4.63e+02 | 4.66e+02 | 4.45e+02 | 4.45e+02 | 4.45e+02 | 0.96 |
| LIPS | AcCoA.c → lipid | 4.34e+02 | 4.29e+02 | 4.34e+02 | 4.32e+02 | 4.32e+02 | 4.32e+02 |  |
| Mixing | cPYR | 0\*PYR.c → PYR.ms | 1.00e+00 | 9.99e-01 | 1.00e+00 | 1.00e-07 | 0.00e+00 | 1.00e+00 |  |
| mPYR | 0\*PYR.m → PYR.ms | 1.00e-07 | 0.00e+00 | 9.83e-04 | 1.00e+00 | 0.00e+00 | 1.00e+00 |  |
| sPYR | PYR.ms → PYR.fix | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 |  |
| EXCH | Transport | MCT | LAC ↔︎ LAC.x | 6.24e-04 | 0.00e+00 | 3.56e+00 | 7.11e+02 | 7.11e+02 | 7.11e+02 | 1139423.08 |
| GLUR | GLU ↔︎ GLU.x | 5.06e+00 | 4.82e+00 | 5.75e+00 | 3.48e+00 | 3.48e+00 | 3.48e+00 | 0.69 |
| Glycolysis | PGI | G6P ↔︎ F6P | 1.40e+06 | 1.39e+06 | Inf | 4.31e+06 | 4.31e+06 | 4.31e+06 |  |
| ALDO | FBP ↔︎ DHAP + GAP | 2.38e+02 | 2.38e+02 | 2.38e+02 | 1.02e+03 | 1.02e+03 | 1.02e+03 | 4.28 |
| TPI | DHAP ↔︎ GAP | 9.99e+06 |  | Inf | 7.57e+03 | 7.57e+03 | 7.57e+03 |  |
| GAPDH | GAP ↔︎ 3PG | 5.81e+02 | 5.81e+02 | 7.25e+02 | 1.09e+02 | 1.07e+02 | 1.09e+02 | 0.19 |
| LDH | PYR.c ↔︎ LAC | 2.65e+03 | 2.58e+03 | 2.65e+03 | 4.92e+01 | 4.91e+01 | 4.94e+01 | 0.02 |
| GPT1 | PYR.c ↔︎ ALA | 1.00e-07 | 0.00e+00 | 5.60e-02 | 2.45e+03 | 2.45e+03 | 2.45e+03 | 24500000000.00 |
| GPT2 | PYR.m ↔︎ ALA | 1.00e-07 | 0.00e+00 | 5.65e-02 | 1.00e-07 | 0.00e+00 | 1.20e-05 |  |
| Pentose phosphate pathway | TK1 | P5P + P5P ↔︎ S7P + GAP | 1.28e+06 | 9.01e+03 | Inf | 1.00e+07 | -Inf | Inf |  |
| TA | S7P + GAP ↔︎ F6P + E4P | 8.89e+00 | 8.88e+00 | 9.53e+00 | 5.10e+01 | 5.10e+01 | 5.10e+01 | 5.74 |
| TK2 | P5P + E4P ↔︎ F6P + GAP | 6.93e+00 | 5.12e+00 | 6.98e+00 | 1.00e-07 | 0.00e+00 | 1.56e-04 | 0.00 |
| Anaplerosis | GLDH | GLU ↔︎ AKG | 5.63e+03 | 4.43e+03 | 5.66e+03 | 1.42e+03 | 1.42e+03 | 1.42e+03 | 0.25 |
| GLS | GLN ↔︎ GLU | 1.27e+00 | 1.20e+00 | 1.50e+00 | 5.52e-01 | 5.51e-01 | 5.55e-01 | 0.43 |
| Tricarboxylic acid cycle | IDH | CIT ↔︎ AKG + CO2 | 3.36e+00 | 3.24e+00 | 3.92e+00 | 4.66e+00 | 4.66e+00 | 4.66e+00 | 1.39 |
| SDH | SUC ↔︎ FUM | 4.30e+02 | 4.30e+02 | 1.46e+06 | 1.04e+04 | 1.04e+04 | 1.04e+04 |  |
| FH | FUM ↔︎ MAL | 7.29e+06 | -Inf | Inf | 4.56e+06 | 4.56e+06 | 4.56e+06 |  |
| MDH | MAL ↔︎ OAC | 5.49e+02 | 5.47e+02 | 5.49e+02 | 1.00e-07 | 0.00e+00 | 6.30e-03 | 0.00 |
| GOT | OAC ↔︎ ASP | 1.04e+02 | 1.04e+02 | 1.04e+02 | 4.76e+05 | 4.76e+05 | 4.76e+05 | 4576.92 |
| Amino acid metabolism | SHT | SER ↔︎ GLY + MEETHF | 1.39e+00 | 1.37e+00 | 1.41e+00 | 1.86e+03 | 1.86e+03 | 1.86e+03 | 1338.13 |
| CYST | SER ↔︎ CYS | 1.25e-07 | 0.00e+00 | 4.22e-02 | 1.33e-01 | 1.33e-01 | 1.33e-01 | 1064000.00 |
| a SSR 393.5 [311.2-416.6] (95% CI, 362 DOF) | | | | | | | | | | |
| b SSR 392.4 [308.4-413.4] (95% CI, 359 DOF) | | | | | | | | | | |

Table 3: PASMC fluxes in 21% and 0.5% oxygen

|  | | | | **21%a** | | | **0.5%b** | | |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Type** | **Pathway** | **ID** | **Reaction** | **Flux** | **LB** | **UB** | **Flux** | **LB** | **UB** | **Ratio** |
| NET | Transport | GLUT | GLC.x → GLC | 4.28e+02 | 4.28e+02 | 4.28e+02 | 3.65e+02 | 3.65e+02 | 3.65e+02 | 0.85 |
| PYRR | PYR.x → PYR.c | 1.04e+02 | 1.02e+02 | 1.09e+02 | 4.53e+01 | 4.31e+01 | 4.57e+01 | 0.44 |
| MCT | LAC ↔︎ LAC.x | 8.01e+02 | 8.01e+02 | 8.04e+02 | 6.49e+02 | 6.49e+02 | 6.49e+02 | 0.81 |
| ALAR | ALA → ALA.x | 1.43e+01 | 1.43e+01 | 1.46e+01 | 7.83e+00 | 7.83e+00 | 8.24e+00 | 0.55 |
| GLNR | GLN.x → GLN | 7.73e+01 | 7.53e+01 | 7.73e+01 | 1.77e+02 | 1.77e+02 | 1.77e+02 | 2.29 |
| GLUR | GLU ↔︎ GLU.x | 2.53e+01 | 2.52e+01 | 2.54e+01 | 1.19e+01 | 1.19e+01 | 1.22e+01 | 0.47 |
| ASPR | ASP → ASP.x | 7.01e+00 | 6.99e+00 | 7.02e+00 | 6.92e+00 | 6.84e+00 | 7.00e+00 |  |
| SERR | SER.x → SER | 2.54e+00 | 2.48e+00 | 2.55e+00 | 2.57e+00 | 2.55e+00 | 2.57e+00 | 1.01 |
| CYSR | CYX.x → CYS + CYS | 6.39e+00 | 6.34e+00 | 6.45e+00 | 3.75e+00 | 3.75e+00 | 3.75e+00 | 0.59 |
| GLYR | GLY → GLY.x | 3.66e-01 | 3.03e-01 | 4.19e-01 | 4.06e-01 | 3.86e-01 | 4.25e-01 |  |
| Glycolysis | HK | GLC → G6P | 4.28e+02 | 4.28e+02 | 4.28e+02 | 3.65e+02 | 3.65e+02 | 3.65e+02 | 0.85 |
| PGI | G6P ↔︎ F6P | 4.06e+02 | 4.06e+02 | 4.07e+02 | 3.62e+02 | 3.62e+02 | 3.63e+02 | 0.89 |
| PFK | F6P → FBP | 4.17e+02 | 4.17e+02 | 4.18e+02 | 3.61e+02 | 3.60e+02 | 3.61e+02 | 0.87 |
| ALDO | FBP ↔︎ DHAP + GAP | 4.17e+02 | 4.17e+02 | 4.18e+02 | 3.61e+02 | 3.60e+02 | 3.61e+02 | 0.87 |
| TPI | DHAP ↔︎ GAP | 4.16e+02 | 4.16e+02 | 4.16e+02 | 3.60e+02 | 3.60e+02 | 3.60e+02 | 0.87 |
| GAPDH | GAP ↔︎ 3PG | 8.39e+02 | 8.39e+02 | 8.41e+02 | 7.21e+02 | 7.21e+02 | 7.21e+02 | 0.86 |
| ENO | 3PG → PEP | 8.36e+02 | 8.35e+02 | 8.53e+02 | 7.20e+02 | 7.20e+02 | 7.20e+02 | 0.86 |
| PK | PEP → PYR.c | 9.31e+02 | 9.30e+02 | 9.31e+02 | 9.24e+02 | 9.24e+02 | 9.24e+02 | 0.99 |
| LDH | PYR.c ↔︎ LAC | 8.01e+02 | 8.01e+02 | 8.04e+02 | 6.49e+02 | 6.49e+02 | 6.49e+02 | 0.81 |
| GPT1 | PYR.c ↔︎ ALA | 1.64e+02 | 1.62e+02 | 1.92e+02 | -1.36e+01 | -1.39e+01 | -1.35e+01 | -0.08 |
| GPT2 | PYR.m ↔︎ ALA | -1.43e+02 | -1.43e+02 | -1.42e+02 | 2.62e+01 | 2.51e+01 | 2.65e+01 | -0.18 |
| Pentose phosphate pathway | G6PD | G6P → P5P + CO2 | 1.89e+01 | 1.57e+01 | 1.93e+01 | 1.16e-07 | 0.00e+00 | 1.10e-03 | 0.00 |
| TK1 | P5P + P5P ↔︎ S7P + GAP | 5.46e+00 | 4.44e+00 | 5.96e+00 | -6.15e-01 | -6.15e-01 | -5.77e-01 | -0.11 |
| TA | S7P + GAP ↔︎ F6P + E4P | 5.46e+00 | 4.44e+00 | 5.96e+00 | -6.15e-01 | -6.15e-01 | -5.77e-01 | -0.11 |
| TK2 | P5P + E4P ↔︎ F6P + GAP | 5.46e+00 | 4.44e+00 | 5.96e+00 | -6.15e-01 | -6.15e-01 | -5.77e-01 | -0.11 |
| Anaplerosis | PYRT | PYR.c → PYR.m | 7.60e+01 | 7.59e+01 | 7.66e+01 | 3.36e+02 | 3.36e+02 | 3.36e+02 | 4.42 |
| PC | PYR.m + CO2 → OAC | 6.30e+01 | 6.29e+01 | 6.59e+01 | 2.37e+02 | 2.36e+02 | 2.37e+02 | 3.76 |
| PEPCK | OAC → PEP + CO2 | 9.51e+01 | 9.51e+01 | 9.53e+01 | 2.03e+02 | 2.03e+02 | 2.04e+02 | 2.14 |
| ME2 | MAL → PYR.m + CO2 | 1.20e-03 | 0.00e+00 | 5.20e-03 | 1.82e+02 | 1.81e+02 | 1.82e+02 | 151517.08 |
| ME1 | MAL → PYR.c + CO2 | 3.29e-05 | 0.00e+00 | 1.15e+00 | 5.91e-05 | 0.00e+00 | 8.06e-02 |  |
| FAO | FAO → AcCoA.m | 1.00e-07 | 0.00e+00 | 1.32e-02 | 1.15e-04 | 0.00e+00 | 1.56e-01 |  |
| GLDH | GLU ↔︎ AKG | 4.43e+01 | 4.42e+01 | 4.45e+01 | 1.59e+02 | 1.59e+02 | 1.59e+02 | 3.60 |
| GLS | GLN ↔︎ GLU | 7.38e+01 | 7.36e+01 | 7.38e+01 | 1.74e+02 | 1.74e+02 | 1.74e+02 | 2.36 |
| Tricarboxylic acid cycle | PDH | PYR.m → AcCoA.m + CO2 | 1.56e+02 | 1.48e+02 | 1.66e+02 | 2.55e+02 | 2.55e+02 | 2.55e+02 | 1.63 |
| CS | AcCoA.m + OAC → CIT | 1.56e+02 | 1.56e+02 | 1.58e+02 | 2.55e+02 | 2.55e+02 | 2.55e+02 | 1.63 |
| IDH | CIT ↔︎ AKG + CO2 | 2.11e+01 | 2.10e+01 | 2.11e+01 | 2.16e+01 | 2.16e+01 | 2.16e+01 | 1.03 |
| OGDH | AKG → SUC + CO2 | 6.54e+01 | 6.51e+01 | 6.59e+01 | 1.81e+02 | 1.80e+02 | 1.81e+02 | 2.77 |
| SDH | SUC ↔︎ FUM | 6.54e+01 | 6.51e+01 | 6.59e+01 | 1.81e+02 | 1.80e+02 | 1.81e+02 | 2.77 |
| FH | FUM ↔︎ MAL | 6.54e+01 | 6.51e+01 | 6.59e+01 | 1.81e+02 | 1.80e+02 | 1.81e+02 | 2.77 |
| MDH | MAL ↔︎ OAC | 2.01e+02 | 2.01e+02 | 2.01e+02 | 2.32e+02 | 2.32e+02 | 2.33e+02 | 1.16 |
| GOT | OAC ↔︎ ASP | 1.22e+01 | 1.17e+01 | 1.24e+01 | 1.07e+01 | 1.06e+01 | 1.07e+01 | 0.87 |
| Amino acid metabolism | PST | 3PG → SER | 2.69e+00 | 2.57e+00 | 2.80e+00 | 7.12e-01 | 7.01e-01 | 7.21e-01 | 0.26 |
| SHT | SER ↔︎ GLY + MEETHF | 5.19e+00 | 5.15e+00 | 5.20e+00 | 3.82e+00 | 3.81e+00 | 3.86e+00 | 0.74 |
| CYST | SER ↔︎ CYS | -1.12e+01 | -1.17e+01 | -1.11e+01 | -6.35e+00 | -6.35e+00 | -6.35e+00 | 0.57 |
| SD | SER → PYR.c | 6.39e+00 | 6.23e+00 | 6.44e+00 | 2.33e+00 | 2.33e+00 | 2.33e+00 | 0.36 |
| GLYS | CO2 + MEETHF → GLY | 2.39e+00 | 2.36e+00 | 2.42e+00 | 1.80e+00 | 1.79e+00 | 1.81e+00 | 0.75 |
| Biomass | BIOMASS | 978\*AcCoA.c + 237.8\*ALA + 187\*ASP + 92.3\*CO2 + 57.46\*CYS + 45.97\*DHAP + 114.5\*G6P + 127.6\*GLN + 153\*GLU + 260.8\*GLY + 101.1\*MEETHF + 92.3\*P5P + 174.8\*SER → biomass | 2.77e-02 | 2.70e-02 | 2.79e-02 | 2.00e-02 | 2.00e-02 | 2.00e-02 | 0.72 |
| ACL | CIT → AcCoA.c + MAL | 1.35e+02 | 1.34e+02 | 1.38e+02 | 2.33e+02 | 2.33e+02 | 2.33e+02 | 1.72 |
| LIPS | AcCoA.c → lipid | 1.08e+02 | 9.99e+01 | 1.08e+02 | 2.14e+02 | 2.14e+02 | 2.14e+02 | 1.98 |
| Mixing | cPYR | 0\*PYR.c → PYR.ms | 5.77e-01 | 5.64e-01 | 5.92e-01 | 1.00e+00 | 9.96e-01 | 1.00e+00 | 1.73 |
| mPYR | 0\*PYR.m → PYR.ms | 4.23e-01 | 4.08e-01 | 4.36e-01 | 1.00e-07 | 0.00e+00 | 4.40e-03 | 0.00 |
| sPYR | PYR.ms → PYR.fix | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 |  |
| EXCH | Transport | MCT | LAC ↔︎ LAC.x | 1.00e-07 | 0.00e+00 | 1.36e+02 | 1.64e+03 | 1.63e+03 | 1.65e+03 | 16400000000.00 |
| GLUR | GLU ↔︎ GLU.x | 1.00e-07 | 0.00e+00 | 2.27e-02 | 5.69e-05 | 0.00e+00 | 1.71e-02 |  |
| Glycolysis | PGI | G6P ↔︎ F6P | 4.88e+06 | 4.88e+06 | Inf | 9.92e+06 | 9.85e+04 | Inf |  |
| ALDO | FBP ↔︎ DHAP + GAP | 2.89e+02 | 2.80e+02 | 2.89e+02 | 2.57e+02 | 2.56e+02 | 2.57e+02 | 0.89 |
| TPI | DHAP ↔︎ GAP | 9.86e+06 | -Inf | Inf | 1.65e+03 | 1.63e+03 | 1.68e+03 |  |
| GAPDH | GAP ↔︎ 3PG | 1.12e+03 | 0.00e+00 | 5.88e+05 | 1.00e-07 | 0.00e+00 | 2.27e-01 |  |
| LDH | PYR.c ↔︎ LAC | 1.47e+03 | 1.39e+03 | 1.47e+03 | 4.49e+02 | 4.49e+02 | 4.49e+02 | 0.31 |
| GPT1 | PYR.c ↔︎ ALA | 2.74e+02 | 2.73e+02 | 2.77e+02 | 1.00e-07 | 0.00e+00 | 4.28e-02 | 0.00 |
| GPT2 | PYR.m ↔︎ ALA | 1.38e+02 | 1.38e+02 | 1.49e+02 | 9.64e+01 | 0.00e+00 | 1.01e+02 | 0.70 |
| Pentose phosphate pathway | TK1 | P5P + P5P ↔︎ S7P + GAP | 7.99e+02 | 7.97e+02 | 8.08e+02 | 3.54e+01 | 3.54e+01 | 3.55e+01 | 0.04 |
| TA | S7P + GAP ↔︎ F6P + E4P | 1.53e-01 | 0.00e+00 | 5.82e-01 | 2.55e+00 | 2.54e+00 | 2.57e+00 | 16.67 |
| TK2 | P5P + E4P ↔︎ F6P + GAP | 3.33e+00 | 2.62e+00 | 3.35e+00 | 1.29e+01 | 1.29e+01 | 1.29e+01 | 3.88 |
| Anaplerosis | GLDH | GLU ↔︎ AKG | 5.36e+02 | 5.34e+02 | 8.37e+02 | 1.23e+03 | 1.23e+03 | 1.23e+03 | 2.29 |
| GLS | GLN ↔︎ GLU | 3.20e-01 | 0.00e+00 | 2.74e+00 | 1.12e+00 | 1.07e+00 | 1.74e+00 |  |
| Tricarboxylic acid cycle | IDH | CIT ↔︎ AKG + CO2 | 1.04e+01 | 1.02e+01 | 1.04e+01 | 6.30e+01 | 6.30e+01 | 6.31e+01 | 6.09 |
| SDH | SUC ↔︎ FUM | 2.78e-01 | 0.00e+00 | Inf | 3.34e+06 | 3.34e+06 | 3.34e+06 |  |
| FH | FUM ↔︎ MAL | 1.03e-04 | 0.00e+00 | 1.58e+01 | 2.18e+02 | 2.18e+02 | 2.18e+02 | 2114238.83 |
| MDH | MAL ↔︎ OAC | 1.01e+03 | 8.27e+02 | 1.01e+03 | 3.67e+03 | 3.67e+03 | 3.69e+03 | 3.63 |
| GOT | OAC ↔︎ ASP | 2.27e+02 | 2.27e+02 | 2.47e+02 | 1.54e+01 | 1.54e+01 | 1.55e+01 | 0.07 |
| Amino acid metabolism | SHT | SER ↔︎ GLY + MEETHF | 3.55e+00 | 3.52e+00 | 3.59e+00 | 1.60e-01 | 1.36e-01 | 1.70e-01 | 0.05 |
| CYST | SER ↔︎ CYS | 1.04e+03 | 1.03e+03 | 1.04e+03 | 2.00e-03 | 0.00e+00 | 2.00e-03 | 0.00 |
| a SSR 575.6 [499.1-630.6] (95% CI, 563 DOF) | | | | | | | | | | |
| b SSR 521.3 [482.2-611.6] (95% CI, 545 DOF) | | | | | | | | | | |