Lactate as substrate for mitochondrial respiration in alveolar epithelial type II cells

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Lactate metabolism in the lung has received little attention in modern investigation, although several landmark studies of whole organ metabolism previously indicated that lactate oxidation occurs in the lung. Wolfe et al. used adult isolated perfused rat lung to demonstrate rapid oxidation of lactate by whole lung tissue, even when the perfusate contained glucose at a concentration several times that of lactate (34). Under these conditions, CO₂ derived from lactate exceeded that from glucose. Later studies by Fox and colleagues using both perfused whole lung and isolated alveolar epithelial cells from fetal rat lung demonstrated rates of CO₂ generation from lactate that were ~20 times the rate of glucose oxidation and the highest rate for any substrate examined (11, 12). In these studies, glucose and lactate showed reciprocal inhibition, indi-
cating similar pathways of metabolism. The observation that labeled lactate in pulmonary circulation is incorporated into lung lipids in both adult (31) and perinatal (28) lung further implicates ATII cells as a site of lactate consumption in the lung, since ATII cells synthesize the majority of lipid components in pulmonary surfactant. Despite this, lactate consumption has not been directly linked to cellular energetics in ATII cells.

Cell consumption of lactate for ATP generation is achieved through conversion of lactate to pyruvate by lactate dehydrogenase (LDH) enzyme activity. Pyruvate is shuttled into the mitochondrond and oxidized through reactions of the TCA cycle, and the reducing equivalents generated serve as electron donors for oxidative phosphorylation. Lactate transport both into and out of the cell is mediated by monocarboxylate transporter (MCT) proteins (17). Although MCT isoforms 1–4 all transport lactate and pyruvate bidirectionally, different isoforms more heavily favor import vs. export. Specifically, the lower-affinity MCT4 is associated mainly with lactate efflux from glycolytic cells (8, 26), whereas the higher-affinity isoform MCT1 is associated with import into oxidative lactate-consuming cells. Both MCT1 and MCT4 have been found in the developing lung (15), and MCT1, -2, and -4 are present in adult whole lung samples (21). However, the specific cell types in the mature lung expressing each MCT isoform have not been determined, and the contribution of MCT-mediated transport to overall control of cell metabolism in the lung is unknown.

We recently reported that ATII cells have a highly oxidative metabolic phenotype and are heavily dependent on mitochondrial function for energy production (24). This metabolic phenotype is characteristic of cells capable of lactate consumption in other tissues (3). This led us to hypothesize that ATII cells import lactate and use it as substrate for mitochondrial energy production. Here, we show that ATII cells can use lactate for oxidative ATP production. Culture in lactate alone induces high levels of oxygen consumption, resulting in a shift to an extremely oxidative, minimally glycolytic phenotype. Also, we provide evidence that the presence of lactate impacts glucose metabolism when both substrates are available. This metabolic strategy is dependent on the function of LDH and MCT proteins, and, for the first time, we define ATII cells as a cell population in the mature lung that expresses MCT1. Finally, we address the impact of hypoxia on lactate-fueled mitochondrial respiration, inspired by the observation that pulmonary hypoxia develops in a number of lung diseases that have been recently associated with lactate build-up in patient lung tissue. Despite the apparent role of lactate in pulmonary disease pathogenesis, a thorough understanding of lactic acid production, consumption, and balance in the healthy lung is lacking. This work therefore represents a critical step toward understanding from a metabolic point of view the normal ATII processes that may be disrupted in, and contribute to, disease pathogenesis.

METHODS

Reagents. Culture media and supplements were obtained from Life Technologies (Grand Island, NY), except for fetal bovine serum (FBS) from Atlanta Biologicals (Lawrenceville, GA). Extracellular flux assay medium and consumables were purchased from Seahorse Bioscience (North Billerica, MA), and general chemicals, including D-glucose and sodium L-lactate, from Sigma-Aldrich (St. Louis, MO).

Cell culture. Cultures of the murine ATII-like cell line MLE-15 were maintained in a humidified incubator under ambient air (21% O_2) and 5% CO_2 at 37°C in HITES medium (glucose-free, pyruvate-free RPMI; 2% FBS, 2 mM Glutamax, 10 μg/ml insulin, 10 μg/ml transferrin, and 0.5 μg/ml sodium selenite; supplemented with 5.5 mM glucose). Cells were plated in HITES medium and allowed to attach to culture dishes. After 1–2 h of incubation, culture medium was replaced with HITES supplemented with 5.5 mM glucose, 5.5 mM lactate, or indicated concentrations. Hypoxia exposures were performed by incubation in a hypoxic chamber (Biospherex, Redfield, NY) with 1.5% O_2, 5% CO_2 at 37°C for 20 h.

Isolation and culture of primary ATII cells. All procedures were conducted under approved Institutional Animal Care and Use Committee protocols. ATII cells were isolated from C57B/6 mouse lungs as described previously (24). Cells were cultured on Matrigel (BD Biosciences, San Jose, CA)-coated plates in glucose-free, pyruvate-free RPMI medium (Life Technologies) with 5% FBS and Small Airway Epithelial Cell supplement (Lonza, Walkersville, MD), formulated with indicated concentrations of lactate and/or glucose.

Extracellular flux analysis. Cellular O_2 consumption and H+ generation were assessed using a Seahorse Bioscience XF24 or XF96 instrument, as previously described (24). Cells were plated in 24-well assay plates at 6.5 × 10^4 cells/well or 96-well assay plates at 1.7 × 10^5 cells/well and assayed in unbuffered XF Base Medium (Seahorse Bioscience) formulated with glucose or lactate and HITES media supplements (as above) except for FBS, which was excluded in MLE-15 cell assay media, but provided at 5% in primary cell media to maintain primary cell phenotype and viability. Glutamax supplement was excluded for indicated oxygen consumption experiments. Data were transformed using “Level (Direct) AKOS” algorithm (14) using the Seahorse XF24 or XF96 software package. Proton production rate (PPR) was calculated from measured extracellular acidification rate with compensation for media-buffering capacity. Media buffering capacities were calculated empirically for each batch. Primary cell assay media buffering capacity was calculated at 2.9 × 10^-3 M. For MLE-15 assay media, values ranged from 1.7 × 10^-3 to 2.3 × 10^-3 M, and correction was done individually for each experiment according to the value calculated on the day of assay. Values were normalized to well protein content.

RNA and protein analyses. RNA harvest, cDNA generation, and qPCR assays were performed as previously described (24). C_{i} values for genes of interest were normalized to β-actin expression. Fold-change values for target genes were calculated using ∆∆C_{i} analysis, and values for biological replicates were averaged.

ATP concentration. ATP content of cultured cells was measured using the CellTitre Glo Luminescent assay (Promega, Madison, WI) following the manufacturer’s instructions.

Cell count and 5-ethyl-2′-deoxyuridine incorporation. MLE-15 were plated onto 12-well plates at 5 × 10^4 cells/well and cultured in...
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Fig. 1. Culture in lactate shifts alveolar epithelial type II (ATII) cells into a highly oxidative metabolic state. Oxygen consumption rates (OCR) and proton production rates (PPR) were measured for primary ATII cells (circles) and MLE-15 cells (diamonds) cultured in either 5.5 mM glucose (closed) or 5.5 mM lactate (open). For MLE-15, 4 individual experiments were performed, and, in each, samples were assayed minimally in triplicate per condition. For primary cultures, 6 single-well experiments were performed for each condition. For each cell type significant difference is indicated as follows: *significant difference (P < 0.05) from glucose condition OCR; †significant difference from glucose condition PPR. Error bars represent ± SD.

HITES medium formulated with glucose, lactate, or combined substrates. At each interval, three individual wells were trypsinized and exposed to the vital dye trypan blue. Cells excluding trypan blue were counted using a hemocytometer. Relative rates of DNA synthesis were determined using 5-ethynyl-2’deoxyuridine (EdU) nucleotide analog incorporation as previously described (30). Each sample well was stained subsequently with ToPRO3 nuclear stain for normalization.

Statistics. Significant difference was assessed using Student’s t-test or ANOVA with Tukey’s post hoc analysis. P values <0.05 were considered significant. All error bars represent ±SD. Statistical details for each experiment are also provided in the legends for Figs. 1–8.

RESULTS

Lactate is a substrate for oxidative ATP production in ATII cells. Metabolic flux analysis was performed using cells cultured in medium containing either lactate or glucose as metabolic substrate. MLE-15 cells cultured in lactate had oxygen consumption rates (OCR) of mitochondrial activity approximately two times those observed for cells metabolizing glucose (Fig. 1). Alternatively, cells in lactate-formulated medium displayed minimal extracellular PPR (a measure of glycolysis) compared with those in glucose. Together, OCR and PPR values demonstrate a shift into a highly oxidative metabolism in the presence of lactate and absence of glucose. Similar results were also obtained with primary mouse ATII cells cultured in lactate vs. those in glucose (Fig. 1).

Numerous processes, including mitochondrial production of ATP and nonmitochondrial oxidation, contribute to total cellular OCR and can be measured by injection of various inhibitors during the flux assay. Following basal measurements, inhibition of ATP synthase via oligomycin injection resulted in a decrease in OCR, indicative of respiration coupled to ATP production. In glucose- and lactate-cultured cells, ~50 and 65% of basal oxygen consumption, respectively, is dedicated to mitochondrial ATP production (Table 1). This indicates a similar degree of coupling of O2 consumption to mitochondrial ATP generation by percentage of total O2 consumed, although in terms of OCR per microgram protein, the amount of oxygen consumed to fuel ATP production is greater in the lactate-cultured cells because of their high basal rates. Similarly, nonmitochondrial oxygen consumption accounted for a similar proportion of total oxygen consumption in glucose- and lactate-grown cells (28% of each respective mean basal value).

Given the greatly increased OCR in cells cultured in lactate compared with those in glucose, we sought to determine if this was the result of a simple increase in mitochondrial activity (use of spare reserve) or an actual increase in functional respiratory capacity of cells grown in lactate. Exposing cells to the mitochondrial uncoupling agent FCCP to force maximal electron transfer/OCR demonstrated that ATII cells grown in glucose, but not lactate, maintained a robust mitochondrial reserve above basal levels, indicating that the greater OCR in lactate is the result of increased usage of existing mitochondrial spare respiratory capacity (Fig. 2).

Lactate must first be converted to pyruvate before it can be utilized as substrate for mitochondrial metabolism. This is achieved by the reverse activity of LDH, which oxidizes lactate to generate pyruvate. Thus, inhibition of LDH would be expected to reduce the ability of cells to respire using lactate as a metabolic substrate. Exposure to the competitive LDH inhibitor oxamate before assay resulted in decreased OCR in lactate-cultured cells, confirming that lactate is consumed by ATII via conversion to pyruvate (Fig. 3). In contrast, OCR was increased in cells cultured in glucose and exposed to LDH inhibitor.

Glutamine, an amino acid commonly supplemented in culture medium, can also be utilized as substrate for mitochondrial respiration via TCA cycle reactions. To ensure that the above results were not the result of glutamine consumption, OCR was measured in cells assayed in XF base medium with and without the addition of glutamine-alanine dipeptide supplement. No significant difference was found between the conditions, suggesting that glutamine oxidation contributes minimally to total flux in ATII in these experiments (data not shown).

Finally, to determine the impact of lactate availability on cellular metabolism when glucose is available, OCR and PPR

Table 1. Oxygen allocation to respiratory functions is proportionally similar in glucose- and lactate-cultured alveolar epithelial type II cells

<table>
<thead>
<tr>
<th>Respiratory Parameter</th>
<th>Glucose</th>
<th>Lactate</th>
<th>Glucose</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol O2·min⁻¹ µg protein⁻¹</td>
<td>Calculated as % mean basal</td>
<td>pmol O2·min⁻¹ µg protein⁻¹</td>
<td>Calculated as % mean basal</td>
</tr>
<tr>
<td>Basal</td>
<td>12.7 ± 1.6</td>
<td>100</td>
<td>22.0 ± 4.9</td>
<td>100</td>
</tr>
<tr>
<td>ATP coupled</td>
<td>6.2 ± 1.3</td>
<td>49</td>
<td>14.2 ± 1.5</td>
<td>65</td>
</tr>
<tr>
<td>Nonmitochondrial</td>
<td>3.6 ± 0.3</td>
<td>28</td>
<td>6.2 ± 1.6</td>
<td>28</td>
</tr>
<tr>
<td>Remainder</td>
<td>2.9</td>
<td>23</td>
<td>1.6</td>
<td>7</td>
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Oxygen consumption rate values are reported ± SD. Percentages were calculated using basal values reported in Fig. 1. Parameters were measured in MLE-15 cultured in 5.5 mM glucose or 5.5 mM lactate. Five single-well experiments were performed per inhibitor.
were measured for MLE-15 cultured in media formulated with 5.5 mM glucose and increasing concentrations of lactate. At 2.75 mM lactate and above, the presence of lactate decreased PPR in a dose-dependent manner (Fig. 4). There was no significant effect of media lactate on OCR, regardless of lactate concentration.

Hypoxia suppresses lactate metabolism. Exposure of MLE-15 cells to 1.5% O$_2$ for 20 h before assay resulted in a similar decrease in OCR to ~50% of normoxic values for cells cultured in either glucose or lactate (Fig. 5A). This indicates that mitochondrial metabolism in general is suppressed by hypoxia to a similar degree regardless of substrate. Similar results were obtained using primary ATII cells cultured in lactate vs. glucose and subjected to hypoxia (Fig. 5B).

Fig. 2. Mitochondrial respiration is performed at maximal rates in MLE-15 cells consuming lactate alone. Following measurements of basal respiration, carbonyl cyanide-p-trifluoromethoxy-phenylhydrazone (FCCP) was added to the assay media to measure uncoupled (maximal) OCR in MLE-15 cells cultured in 5.5 mM glucose or 5.5 mM lactate. Five single-well experiments were performed per condition. *Significant difference ($P < 0.05$) from basal OCR for each condition, error bars represent ± SD.

Fig. 3. Inhibition of lactate dehydrogenase (LDH) reduces lactate-fueled respiration in MLE-15. OCR was measured for MLE-15 cells cultured in 5.5 mM glucose or 5.5 mM lactate and exposed to the LDH inhibitor oxamate. Oxamate was dissolved in assay media to a final concentration of 20 mM and added to cultures 1 h before assay. Control wells received normal assay media. Data are represented as percentage of glucose or lactate controls unexposed to oxamate. Four single-well control and 5 single-well exposures were performed per substrate condition. *Significant difference ($P < 0.05$) from unexposed controls, error bars represent ± SD.

Fig. 4. Extracellular lactate concentration regulates glycolytic output. OCR and PPR were measured for MLE-15 cultured in 5.5 mM glucose and increasing concentrations of lactate. Data are represented as %glucose-only control. Twelve single-well experiments were assayed per substrate condition. *Significant difference ($P < 0.05$) from glucose-only control. Error bars represent ± SD.

Fig. 5. Exposure to hypoxia suppresses ATII cell oxidative metabolism of glucose and lactate. OCR was measured for MLE-15 cells (A) and primary ATII cells (B) cultured in 5.5 mM glucose or 5.5 mM lactate and incubated in normoxia (21% O$_2$) or hypoxia (1.5% O$_2$) for 20 h. For MLE-15 cultures, 3 individual experiments were performed, and, in each, samples were assayed minimally in triplicate per condition. For primary cultures, 6 single-well experiments were performed for each condition. *Significant difference ($P < 0.05$) from normoxic OCR for each condition. Error bars represent ± SD.
Hypoxic exposure leads to decreased use of both lactate and glucose for mitochondrial metabolism in ATII.

Lactate oxidation by ATII cells is dependent on MCT transport function. CHC inhibits MCT protein isoforms at the cytosolic membrane, leading to reduced lactate transport into and out of the cell. Addition of CHC to medium during flux assays resulted in decreased PPR by MLE-15 cells regardless of substrate, with PPR of lactate-grown cells decreased to essentially zero (Fig. 6A). However, CHC treatment led to a decrease of OCR by ~50% for cells cultured in lactate, whereas in glucose-grown MLE-15, OCR was unaffected by CHC (Fig. 6B).

ATII cells express the lactate transporter MCT1. The MCT1 transporter mediates lactate import in lactate-consuming skeletal muscle and, in some cell types, is hypoxia inducible. MCT1 mRNA expression was measured in lysates from MLE-15 and isolated primary ATII cells, and protein expression was assessed in MLE-15 lysates compared with mouse skeletal muscle tissue as positive control. Both MLE-15 and primary ATII cells express MCT1 mRNA. We evaluated the impact of hypoxic exposure on expression of MCT1 expression and found no significant difference in mRNA expression in response to hypoxia in either MLE-15 or primary ATII cells (Fig. 7A). Expression of MCT1 protein was confirmed in MLE-15 (Fig. 7B), and cultures exposed to hypoxia showed no significant difference in MCT1 protein level compared with normoxic cultures (Fig. 7B). Altogether, these data support the idea that hypoxic exposure leads to decreased use of both lactate and glucose for mitochondrial metabolism in ATII.

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indicate that the expression of MCT1 is not hypoxia responsive in ATII cells.

Lactate metabolism alone is sufficient to maintain bioenergetic homeostasis but not cell growth. ATP concentrations of MLE-15 cells cultured in lactate, both those cultured under normoxic and hypoxic conditions, did not differ significantly from glucose-grown controls (Fig. 8A). Thus, lactate consumption provides sufficient substrate for mitochondrial respiration to maintain ATP homeostasis. However, MLE-15 cells cultured in lactate alone were not able to maintain cell growth. In medium containing only lactate, MLE-15 cell DNA synthesis (based on EdU nucleotide analog incorporation) occurred at approximately one-half the rate of cells cultured in glucose (Fig. 8B). Culture in medium containing both substrates did not result in rates significantly different from culture in glucose alone over the 20-h growth period. These findings were reiterated in cell counts over 5 days of culture. Compared with cultures maintained in glucose, those in lactate alone showed very little change in cell number over the growth period of 5 days (Fig. 8C). The glucose-only and combined substrate conditions resulted in similar increases in cell number over time, confirming that the presence of 5.5 mM lactate did not inhibit growth. While the combined substrate condition showed higher mean cell count at day 5, this difference was not quite statistically (P < 0.05) significant until day 5, when the combined substrate condition showed a trend toward higher cell numbers than glucose alone, although this did not reach significance.

DISCUSSION

A series of landmark studies utilizing the isolated perfused whole lung experimental model demonstrated that lactate oxidation occurs in the lung tissue and suggested that lactate serves as an important precursor for both pulmonary cell energy production and lipid synthesis (7). Despite significant insight into pulmonary metabolism gained by whole organ studies, investigators stressed that the model is limited in that it provide no information about the function of specific cell types (7), and therefore the importance of lactate to lung bioenergetics at the cell-specific level was previously unknown. Furthermore, while oxidation of lactate to CO₂ was observed in these studies, the actual contribution of lactate to cellular oxygen consumption or ATP production was not demonstrated or quantified. Here, we have examined the utilization of lactate by isolated primary and model ATII cells for oxidative energy production and demonstrated that ATII cells consume lactate for use as substrate for rapid mitochondrial ATP generation. Using MLE-15 cells as a model for ATII metabolism, we additionally demonstrate that the availability of lactate regulates glucose metabolism. Also, we show that mature ATII cells specifically express the MCT1 isofrom of the monocarboxylate transporter, often associated with lactate import, and MCT-mediated transport governs both lactacid import and export in these cells. Overall, this work further demonstrates the metabolic adaptability of ATII cells to changing extracellular conditions and provides the first detailed assessments of mitochondrial metabolism in cells consuming lactate.

Substrate availability created a dramatic shift in metabolic phenotype, since ATII cells cultured in medium containing lactate in the absence of glucose adopted a highly oxidative metabolism, consuming oxygen at rates approximately double that of cells cultured in glucose and performing minimal glycolysis as indicated by very low acid generation. Also, cells cultured in lactate maintained ATP homeostasis, even when exposed to hypoxia, despite the loss of glycolytic function. The need to compensate for the loss of glycolysis-derived ATP likely contributes to the rapid rates of O₂ consumption observed, since lactate is not metabolized through the glycolytic pathway. Although inhibition of cell replication and division in lactate culture would be expected to limit some of the major energy-demanding functions, it remains possible that increased ATP turnover resulting from other processes such as surfactant production or ion transport could also contribute to increased respiration.

Fig. 8. Lactate alone is sufficient to maintain ATP homeostasis but not cell growth in MLE-15 cells. A: ATP was measured in MLE-15 cells cultured in media formulated with 5.5 mM glucose or 5.5 mM lactate, via luminescence-based assay. Three individual experiments were performed, and, in each, samples were assayed in triplicate. Error bars represent ± SD. B: EdU nucleotide analog incorporation into synthesized DNA was assessed fluorimetrically. Data represent fluorescent signal from Cy7 normalized to ToPro3 signal for each well. Three individual experiments were performed for each condition. *Significantly different (P < 0.05) from glucose-only control. Error bars represent ± SD. C: MLE-15 cell count in culture was assessed over a growth period of 6 days, or until cultures reached 100% confluence, for cultures grown in 5.5 mM glucose, 5.5 mM lactate, or 5.5 mM of both substrates combined. Data represent total cells per well determined via hemocytometer count of only cells excluding the vital dye trypan blue. Three individually cultured wells were assessed for each condition at each time point. *Significantly different (P < 0.05) from glucose-only control. Error bars represent ± SD.
Addition of FCCP to flux assay medium was unable to stimulate increased O2 consumption by cells cultured in lactate, indicating that respiration in lactate-fed cells is performed essentially at maximal mitochondrial capacity. Because cells in lactate alone are unresponsive to FCCP, and because OCR of lactate-cultured MLE-15 was similar to FCCP-stimulated glucose-fed cells, we conclude that the increased OCR during lactate-fueled respiration was not the result of enhanced mitochondrial capacity. For glucose- and lactate-cultured cells, ~50 and 65% of total basal O2 consumption, respectively, was coupled to ATP generation. The difference between lactate- and glucose-cultured cells is subtle and not statistically significant. Although the proportion of oxygen consumption dedicated to energy production is similar between the conditions in terms of percentage, this translates in absolute quantity to a much greater amount of oxygen consumed for ATP generation in the lactate condition, with approximately two times the amount of O2 dedicated to ATP generation in lactate-cultured cells vs. those in glucose. Although the ATP-coupled value for lactate-cultured cells was not significantly higher than that of glucose, this results in an apparently lower percentage of oxygen consumption that is generally attributed to “remainder” processes other than ATP production and nonmitochondrial respiration (e.g., mitochondrial membrane proton leak).

This work also demonstrates that the availability of lactate modulates ATII cell glucose metabolism, since the addition of increasing concentrations of lactate to glucose-formulated medium decreased extracellular acidification, demonstrating decreased lactic acid generation. This finding supports the conclusion that elevated extracellular lactate concentration leads to increased use of lactate in place of glucose to fuel mitochondrial respiration, resulting in decreased glucose use and the observed decrease in glycolytic acid generation. This supports, at the cellular level, previous findings of whole lung lactate consumption from Fisher and Dodia using the isolated perfused rat lung model wherein the addition of lactate to perfusate suppressed both glucose utilization and lactic acid output (10). Combined with multiple studies showing that lactate oxidation occurs in the pulmonary tissue (7, 31, 34), this provided strong evidence that lactate competes with glucose as mitochondrial substrate in the lung, and our investigation supports and extends these findings by identifying ATII cells as a specific subset of pulmonary cells that readily utilize lactate. Further study will be necessary to confirm the extent to which lactate is taken up and utilized by ATII cells when glucose is also present; however, this work establishes that ATII cell glucose metabolism is responsive to extracellular lactate. This may have considerable implications for ATII cell function in disease, since plasma lactate can exceed 20 mM in extreme cases of hypoxia or hypotension (25), and pulmonary tissue concentrations increase more than twofold from normal levels in idiopathic pulmonary fibrosis (IPF) patient parenchymal tissue (22) and possibly in cystic fibrosis, based on elevated lactate measured in patient sputum samples (1).

Lactate must be converted to pyruvate before it can be oxidized via TCA cycle reactions, and we demonstrated that LDH activity is necessary for ATII cell lactate respiration. The marked decrease in OCR resulting from LDH inhibition, with the support of observations that withholding glutamine from media did not appreciably change OCR, confirms that lactate is utilized as fuel for rapid respiration. In these experiments, significant cellular OCR remained following exposure to oxamate. This was not unexpected due to oxamate being a competitive inhibitor of LDH. Furthermore, a significant proportion of OCR (~50–60%) is not linked to ATP production in ATII cells, and is, therefore, not expected to be impacted by reduced LDH turnover. The opposite effect was observed for glucose-fed MLE-15 cultures, since oxamate exposure resulted in modestly increased OCR. This is likely to be an effect of reduced pyruvate-to-lactate conversion, which would also be inhibited by oxamate, leading to enhanced pyruvate available to the mitochondria.

Our findings also indicate that utilization of lactate is suppressed by hypoxia because of decreased mitochondrial substrate demand. The ~50% decrease in lactate-fueled OCR in hypoxia-exposed cells compared with normoxic controls is similar to the degree of decrease measured in glucose-cultured cells, reported here and in a previous publication (24). The clinical significance of this finding rests in the relationship of pulmonary hypoxia with several lung diseases, including IPF. Based on our findings, ATII cells under normoxic conditions can readily remove lactate from the extracellular milieu, facilitated by cotransport with extracellular protons. We propose that this process acts as a lactate and H+ sink in the healthy lung, simultaneously controlling lung tissue pH by preventing lactic acid build-up and providing rapidly respiring ATII with oxidizable substrate for ATP generation and surfactant lipid production. Under hypoxic conditions we observed significant reduction in mitochondrial respiration by ATII cells cultured in lactate, indicating that decreased mitochondrial metabolism limits the ability of ATII cells to consume lactate and therefore to regulate lactate and pH balance in the tissue. Previous clinical investigations have observed lactate build-up and/or release from the lungs of patients suffering from diseases associated with development of pulmonary hypoxia. We suggest that this could be due in part to reduced capacity for lactate uptake and consumption by ATII cells.

The finding that lactate alone is insufficient to support MLE-15 cell growth at the same rate as glucose is not surprising, because multiple glycolytic intermediates are crucial for DNA synthesis. These critical intermediates will not be generated by cells metabolizing lactate alone. We likewise demonstrate that it is the absence of glucose, and not the presence of lactate, that limits cell growth, since the combined substrate condition did not lead to reduced rate of growth or DNA synthesis. Over time, the presence of lactate may enhance growth, since culture in combined substrate leads to apparently higher mean cell count and DNA incorporation, although these differences did not reach significance.

We report that MCT1, previously associated with import of lactate into cells capable of lactate oxidation, is also expressed by ATII cells. MCT1 and MCT4 proteins are both found in lysates of whole lung tissue, but cell-specific expression and the overall role of MCTs in lung cell metabolism was previously undetermined (21). This report shows that MCTs are important for both lactate import and export by ATII cells, since inhibition altered extracellular acidification and/or mitochondrial function, depending on the available substrate. Specific MCT1 expression by ATII cells supports the finding that they consume extracellular lactate, and the importance of MCT to lactate oxidation is apparent given that MCT inhibition suppressed oxygen consumption in cells cultured in lactate.
 Whereas MCT4 contains functional hypoxia-response elements and is a hypoxia-inducible HIF1 target, the inducibility of MCT1 appears to differ by cell type. In adipocytes, hypoxic exposure induces MCT1 expression (29), whereas in vitro studies using multiple cell lines showed a similar expression pattern to that observed here, with no change in hypoxia (33). In our hands, MCT1 was not induced by 20-h hypoxic exposure.

Given that there was no measured change in either mRNA or protein under hypoxia, it is unlikely that the decreased respiration in lactate-cultured cells exposed to hypoxia is controlled at the level of substrate transport.

The capacity of ATII cells to consume lactate from the extracellular space has important implications for whole lung homeostasis. Based on our findings, ATII cells may take up lactate produced by neighboring cells in a relationship analogous to white glycolytic and red oxidative fibers in skeletal muscle. Metabolic flux has not been measured for other pulmonary cell types like ATI cells and pulmonary fibroblasts; however, in fibrotic lung disease, activated myofibroblasts generate high levels of lactic acid (22), and rough comparison of ATII flux measurements with those for normal human dermal fibroblasts indicate that ATII cells likely have higher mitochondrial metabolism (35). Based on perfused lung studies, ATII cells may also utilize lactate delivered in pulmonary circulation. Multiple investigators have demonstrated lactic acid uptake from pulmonary circulation (20), oxidation (7, 31, 34), and incorporation into lung lipids (28, 31). In this manner, local metabolic cooperation between lung cell phenotypes may form a key component of normal alveolar tissue homeostasis, providing substrate for ATII cell energy and lipid production while also preventing lactic acid build-up.

Likewise, dysregulation of local metabolic cooperation between cell types may lead to conditions associated with certain diseases. Recent investigation into profibrotic processes that contribute to myofibroblast activation in IPF have highlighted the importance of lactate balance in the lung. Kottmann and colleagues determined that not only is lactate elevated in the lungs of IPF patients, but lactic acid directly contributes to tissue fibrosis (22). By lowering extracellular pH, lactic acid build-up activates latent transforming growth factor-β (TGF-β). TGF-β is a cytokine responsible for initiating conversion of fibroblasts into the myofibroblast phenotype, and the study determined that increased lactic acid enhances myofibroblast conversion and matrix deposition. Increased lactate generation was associated with increased tissue expression of LDH5, the isoform most strongly favoring pyruvate-to-lactate conversion (and not the reverse). Enhanced expression was localized to the epithelium near fibrotic foci, although the cells responsible have yet to be conclusively determined. While it is now clear that lactic acid build-up is involved in IPF pathogenesis, the cells and molecular processes responsible for lactic acid build-up in diseased tissue are unknown. Based on these findings, it is possible that altered ATII mitochondrial metabolism leading to reduced ability to act as a sink for lactate and H+ is a contributing factor.

While this study examined only the impact of hypoxia on lactate metabolism, any condition that suppresses respiration would be expected to limit lactate consumption. Genetic aberrations, damage by reactive oxygen species, and other challenges besides oxygen deprivation can affect mitochondrial function and, therefore, lactate consumption. Future study may be aimed at addressing the role of ATII cell lactate metabolism in epithelial dysfunction, tissue acidification, and pathogenesis of diseases, including IPF.

DISCLOSURES
No conflicts of interest, financial or otherwise are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


