Triiodo-L-thyronine Rapidly Stimulates Alveolar Fluid Clearance in Normal and Hyperoxia-injured Lungs

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Rationale: Edema fluid resorption is critical for gas exchange and requires active epithelial ion transport by Na,K-ATPase and other ion transport proteins.

Objectives: In this study, we sought to determine if alveolar fluid clearance (AFC) is stimulated by 3,3',5 triiodo-L-thyronine (T₃).

Methods: AFC was measured in *in situ* ventilated lungs and *ex vivo* isolated lungs by instilling isosmolar 5% bovine serum albumin solution with fluorescein-labeled albumin tracer and measuring the change in fluorescein isothiocyanate-albumin concentration over time.

Measurements and Main Results: Systemic treatment with intraperitoneal injections of T₃ for 3 consecutive days increased AFC by 52.7% compared with phosphate-buffered saline-injected control rats. Membranes prepared from alveolar epithelial cells from T₃-treated rats had higher Na,K-ATPase hydrolytic activity. T₃ (10⁻⁶ M), but not reverse T₃ (3,3',5' triiodo-L-thyronine), applied to the alveolar space increased AFC by 31.8% within 1.5 hours. A 61.5% increase in AFC also occurred by airspace instillation of T₃ in *ex vivo* isolated lungs, suggesting a direct effect of T₃ on the alveolar epithelium. Exposure of rats to an oxygen concentration of greater than 95% for 60 hours increased wet-to-dry lung weights and decreased AFC, whereas the expression of thyroid receptor was not markedly changed. Airspace T₃ rapidly restored the AFC in rat lungs with hyperoxia-induced lung injury.

Conclusions: Airspace T₃ rapidly stimulates AFC by direct effects on the alveolar epithelium in rat lungs with and without lung injury.

Keywords: alveolar fluid clearance; acute respiratory distress syndrome; alveolar Na,K-ATPase; type 2 cell; thyroid hormone

Fluid and solute resorption from the alveolus is critical in clearing fluid from lungs at birth and in pathologic conditions, such as lung injury or acute respiratory distress syndrome, hydrostatic pulmonary edema, infant respiratory distress syndrome, and reperfusion injury after lung transplantation. Active alveolar epithelial ion transport is well established as the primary mechanism of fluid clearance from distal airspaces. In the current model of fluid balance in the distal lung, Na⁺ ions enter alveolar type 2 epithelial (AT2) cells at the apical surface primarily through amiloride-sensitive sodium channels and are pumped out on the basolateral surface by Na,K-ATPase (sodium pump). This solute transport drives osmotic water transport (1). Other ion transporters on AT2 and alveolar type 1 (AT1) epithelial

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Alveolar fluid clearance is decreased in conditions associated with pulmonary edema. Stimulation of pulmonary edema clearance might improve outcomes in lung injury and acute respiratory distress syndrome.

What This Study Adds to the Field

3,3',5 Triiodo-L-thyronine (T₃) stimulates alveolar fluid clearance. Airspace T₃ rapidly restores the decreased alveolar fluid clearance in rat lungs with hyperoxia-induced lung injury.

cells participate in the coordinated movement of solutes and water under a variety of conditions (2–5). Thyroid hormone stimulates Na,K-ATPase activity in many tissues (6). We previously reported that 3,3',5 triiodo-L-thyronine (T₃) rapidly increased Na,K-ATPase activity *in vitro* in primary rat AT2 cells and alveolar type 2 epithelial (MP 48 and RLE) cell lines (7–9). Parenteral T₃ given intramuscularly for 3 days increased alveolar fluid clearance (AFC) in rats (10) with intact lungs.

Although subacute treatment with thyroid hormone systemically stimulates the removal of fluid from intact lung (10), it is unknown if this is secondary to indirect effects on other organ systems or if T₃ has a direct effect on the lungs. Identification of new molecules that stimulate AFC is important, because currently there is no established treatment for acute lung injury, a pathologic condition with alveolar flooding, impairment in gas exchange, and an unacceptably high mortality. Some of the rapid, nongenomic effects seen with T₄ (which is deiodinated to T₃) are triggered at the plasma membrane and do not require entry of T₄ into the cytoplasm (11). Thus, if T₃, directly instilled into the lungs, increases AFC, systemic side effects may be avoided and it could be a potential drug for further testing in clinical ARDS.

The present study tested the hypothesis that administration of T₃ systemically or directly in the alveolar space will increase AFC in intact and injured rat lungs. Our secondary hypothesis was that augmented AFC would also be associated with alveolar epithelial cell Na,K-ATPase stimulation after treatment with T₃. Some of the results of these studies have been previously reported in the form of abstracts (12, 13).

METHODS

A detailed description of the experimental procedure is provided in the online supplement. Most of the methods have been previously published by our group (7, 8, 14–17). The University of Minnesota Animal Care and Use Committee approved all experimental protocols. Specific pathogen-free (SPF) male Sprague Dawley rats (200–300 g) were used for this study. AFC was measured as previously described (10, 16, 18,

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19) by instilling an isosmolar 5% bovine serum albumin solution with fluorescein-labeled albumin tracer and measuring the change in fluorescein isothiocyanate-albumin concentration over time. AFC measurement was performed using a ventilated lung model for (1) control unmanipulated rats, (2) rats receiving intraperitoneal injections of either saline or 30 μg of T_3 , 24 hours apart for 3 consecutive days, and (3) rats with airspace instillation of T_3 as described below. On the basis of our *in vitro* data (7, 8), 10^{-6} M T_3 or reverse T_3 (rT_3) was included in the instillate to determine the effects of airspace instillation of T_3 . Because a number of extrapulmonary changes could affect AFC, for all subsequent experiments AFC was measured in *ex vivo* rat lungs as described previously (19), with some modifications. The direct effect of airspace instillation of T_3 was studied in both normal lung and injured lungs, with and without 10^{-6} M T_3 in the instillate. Injury was accomplished by 60 hours of hyperoxia ($\text{FiO}_2 > 95\%$) exposure (14, 20–22). This model is well characterized by other investigators and in our laboratory. Solute permeability is increased, whereas the AFC change is heterogeneous at 60 hours. This duration of hyperoxia was chosen because it is nonlethal and well characterized with physiologic disruption of alveolar epithelial permeability barrier (14). Extravascular lung water was measured using a gravimetric method (16).

To assess whether T_3 affects AFC by increasing Na,K-ATPase *in vivo*, rats were treated with intraperitoneal injections of saline with or without 30 μg of T_3 . AT2 cells were isolated (14, 23) and used for measurement of Na,K-ATPase hydrolytic activity as well as steady-state mRNA and protein levels. Cell membranes (CMs) were prepared from freshly isolated AT2 cells (7), and Na,K-ATPase hydrolytic activity was measured as ouabain-sensitive inorganic phosphate generated from exogenous ATP hydrolysis under maximal velocity conditions as previously described (7, 24), with some modifications. Na,K-ATPase α_1 and β_1 steady-state mRNA levels were quantified by real-time polymerase chain reaction. Western blotting was performed to evaluate the changes in the steady-state protein levels. To optimize the detection of the β subunit (25), deglycosylation was performed on cell lysate using peptide N-glycosidase F. Aliquots of cell membrane for α_1 subunit protein or deglycosylated samples were used for immunodetection with primary mouse monoclonal antibodies against the Na,K-ATPase α_1 or β_1 (UBI) subunit (7, 25). Expression of thyroid hormone receptors in AT2 cells isolated after lung injury was also determined on freshly isolated AT2 cells by Western blotting and immunofluorescence of cytospin preparations.

Statistics

Data are presented as medians. The AFC data are presented as box and whiskers plot showing the median value, the 25th and 75th percentile, and the range. Because the data likely were not normally distributed, the statistical significance was assessed using a Mann-Whitney *U* test and a *P* value of less than 0.05 indicated statistical significance. For comparison of multiple groups, the data were log-transformed to minimize the variance within groups. Analysis of variance (ANOVA) was performed on the transformed data and *post hoc* testing used the least significant difference (LSD) method when the ANOVA showed a significant effect. All experiments were performed three or more times, except for β_1 protein assessment.

RESULTS

In this study, we evaluated the effects on AFC of systemic T_3 given intraperitoneally, of airspace T_3 and rT_3 in intact ventilated rats, and of airspace T_3 in *ex vivo* normal and injured lungs.

Intraperitoneal injection of T_3 for 3 days stimulated the AFC by 52.7% when compared with controls injected with intraperitoneal phosphate-buffered saline (PBS) (27.5 ± 1.75 vs. $18 \pm 2.0\%$ per h, $P = 0.02$) (Figure 1). We then determined the Na,K-ATPase hydrolytic activity in CMs prepared from freshly isolated AT2 cells from rats similarly treated with intraperitoneal T_3 for 3 consecutive days and from control rats treated with intraperitoneal PBS. CMs prepared from AT2 cells isolated after T_3 treatment had a higher peak Na,K-ATPase hydrolytic activity ($P = 0.01$; Figure 2). This increase in the Na,K-ATPase activity was associated with modest increases in steady-state α_1

Figure 1. Effect of intraperitoneal injections of 30 μg triiodo-L-thyronine (T_3) for 3 consecutive days on alveolar fluid clearance from rat lungs. Alveolar fluid clearance was measured over 90 minutes in ventilated animals. Data are presented as a box and whiskers plot showing median, 25th percentile, and 75th percentile, with the whiskers demonstrating the range. * $P < 0.05$ versus control. PBS = phosphate-buffered saline.

and deglycosylated β_1 subunit protein levels (Figures 3 and 4). However, the increase in the CM steady-state α_1 subunit protein levels did not reach statistical significance ($P = 0.11$; Figure 3). With deglycosylation, the expected decrease was seen in the molecular weight of the β subunit. Higher levels of β subunit protein levels in AT2 cell membranes were found in two of two independent experiments. In contrast to the AT2 cell protein levels, the steady-state α_1 subunit mRNA levels were significantly lower after *in vivo* T_3 treatment, whereas the β_1 subunit mRNA levels were unchanged (Figure 5). Thus, sys-

Figure 2. Effect of intraperitoneal (IP) injections of 30 μg of triiodo-L-thyronine (T_3) for 3 consecutive days on peak Na,K-ATPase hydrolytic activity. Type 2 alveolar epithelial cells were isolated from T_3 -treated rats. Na,K-ATPase activity was measured on cell membranes preparations on the same day by measuring the generation of inorganic phosphate under V_{max} conditions. Cell isolation and Na,K-ATPase activity were measured in two animals in each group on the same day. Each data point represents the ratio of individual value to mean of controls for that day. The solid lines represent the median value. * $P < 0.05$ versus controls (IP phosphate-buffered saline [PBS]).

Figure 3. Effect of intraperitoneal triiodo-L-thyronine (T_3) on steady-state Na,K-ATPase α_1 subunit protein levels. Alveolar epithelial cell isolation and cell membrane preparation as in Figure 2. Protein separation was done on 7.5% sodium dodecyl sulfate gel under denaturing condition after equal loading. Data are presented as medians, with individual time point presented as ratio to tubulin, $P = 0.11$ versus control. IP = intraperitoneal; PBS = phosphate-buffered saline.

temic treatment with T_3 increased the rate of fluid removal from intact rat lungs and was associated with increased hydrolytic activity of Na,K-ATPase, specifically in AT2 cells.

Because we previously demonstrated that *in vitro* T_3 increases AT2 Na,K-ATPase activity very rapidly, within 30 minutes (8), we sought to determine if short-term direct exposure of alveolar cells to T_3 in the airspace would increase the rate of removal of alveolar fluid. On the basis of the dose and time response in the *in vitro* experiments, the AFC was measured over 90 minutes with or without inclusion of 10^{-6} M T_3 in the alveolar instillate. The presence of T_3 in the instillate rapidly increased the AFC when compared with controls (29 ± 1.97 vs. $21.08 \pm 1.35\%/h$; $P = 0.004$). In contrast, the inclusion of rT_3 in the instillate did not alter the rate of fluid resorption (22 ± 2.08 vs. $21.08 \pm 1.35\%/h$, $P = 0.47$; Figure 6).

Next, we used an *ex vivo* lung model to assess if the T_3 -induced stimulation of fluid clearance was a direct effect on the alveolar epithelium and was not a secondary indirect systemic effect of T_3 . Lungs from unmanipulated rats were isolated and fluid clearance was measured *ex vivo*. As in the ventilated *in situ* experiments, inclusion of T_3 in the alveolar instillate rapidly increased AFC by 61.5% (21 ± 1.4 vs. $13 \pm 1.65\%/h$; $P < 0.05$; Figure 7).

Figure 4. Effect of intraperitoneal (IP) triiodo-L-thyronine (T_3) on steady-state Na,K-ATPase β_1 subunit protein levels. Western blot of Na,K-ATPase β_1 subunit protein was performed after cell isolation from rats with or without *in vivo* T_3 treatment as in Figure 2. Enzymatic deglycosylation was performed using peptide-N-glycosidase (PNGase) F and separated on 10% sodium dodecyl sulfate gel under denaturing conditions. Protein isolates from kidney were used as positive controls. As expected, with deglycosylation (Dgly), the size of the α_1 subunit protein shifts from 45–50 kD (arrows) to 30 kD (arrows). For alveolar epithelial cell

The same *ex vivo* method was used to determine the AFC in rat lungs injured by exposure to an oxygen concentration of more than 95%. In rat lungs injured by 60 hours of exposure to hyperoxia, the median AFC was $11 \pm 0.8\%$ per hour compared with $13 \pm 1.62\%$ per hour for uninjured lungs ($P = 0.09$ by ANOVA with *post hoc* LSD test; Figure 7) and the wet-to-dry lung weight of hyperoxia-exposed lungs was greater than that of uninjured lungs (6.49 ± 0.27 vs. 5.3 ± 0.16 , $P = 0.004$; Figure 8).

With injury, a statistically significant increase in wet-to-dry lung weight and a trend toward a numerically significant decrease in AFC were seen, consistent with an increase in extravascular lung water with exposure to an oxygen concentration of more than 95%. In the presence of lung injury, airspace T_3 stimulated AFC within 90 minutes (Figure 7).

Expression of thyroid receptors in freshly isolated AT2 cells from normal and hyperoxia-exposed rats was determined. Type 2 alveolar epithelial cells were fixed in methanol and cytospun. There was no significant change in the pattern of thyroid receptor immunofluorescence (Figure 9A). No statistically significant quantitative difference was noted by Western blotting in either α_1 or the β_1 isoforms of the thyroid receptor (Figures 9B and 9C).

DISCUSSION

This study provides evidence that thyroid hormone plays a significant role in regulating AFC in rats, with T_3 stimulating fluid clearance. Our study confirms and significantly extends the observation of one prior study (10), with multiple new observations. Parenteral T_3 increased the rate of clearance of alveolar fluid and sodium pump activity in AT2 cells. More interestingly, this stimulation occurred very rapidly on direct instillation of T_3 into the airspace in injured lungs and was specific, because the inactive form of the hormone rT_3 had no effect. Airspace administration of T_3 also increased AFC in hyperoxia-injured rat lungs.

Earlier studies show the critical role of thyroid hormone in lung development (26–28). Synergistic effects on alveolar fluid resorption of T_3 and hydrocortisone have been observed in developing lambs (29) and adult rats (10). Here we demonstrate the role of T_3 in affecting AFC in adult rats in two different model systems, one in which T_3 is given systemically and one in which it is applied directly to the alveolar surface. The model with repeated intraperitoneal T_3 injections involves an intact animal and more prolonged (3 d) elevation of thyroid hormone levels. Thus, the changes in alveolar epithelial ion transport could occur due to direct action on the alveolar epithelium and/or due to secondary systemic changes induced by high T_3 levels. We do not believe that these changes are due to systemic effects, because stimulation of AFC by T_3 in our study was seen in isolated lungs without any perfusion. It is not known if increasing cardiac output alone will increase AFC; other inves-

(but not kidney) lysates, deglycosylation was not complete and there was a decrement in the total immunoreactivity, as previously reported (25). A modest increase in the levels of total and deglycosylated Na,K-ATPase β_1 subunit protein was seen in two independent experiments.

Figure 5. Effect of intraperitoneal triiodo-L-thyronine (T_3) treatment on Na,K-ATPase mRNA levels. T_3 treatment and cell isolation were performed as in Figure 2. Steady-state mRNA levels measured using reverse transcriptase-polymerase chain reaction with α_1 and β_1 Na,K-ATPase-specific primers. Each time point represents ratio to tubulin. * $P < 0.05$ versus controls. PBS = phosphate-buffered saline.

tigators did not observe an increase in lymph flow with nitroprusside-induced increase in cardiac output (30). In our intraperitoneal T_3 treatment experiments, the finding of increased AFC with intraperitoneal T_3 is similar to results previously reported with multiple days of intramuscular T_3 administration (10). In the present study, we also examined the molecular aspects of changes in Na,K-ATPase activity and subunit protein levels in the alveolar epithelial cells. We measured changes in AT2 cell Na,K-ATPase because thyroid hormone is well known to stimulate Na,K-ATPase in many cell types, Na,K-ATPase is the primary mechanism for active basolateral Na^+ extrusion from alveolar epithelial cells, and increasing Na,K-ATPase expression is sufficient to augment AFC (31). We found that 3 days of exposure to T_3 produced a significant increase in Na,K-ATPase activity and modest, but not statistically significant, increases in Na,K-ATPase α_1 and β_1 protein levels. This is consistent with our prior studies on alveolar epithelial cells *in vitro*, in which the primary mechanism of Na,K-ATPase stimulation by T_3 was increased plasma membrane quantities of Na,K-ATPase. The past studies did not observe changes in Na,K-ATPase protein levels with short-term (12 h) exposure and longer term exposures were not studied (8). Although we do not provide direct evidence in this study, we speculate that there is both short- and long-term regulation of Na,K-ATPase by thyroid hormone, as is seen with aldosterone and insulin (32). For the rapid increase in AFC observed within 90 minutes of T_3 in-

stillation into the lung, we believe that the most likely explanation is that Na,K-ATPase protein is translocated from existing intracellular pools to the plasma membrane. This is the mechanism that our group has established in the response of isolated primary rat AT2 cells to T_3 *in vitro* (7, 8). In these studies, we demonstrated a rapid increase in the plasma membrane quantity of Na,K-ATPase, without any change in the total cellular content of this protein. For the intraperitoneal T_3 treatment model, the exact mechanism(s) by which T_3 stimulates AFC and increases Na,K-ATPase activity in AT2 cells has not yet been fully determined. There are a number of possible mechanisms, including increased numbers of AT2 cells, increased steady-state Na,K-ATPase enzyme activity, or increased quantity of plasma membrane sodium pump molecules per AT2 cells.

Na,K-ATPase is the predominant basolateral surface transport protein on both AT1 (33) and AT2 cells. Up-regulation of Na,K-ATPase alone (presumably with secondary changes in other transporters) is sufficient to increase AFC (31, 34, 35); T_3 stimulation of Na,K-ATPase activity may be sufficient to

Figure 6. Effect of airspace instillation of triiodo-L-thyronine (T_3) on alveolar fluid clearance in *in situ* ventilated rat lungs. Alveolar fluid clearance was measured in ventilated rats and data are presented as median and range as in Figure 1. A concentration of 10^{-6} M T_3 or reverse T_3 (rT_3) was included in the isosmolar bovine serum albumin solution to determine the effect on alveolar fluid clearance by direct instillation into the airspace. The data were log-transformed and analysis of variance was performed on the transformed data. *Post hoc* testing used the least significant difference method; * $P < 0.05$, airspace T_3 versus control or rT_3 .

Figure 7. Airspace instillation of triiodo-L-thyronine (T_3) increases alveolar fluid clearance in normal and injured *ex vivo* rat lungs. Alveolar fluid clearance was measured in isolated *ex vivo* lungs; data are presented as median and range as in Figure 1. Statistical analysis performed was similar to Figure 6. Intact rat lungs had a higher rate of removal of alveolar fluid when 10^{-6} M T_3 was included in the instillate (* $P < 0.05$ vs. control intact lungs). With exposure to an oxygen concentration of more than 95%, there is a numerical trend toward a decrease in alveolar fluid clearance ($P = 0.09$), but inclusion of 10^{-6} M T_3 in the instillate increased alveolar fluid clearance; solid diamond, $P < 0.05$ versus hyperoxia.

Figure 8. Increase in wet:dry lung weight with 60 hours of exposure to hyperoxia. Rats were exposed to an oxygen concentration of more than 95% for 60 hours and wet-to-dry lung weights were measured. Each data point represents individual wet:dry weight. Solid lines represent the median value. * $P < 0.05$ versus no hyperoxia exposure.

explain the increased AFC we observed. However, it is likely that parallel changes in other ion transporters are also involved. We believe the rapid stimulation of AFC that occurred in *ex vivo* isolated lungs mimics the rapid *in vitro* effects of T_3 seen in our prior cell culture experiments (8). Interestingly, the change in α_1 subunit protein level is not associated with a simultaneous increase in the steady-state mRNA level. In fact, in this study, α_1 subunit mRNA was significantly decreased. These findings are consistent with an effect of T_3 on the regulation of sodium pump α subunit translation, but the rate of translation has not been directly determined. We also have not yet determined the

mechanism of decrease in α_1 mRNA level, but a repressive effect of thyroid response elements on gene transcription is a possible mechanism, as has been observed in response to T_3 for Na,K-ATPase α_3 subunit by He and colleagues (36). Although we demonstrate that thyroid receptor expression is not changed in hyperoxia, the role of these receptors in regulation of AFC needs to be determined.

Active alveolar epithelial ion transport is a well-established mechanism of fluid removal from distal airspaces, even in presence of lung injury (37). Decreases in AFC occur in acute lung injury/acute respiratory distress syndrome (38), cardiogenic pulmonary edema (39), reperfusion injury after lung transplantation (40), and other less common forms of pulmonary edema. Stimulation of AFC by β -adrenergic agonists in normal and injured lungs is well described (16, 19, 41, 42), but critical illness is associated with high endogenous catecholamine levels and there may be desensitized and/or down-regulated β -adrenergic receptors in the alveolar epithelium (19). There is a potential major benefit to identifying other agents that could be applied directly to the distal lung either individually or in combination with β -adrenergic agonists to increase AFC. T_3 is of particular interest because it has been administered safely to humans even in critical illness without significant systemic side effects (43–45), and our data suggest that it rapidly stimulates AFC in lung injury. Delivery of T_3 via the airspace may be effective and could decrease potential systemic side effects. Thus, the finding that T_3 rapidly stimulated AFC even in the presence of hyperoxia-induced lung injury in the *ex vivo* model is a particularly important observation in the current study.

Although the findings in this study are encouraging for further exploration of this treatment option and its mechanisms, there are some potential limitations of our study. The fluid

Figure 9. Lung injury caused by exposure to an oxygen concentration of more than 95% for 60 hours does not alter the expression of thyroid receptors. Type 2 alveolar epithelial cells were isolated from rats exposed to an oxygen concentration of more than 95%. Type 2 alveolar epithelial cells from rats maintained on room air were used as controls. (A) Freshly isolated cells were cytospun on glass slides for immunofluorescence (higher dilutions were used for injured cells). No primary controls had any immunofluorescence (data not shown). (B) Cell lysates from these cells were separated on 12.5% sodium dodecyl sulfate gel under denaturing conditions. Protein isolates from the liver and heart were used as positive controls and HeLa cells were used as negative controls because they have minimal protein expression for the thyroid hormone receptors. (C) More than one isoform of thyroid receptor (TR) α and β were detected by Western blotting, but no significant difference in the total amount of thyroid receptor was detected by densitometry. H = hyperoxia; N = normoxia.

clearance measurements in our study have variability within groups, as we previously reported. We believe that this reflects the normal distribution of AFC across a population of animals and other investigators have also observed this variability. With hyperoxia for 60 hours, the heterogeneity of response is increased, and some rats have augmented AFC, whereas others have diminished AFC (22). This heterogeneity makes it difficult to demonstrate the impact of T₃ in restoring AFC, because each animal is not used as its own control. Also, in this study, we did not attempt to determine the exact role of all the potentially involved ion transporter proteins. Inhibitor studies in the *in situ* lung will be important to determine the exact mechanisms by which airspace T₃ alters ion transport. The discrepancies between the changes in Na,K-ATPase activity, protein amount, and mRNA level are of uncertain significance. The Na,K-ATPase in primary rat AT2 cells usually tracks with the level of β_1 protein expression and this was augmented by T₃ in two of two experiments. The decrease in the AT2 α_1 subunit mRNA with T₃ treatment is interesting, and discordance between changes in the protein and mRNA levels will also need further studies to define their mechanism and significance.

In conclusion, T₃ instilled directly into the distal lungs rapidly improves clearance of lung liquid, acting at least partially through stimulation of Na,K-ATPase. This stimulation of fluid clearance occurs even in face of lung injury with disruption of the epithelial permeability barrier. Thus, treatment of alveolar epithelium with thyroid hormone in critically ill patients with lung injury and pulmonary edema has the potential to augment lung edema clearance, and this strategy should be tested in pulmonary edema and lung injury.

Conflict of Interest Statement: M.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.R.R. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. D.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. J.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. T.J.G. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. C.N.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. O.D.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. D.H.I. received a grant from the Thyroid Research Council of Knoll Pharmaceuticals for initial exploratory studies of T₃ on lung ion transport; this support ended in 2001.

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