Metabolic shift in lung alveolar cell mitochondria following acrolein exposure

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Agarwal AR, Yin F, Cadenas E. Metabolic shift in lung alveolar cell mitochondria following acrolein exposure. Am J Physiol Lung Cell Mol Physiol 305: L764–L773, 2013. First published September 20, 2013; doi:10.1152/ajplung.00165.2013.—Acrolein, an α,β unsaturated aldehyde, is an environmental pollutant released in ambient air from diesel exhausts and cooking oils. This study examines the role of acrolein in altering mitochondrial function and metabolism in lung-specific cells. RLE-6TN, H441, and primary alveolar type II (pAT2) cells were exposed to acrolein for 4 h, and its effect on mitochondrial oxygen consumption rates was studied by XF Extracellular Flux analysis. Low-dose acrolein exposure decreased mitochondrial respiration in a dose-dependent manner because of alteration in the metabolism of glucose in all the three cell types. Acrolein inhibited glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity, leading to decreased substrate availability for mitochondrial respiration in RLE-6TN, H441, and pAT2 cells; the reduced GAPDH activity was compensated in pAT2 cells by an increase in the activity of glucose-6-phosphate dehydrogenase, the regulatory control of the pentose phosphate pathway. The decrease in pyruvate from glucose metabolism resulted in utilization of alternative sources to support mitochondrial energy production: palmitate-BSA complex increased mitochondrial respiration in RLE-6TN and pAT2 cells. The presence of palmitate in alveolar cells for surfactant biosynthesis may prove to be the alternative fuel source for mitochondrial respiration. Accordingly, a decrease in phosphatidylcholine levels and an increase in phospholipase A2 activity were found in the alveolar cells after acrolein exposure. These findings have implications for understanding the decreased surfactant levels frequently observed in pathophysiological situations with altered lung function following exposure to environmental toxicants.

glycolysis; pulmonary surfactant; palmitate

ACROLEIN, an α,β unsaturated electrophilic aldehyde, is produced in ambient air through cooking oils, combustion of fuels, biomass, and tobacco smoking. Glycerin and sugars form the major sources of acrolein from cigarettes, generating ~60 and 97 μg acrolein/cigarette, respectively, depending on the brand. Acrolein is also formed endogenously as a product of lipid peroxidation, metabolic by-product from amino acids (methionine and threonine), and polyamines (45).

The high electrophilicity of acrolein renders cellular nucleophiles such as thiols particularly susceptible to the oxidative modification, and its ability to form adducts with glutathione leading to its subsequent depletion is well documented (18, 22, 41, 44). Apoptosis induction after low-level acrolein exposure occurs via both the intrinsic (47, 48) and the extrinsic pathways (40). Acrolein also inhibits apoptosis in neutrophils (13) and is a weak apoptosis inducer in proB lymphocytes, causing predominantly necrosis at higher doses (21), thus indicating its cell type-specific effects. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a redox-sensitive cysteine-containing glycolytic enzyme, is also susceptible to adduct formation and functional inhibition (27), eventually contributing to acrolein-induced cytotoxicity (31). Its effects on mitochondria are more pronounced, not only in initiating apoptosis and forming adducts with mitochondrial membrane proteins such as adenine nucleotide translocase and ATP synthase δ-chain (50), but also as a direct effect on the mitochondrial pyruvate dehydrogenase complex and tricarboxylic acid enzymes, such as α-ketoglutarate dehydrogenase, as shown in rat hepatocytes (46) and with purified enzyme preparations (35). The specific inhibition of mitochondrial complexes I and II is also known to contribute to the mitochondrial dysfunction and loss of energy production in rat hepatocytes (46) and isolated brain mitochondria (34) after acrolein exposure. In addition to these effects, acrolein is also known to conjugate with nucleic acid bases like deoxyguanosine (6) and inhibit NF-κB-mediated gene expression and stress responses (23, 29).

Lungs are the foremost organs to be exposed to environmental acrolein and, as such, it may have a direct effect on the epithelial cells lining the bronchi and alveoli. Type II alveolar cells, which form 60% of the alveolar epithelium (7), are responsible for surfactant secretion (26) and protection of the alveolus by differentiating into type I alveolar cells following acute injury (11). Glucose, the preferred substrate used by the lungs for energy production under normal conditions (5, 14), also provides the glycerol backbone for phosphatidylcholine synthesis, a major surfactant phospholipid (38). The pentose phosphate pathway provides an alternative route for glucose metabolism without consuming ATP while generating NADPH, the reducing power that supports the detoxification of electrophilic and oxidative stressors (33). Biosynthesis of lung surfactant is one of the major functions of type II alveolar cells, and thus any alterations in the processing or secretion can lead to diseases such as respiratory distress syndrome (4) and other obstructive lung diseases (17). Palmitate has also been shown to be preferred over glucose for energy production under conditions of starvation (37) and thus could be expected to support ATP production in the event of altered glycolytic breakdown of glucose in lungs.

This study is aimed at establishing changes in energy metabolism when cells are exposed to acrolein under controlled conditions; experiments were performed on isolated mouse primary type II alveolar cells (pAT2), rat lung epithelial cells (RLE-6TN), and human lung adenocarcinoma cells (H441).

EXPERIMENTAL PROCEDURES

Cell lines. Experiments in this study were carried out in RLE-6TN (CRL-2300) and H441 (HTB-174) cell lines obtained from American Type Culture Collection. RLE-6TN cell line, derived from type II alveolar cells, was cultured in Ham’s F-12 media supplemented with 2 mM l-glutamine, 0.01 mg/ml bovine pituitary extract, 5 μg/ml insulin, 0.0025 μg/ml insulin-like growth factor, 1.25 μg/ml transferrin, 2.5 ng/ml epidermal growth factor, 5% penicillin-streptomycin, and 10% fetal bovine serum. The Clara cell-like human bronchial epithelial (H441) cell line, derived from pericardial fluid of a patient...
with papillary adenocarcinoma, was cultured in RPMI-1640 media supplemented with 5% penicillin-streptomycin and 10% fetal bovine serum. All the supplements were obtained from Sigma Aldrich (St. Louis, MO).

Isolation of pAT2 cells. pAT2 cells were isolated from male A/J mice (Jackson Laboratories) 8–12 wk of age using the dispase (BD Biosciences, Bedford, MA) digestion-agar instillation method (8): mice were killed after pentobarbital sodium overdose, and the abdominal cavity was opened to sever the renal artery and drain blood. PBS was then perfused through the veins, followed by 0.5 ml displace and 0.5 ml of 1% low-melting-point agarose (Sigma) in the lungs through the trachea. Lungs were then excised and incubated in 3 ml of dispase for 45 min following which they were chopped in wash medium containing a 1:1 mixture of Dulbecco’s modified Eagle’s medium and Ham’s F-12 (DMEM/F-12; Sigma) supplemented with 0.01% DNase, 1 mM L-glutamine, 100 U/ml sodium penicillin G, and 100 μg/ml streptomycin. The cell mixture was then passed through cell strainers of pore size 100, 40, 20, 15, and 10 μm, and the resulting cell suspension was then centrifuged at 300 g for 10 min at 10°C. The pellet was resuspended in the wash medium supplemented with 10% fetal bovine serum (Gemini Bio-products, West Sacramento, CA) and incubated with biotinylated antimacrophage antibodies (anti-CD45, anti-CD45.1, anti-CD45.2, anti-Ter 119, and anti-CD16/32; BD Biosciences) for 30 min. The antibody-bound macrophages were separated using streptavidin-conjugated magnetic beads (Promega, Madison, WI), and the cells were incubated on petri dishes previously coated with mouse IgG (Sigma) for 2 h at 37°C. The unattached cells were then seeded on plates coated with Laminin-1 (Trevigen, Gaithersburg, MD) in Complete Mouse Medium (CMM) containing DMEM/F-12, 1 mM L-glutamine, 0.25% bovine serum albumin (BD Biosciences), 10 mM HEPES, 0.1 mM nonessential amino acids, 0.05% insulin-transferrin-sodium selenite (Roche, Basel, Switzerland), and 100 μg/ml Primocin (Invitrogen, Carlsbad, CA) supplemented with 10% newborn bovine serum (Omega Scientific, Tarzana, CA). After 3 days of seeding, the medium was changed to serumless CMM to remove any contaminating fibroblasts.

Acrolein treatments. All acrolein exposures were done in the respective cell-culture medium for 4 h in a humidified 5% CO2-95% air incubator at 37°C. The exposure period of 4 h was based on time course experiments and the cell-doubleting time where longer duration of exposure showed higher cytotoxicity and cell proliferation.

Cell viability assays. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)-cell viability was assessed by measuring the ability of the cells to reduce MTT after acrolein exposure. After the exposure period the medium was removed, and the cells were washed two times with PBS and then replaced with HEPES-buffered toxicity medium containing 5 mM HEPES, 154 mM NaCl, 4.6 mM KCl, 2.3 mM CaCl2, 1.1 mM MgCl2, 33 mM glucose, 5 mM NaHCO3, 1.2 mM Na2HPO4, pH 7.4, and 0.5 mg/ml of MTT (Sigma Aldrich). The cells were incubated for 90 min in the above media, and after incubation the resulting formation of formazan crystals was measured by dissolving in DMSO and reading the absorbance at 490 nm in a microplate spectrophotometer.

FACS. Cells were incubated with FITC-conjugated annexin V (apoptosis marker) for 15 min at room temperature and propidium iodide (necrosis marker) following acrolein treatment using the annexin V FITC apoptosis detection kit from Calbiochem (San Diego, CA) according to the manufacturer’s instructions. Flow cytometric analysis was performed using FACSDiva from BD Biosciences (San Jose, CA).

XF extracellular metabolic flux analysis. Mitochondrial respiration was measured using the XF Extracellular Flux Analyzer from Seahorse Biosciences (North Billerica, MA) according to the manufacturer’s protocol. Cells were seeded in XF-24 or XF-96 plates 1 day before the experiment and treated with acrolein the next morning. For basal mitochondrial respiration on glucose and pyruvate, the cells were washed with DMEM buffer, pH 7.4, containing 25 mM glucose and 2 mM pyruvate, and the decrease in O2 levels was measured in the medium immediately surrounding the cells. Oligomycin (4 μM) and 1 μM carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) were added through one of the ports to measure the H+ leak and maximal respiration capacity, respectively. For mitochondrial respiration on glucose/pyruvate, the initial respiration was measured in DMEM medium without glucose/pyruvate to get baseline respiration, and the increase/decrease in mitochondrial respiration after addition of substrates was measured to obtain basal respiration values. The respiration on palmitate-BSA/BSA was measured similarly in Krebs-Henseleit buffered medium, pH 7.4, containing 2.5 mM glucose and 0.5 mM carnitine to facilitate palmitate-BSA uptake; palmitate-BSA was added to a final concentration of 200 μM. Extracellular acidification rate (ECAR) data were validated by using 2-deoxyglucose. All values were normalized to control by measuring the protein concentrations using Bradford assay post-XF Extracellular Flux analysis.

GAPDH and glucose-6-phosphate dehydrogenase activities. The activities for GAPDH and glucose-6-phosphate dehydrogenase (G-6-PDH) were measured in buffer containing 100 mM Tris-HCl and 5 mM sodium arsenate, pH 8.6. Cell lysate (100 μg) was added along with 100 μM NADP and 3.4 mM glucose-6-phosphate in a final reaction volume of 1 ml for G-6-PDH activity. The formation of NADPH was monitored spectrophotometrically at 340 nm. For GAPDH activity, 100 μg of cell lysate were added along with 250 μM NAD+ and 15 μl of 50 mg/ml of glyceraldehyde-3-phosphate to a final reaction volume of 1 ml (I). The formation of NADH was monitored spectrophotometrically at 340 nm, and the enzyme activity was calculated from the slopes obtained with an extinction coefficient of 6.22.

Total phosphatidylcholine assay. The levels of phosphatidylcholine were measured using kits available from Abnova (Taipei, Taiwan) using the manufacturer’s protocol. Cell lysates were centrifuged at 14,000 g to prevent contamination from the cell membrane, and the supernatant was used for analysis. The OxiRed probe generated after phosphatidylcholine hydrolysis and its subsequent oxidation were measured calorimetrically at 570 nm.

Phospholipase A2 activity assay. Phospholipase A2 (PLA2) activity was measured in cell lysates after acrolein treatment using kits available from Cayman Chemicals (Ann Arbor, MI) according to the manufacturer’s protocol. Cell lysates were centrifuged at 14,000 g to prevent contamination from cell membrane, and the supernatant was used for analysis. The free thiol released after hydrolysis of arachidonyl thio-PC at the sn-2 position by PLA2 was detected at 414 nm using 5,5’-dithiobis-(2-nitrobenzoic acid).

Statistical analyses. Students t-test assuming unequal variances was performed along with ANOVA to determine statistical significance as indicated in the legends for Figs. 1–9. Results are means ± SD from a minimum of three experiments.

RESULTS

Acrolein induces cytotoxicity in RLE-6TN, H441, and pAT2 cells. The effect of acrolein on cell viability was measured by studying the ability of cells to reduce MTT after 4 h acrolein exposure (Fig. 1A). RLE-6TN and pAT2 cells were more susceptible to acrolein-induced cell death than H441 cells. The IC50 for RLE-6TN and pAT2 cells was ~40 and ~50 μM acrolein, respectively, whereas that for H441 cells was ~200 μM. This indicates that H441 cells are more resistant to acrolein than the alveolar type II cells (RLE-6TN and pAT2). The higher resistance of H441 cells to acrolein may be partly accounted for their having club cell (Clara cell)-like morphology, mostly found in the bronchial epithelium, therefore more resistant to environmental toxicants, since they would be exposed to higher concentrations compared with the distal alveolar cells.
did not completely inhibit the OCR as a result of H
respiration by
The concentration of acrolein needed to reduce mitochondrial
type II cells (RLE-6TN and pAT2). Addition of 4
iodide were quantified and plotted as shown in
with FITC-conjugated annexin V and propidium
acrolein (C).
The no. of cells that did not label
D
20
pAT2 cells showing control (B)
acrolein (C). The no. of cells that did not label
with FITC-conjugated annexin V and propidium
iodide were quantified and plotted as shown in D.

olar epithelial cells. These cytotoxicity results were also con-

Figs. 2, A and B, 3A, and 4A. The spare respiratory capacity
(maximal respiration − basal respiration) of the cells did not change significantly in RLE-6TN, pAT2, and H441 cells after 4 h acrolein exposure. Of note, basal OCR values differed with cell type: H441 cells are the largest in size and thus showed the highest basal OCR values compared with RLE-6TN and pAT2 cells, whereas pAT2 cells showed the lowest basal OCR values possibly because of their primary nature.

The increase in basal ECAR values in the two cancerous cell
lines [RLE-6TN (Fig. 2C) and H441 (Fig. 4B)] upon acrolein exposure was not statistically significant, whereas the decrease in basal ECAR in pAT2 cells (Fig. 3B) upon exposure to 20 μM acrolein was statistically significant, indicating altered glycolysis. These data were validated by supplementing RLE-
Acrolein induces metabolic shift in alveolar cells

6TN cells with 2-deoxyglucose, an inhibitor of glycolysis, which decreased basal ECAR values by ~80% (data not shown).

Acrolein mediated inhibition of glycolysis in RLE-6TN, H441, and pAT2 cells. Four hours acrolein exposure resulted in a decrease of basal respiration on glucose by 25, 12, and 20% in RLE-6TN, pAT2, and H441 cells (compared with their respective controls), respectively (Fig. 5, A–C). Basal respiration when cells were metabolizing pyruvate increased by 22 and 20% in RLE-6TN and pAT2 cells, respectively, after 15 μM acrolein exposure for 4 h (Fig. 5, A and B). The increase in basal respiration on pyruvate was not observed in H441 cells after 100 μM acrolein exposure for 4 h (Fig. 5C). This indicated an alteration of glucose metabolism in all three cell types, leading to decrease substrate (glucose) availability for mitochondrial respiration; bypassing glycolysis by providing mitochondrial substrate (pyruvate) resulted in an OCR increase.

Acrolein inhibits GAPDH activity and upregulates G-6-PDH activity. The decrease in glucose metabolism observed in Fig. 5 was partly because of a dose-dependent inhibition of GAPDH activity in RLE-6TN, pAT2, and H441 cells after 4 h acrolein exposure (Fig. 6, A–C). Type II alveolar cells were more susceptible to acrolein toxicity, entailing a decrease by ~72 and 50% in RLE-6TN and pAT2 cells, respectively (following 4 h exposure to 15 μM acrolein). The activity of G-6-PDH, the only regulatory control of the pentose phosphate pathway, increased in the pAT2 cells (Fig. 6B) with decreasing GAPDH activity but not in the two immortalized cell lines (RLE-6TN and H441) (Fig. 6, A and C). This may be because of the pentose phosphate pathway providing ribose 5-phosphate for nucleic acid synthesis and continuous proliferation, already high in the two immortalized cell lines. As mentioned above, the decrease in GAPDH activity may account for the decrease in basal respiration on glucose in the three cell types.

Acrolein exposure leads to an increase in mitochondrial metabolism of palmitate in type II alveolar cells. Mitochondrial respiration on glucose was compared with that on 200 μM palmitate-BSA complex following acrolein exposure. RLE-6TN and pAT2 cells showed an increase in oxygen consump-
tion on palmitate-BSA (Fig. 7, A and B) by 65 and 29%, respectively, compared with control after 10 μM acrolein exposure, whereas H441 cells respiring on palmitate-BSA showed a dose-dependent decrease in OCR (Fig. 7C) upon acrolein exposure. The OCR on glucose was found to decrease in RLE-6TN, pAT2, and H441 cells (Fig. 7, A–C). This indicated that the rate of β-oxidation increased in type II alveolar cells after acrolein exposure but not in bronchoalveolar (H441) cells. Etomoxir, an inhibitor of carnitine palmitoyltransferase I, inhibited OCR (basal respiration, ATP turnover, and maximal respiratory capacity) (data not shown). This supports the β-oxidation of palmitate in these cells; the inhibition of the oligomycin effect indicated that palmitate metabolism was ATP linked.

**Effect of acrolein on levels of surfactant lipids.** The biosynthesis of surfactants takes place exclusively in type II alveolar cells, and thus the levels of phosphatidylcholine [major surfactant phospholipid (2)] were measured following acrolein exposure. The levels of phosphatidylcholine were found to decrease by 43 and 58% in RLE-6TN (Fig. 8A) and pAT2 (Fig. 8B) cells, respectively, after exposure to 15 μM acrolein.

**Effect of acrolein on PLA2 activity.** PLA2 catalyzes the release of fatty acids from the sn-2 position of phosphatidylcholine; acrolein exposure (15 μM) resulted in a 2.5- and 1.5-fold increase in PLA2 activity in RLE-6TN and pAT2 cells, respectively (Fig. 9).

**DISCUSSION**

The doses of acrolein used in the study were based on the MTT cell viability assay and the OCR values for the three cell types. Physiologically, it has been shown that acrolein may reach the concentrations of 80 μM in the respiratory tract lining fluid in smokers (10) and up to 180 μM in plasma of patients with renal failure (42). Low dose of acrolein (maximum 20 μM) did not decrease the cell viability by more than 10% in type II cells (Fig. 1A) but decreased the mitochondrial respiration by ~50% (Figs. 2, A and B, and 3A). In H441 cells, the maximum dose used (120 μM) decreased the cell viability by ~20% and mitochondrial respiration by ~27% (Fig. 4A). The reduction of MTT is catalyzed by mitochondrial aldehyde...
because of the ability of acrolein to deplete glutathione without glutathionylation as shown before (1). This pentose phosphate pathway, G-6-PDH, in pAT2 (Fig. 6), cells. The decrease in OCR in RLE-6TN and pAT2 cells respiring on glucose and the increase in its oxidation to glutathione disulfide (22). The metabolism of palmitate, present in the form of phosphatidylcholine, a major surfactant, may meet the energy demands in alveolar type II cells (Fig. 7, A and B).

The basal and maximal respiration values were found to decrease in a dose-dependent manner following acrolein exposure for 4 h in RLE-6TN, pAT2, and H441 cells. ATP production (oligomycin-sensitive respiration) was also found to decrease as a result of a decrease in substrate availability after acrolein exposure in RLE-6TN (Fig. 2, A and B), pAT2 (Fig. 3A), and H441 (Fig. 4A) cells. The decrease in OCR in RLE-6TN and pAT2 cells respiring on glucose and the increase

Fig. 6. Acrolein induced glyceraldehyde-3-phosphate dehydrogenase (GAPDH) inhibition in RLE-6TN, pAT2, and H441 cells. Changes in GAPDH (3) and glucose-6-phosphate dehydrogenase (G6PDH, ■) activity, measured by monitoring the consumption and formation of NADH and NADPH, respectively, at 340 nm using an ultraviolet spectrophotometer as described in EXPERIMENTAL PROCEDURES.*

changes as a result of a decrease in substrate availability after acrolein exposure in RLE-6TN (Fig. 2, A and B), pAT2 (Fig. 3A), and H441 (Fig. 4A) cells. The decrease in OCR in RLE-6TN and pAT2 cells respiring on glucose and the increase

Fig. 7. Acrolein induced increase in β-oxidation in type II alveolar cells. Changes in OCR on glucose (dark gray bars) or palmitate-BSA (light gray bars), measured in RLE-6TN (A), pAT2 (B), and H441 (C) cells after 4 h acrolein exposure, measured using the XF Extracellular Flux Analyzer as described in EXPERIMENTAL PROCEDURES. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with control of the respective cells was found as evaluated using t-test. Black bars, control, BSA alone. ANOVA statistical analysis was also performed, and P < 0.01 for RLE-6TN cells (glucose), P < 0.01 for pAT2 cells (glucose), P < 0.001 for pAT2 cells (palmitate-BSA), P < 0.001 for H441 cells (glucose), and P < 0.001 for H441 cells (palmitate-BSA) was found.

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The increase in G-6-PDH activity in pAT2 cells after acrolein exposure indicated a shift of glucose metabolism toward the pentose phosphate pathway (Figs. 6A and 10). This upregulation of the pentose phosphate pathway would also support an increase in the levels of NADPH to counteract acrolein-induced oxidative stress (1, 36). The immortalized cell lines (RLE-6TN and H441) would require a continuous supply of ribose 5-phosphate for the synthesis of nucleic acid bases and nucleotides, provided by the pentose phosphate pathway. Thus the G-6-PDH activity in RLE-6TN and H441 cells did not change significantly following acrolein exposure (Fig. 6, A and C).

The downregulation of GAPDH activity would be expected to promote surfactant biosynthesis through the glycerol 3-phosphate pathway (Fig. 10). However, the levels of phosphatidylcholine decreased with increasing acrolein exposure, thus indicating a decrease in the levels of surfactant, since phosphatidylcholine represents ~80% of the surfactant phospholipid (38, 52). This decrease in the levels of phosphatidylcholine may be attributed either to the downregulation of choline-phosphate cytidylyltransferase, the rate-limiting enzyme in the biosynthesis of phosphatidylcholine (2, 39), or its degradation by cytosolic PLA2 to furnish fatty acids for mitochondrial β-oxidation and energy production. Acrolein has been shown to downregulate ANX1 gene, which would lead to an upregulation of PLA2 activity in rat lung epithelial cells (43). This is also supported by the increase in PLA2 activity observed in sheep after lung injury (15). In accordance with these results, we found a substantial increase in PLA2 activity after acrolein exposure in RLE-6TN and pAT2 cells (Fig. 9). The released palmitate converted to palmitoyl-CoA by the activity of palmitoyl-CoA synthase can then be translocated to the mitochondrion by carnitine-palmitoyl transferases I and II. Alterna-
tively, inhibition of glycolysis alone may be sufficient to increase fatty acid oxidation and utilization of palmitate (37), since it can be surmised by the substantial increase in fatty acid oxidation in RLE-6TN cells after inhibiting glycolysis with 2-deoxyglucose for 1 h (data not shown).

Chronic obstructive pulmonary disease is characterized by surfactant impairment that may be the result of alterations in the levels of phospholipids or inactivation of surfactant proteins (30, 32). Cigarette smoke exposure has also been shown to affect the levels of surfactant protein in mice and in A549 cells (16). A number of studies have focused on mitochondria because of the toxicity mediated by acrolein-induced oxidative stress in brain (9, 25, 34), heart (3, 50), liver (28, 46, 49), eyes (12, 19, 24), and lungs (20, 40); however, the effect of acrolein on energy metabolism, specifically in lung alveolar cells, has not been highlighted before. The findings from this study, summarized in Fig. 10, have implications for understanding the acrolein-mediated bioenergetic alterations in alveolar cells that may affect the surfactant biosynthesis pathway and energy production, possibly providing a mechanism for reduced surfactant secretion in respiratory diseases.

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DISCLOSURES

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


