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L. J. Huffman , D. J. Judy , K. M. K. Rao , D. G. Frazer & W. T. Goldsmith

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# LUNG RESPONSES TO HYPOTHYROIDISM, HYPERTHYROIDISM, AND LIPOPOLYSACCHARIDE CHALLENGE IN RATS

**L. J. Huffman**

Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia, and Department of Physiology, West Virginia University School of Medicine, Morgantown, West Virginia, USA

**D. J. Judy, K. M. K. Rao**

Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA

**D. G. Frazer**

Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia, and Department of Physiology, West Virginia University School of Medicine, Morgantown, West Virginia, USA

**W. T. Goldsmith**

Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA

*The objectives of this investigation were to study the effects of hypo- and hyperthyroidism on some factors involved in lung injury under basal conditions (air exposure) and during an inflammatory response induced by inhalation exposure to lipopolysaccharide (LPS; 100 μg/ml; 3 h) in adult rats. Thyroid status was altered by thyroidectomy or thyroxine injections for 15 d. Hyperthyroidism alone caused a greater degree of lung cell damage, an increase in the permeability of the alveolar–capillary barrier, a rise in the total number of phagocytic cells obtained by bronchoalveolar lavage (BAL), and enhanced nitric oxide (NO) release by phagocytic cells relative to that in euthyroid control animals. Hypothyroidism alone was associated with opposite effects. Exposure of animals to LPS produced inflammatory responses, which included significant increases in lung cell damage, permeability of the alveolar–capillary barrier, number of phagocytic cells obtained by BAL, and NO production by the phagocytic cells. In general, hyperthyroidism enhanced the effects of LPS, while hypothyroidism reduced LPS-induced responses. These results suggest that thyroid status alone can affect some of the factors involved in lung injury and also modulate some of the inflammatory effects of LPS. Hyperthyroidism tends to enhance lung injury, while hypothyroidism seems to reduce lung injury.*

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Address correspondence to Linda Huffman, PhD, M/S 2015, NIOSH, 1095 Willowdale Road, Morgantown, WV 26505, USA. E-mail: ljh3@cdc.gov

One of the most important types of injury to the lungs is oxidant-induced injury. Such injury is thought to be caused by an increased local production of reactive oxygen and nitrogen species, such as superoxide anion radicals, hydrogen peroxide, nitric oxide, and hydroxyl radicals. In general, there are two sources of reactive species in the lungs: (1) Reactive oxygen species are generated as a by-product of metabolism during mitochondrial respiration, and (2) reactive species are produced by phagocytic cells, such as alveolar macrophages and neutrophils (Freeman & Crapo, 1981; Weiss & LoBuglio, 1982). Oxidant-induced lung injury is characterized by damage to cells in the alveolar region, an increase in the permeability of the alveolar–capillary barrier, and an increase in the number of phagocytic cells in the alveoli (Johnson et al., 1981). This type of injury can occur as a result of several different types of insults. One example of such an insult is a pulmonary inflammatory response. An inflammatory response may result, for example, from inhalation of microbial products, such as lipopolysaccharide (LPS) or endotoxin (Repine & Parsons, 1994).

It is possible that the thyroid status of an animal could affect some of the factors involved in the generation of reactive species, and thus oxidant injury, in the lungs. It is well known that thyroid status affects the basal rate of metabolism. For example, the rate of oxygen consumption by lung tissue can be altered when circulating thyroid hormone levels change (Joyner et al., 1976). As might be expected, increased thyroid hormone levels are associated with increased oxygen consumption by lung tissue slices, whereas lung tissue oxygen consumption is decreased when thyroid hormone levels are low. Such thyroid hormone-linked changes in oxygen consumption could alter the baseline level of production of reactive oxygen species. In fact, it has been shown that when pulmonary oxygen consumption is increased, there is an increase in the production of reactive oxygen radicals (Freeman & Crapo, 1981). Furthermore, any damage produced by these thyroid-induced alterations in reactive oxygen species may lead to activation of alveolar macrophages and the appearance of neutrophils on the alveolar surface. In addition, thyroid status may have effects on phagocytic cells. For instance, systemic hypothyroidism in the rat is associated with a decrease in subcellular lysosomal enzyme activity in alveolar macrophages (Starling & Weese, 1985). Taken together, this information suggests that thyroid status may have an impact on susceptibility to lung injury. In addition, it is possible that lung injury caused by insults, such as inflammatory toxins, may be influenced by thyroid status.

The objective of this investigation was to determine whether or not alterations in thyroid status of the rat have any effect on some of the factors involved in lung inflammation/injury. Hypo- and hyperthyroid rat animal models were used to evaluate lung responses under normal conditions (following inhalation exposure to air) and during an inflammatory response induced by inhalation of LPS. The results suggest that alterations in thyroid status affect lung cell damage, the permeability of the alveolar–capillary

barrier, the number of lung phagocytes, and the production of nitric oxide by lung phagocytes. Further, thyroid status affects the magnitude of lung inflammatory responses to LPS.

## METHODS

### Animal Treatments to Alter Thyroid Status

Specific-pathogen-free male Sprague-Dawley rats (Hilltop, Scottdale, PA) of 37–40 d of age, with initial body weights in the range of 180–210 g, were used in this study. The animals were maintained on standard laboratory rat feed and tap water ad libitum, and housed in laminar flow hoods under controlled light (12 h light, 12 h darkness) and temperature (22–24°C) conditions. To create a hypothyroid condition, rats were surgically modified by the commercial supplier and underwent thyroidectomy with parathyroid transplant (THx). Age-matched control rats underwent sham surgery. Rats were shipped to our animal facility on d 7 or 8 (day of surgery = d 1). Inhalation exposures (described later) for groups of thyroidectomized or sham-operated control rats occurred on d 15. Hyperthyroidism was induced in thyroid-intact rats by daily injections of thyroxine ($T_4$; 0.1 mg/0.2 ml/100 g body weight; sc) for 15 d (start of injections = d 1). To facilitate solubility, $T_4$ was initially dissolved in 0.01 *N* NaOH. The $T_4$ solution was neutralized with an equal volume of Dulbecco's phosphate-buffered saline (pH 6.8; 10 × solution; Sigma, St. Louis, MO) just before injection. Age-matched control rats received daily injections of neutralized NaOH (0.2 ml/100 g body weight; sc) for 15 d. Inhalation exposures (described later) for thyroxine-treated or vehicle-treated control rats occurred on d 15.

### Inhalation Exposures

A whole-body inhalation exposure system was used to expose the rats to LPS (Frazer et al., 1996). LPS from *Escherichia coli* serotype 055:B5 (Difco Laboratories, Inc., Detroit, MI) was diluted in endotoxin-free sterile saline (0.9% NaCl, Baxter Healthcare Corp., Deerfield, IL) to a final concentration of 100 μg/ml. The solution was aerosolized with an ultrasonic nebulizer (DeVilbiss, model Ultra Neb 99, Somerset, PA) and mixed with air that had been passed through a high-efficiency particulate air (HEPA) filter. The diluted aerosol was then introduced into the exposure chamber (Plas Labs, Metabolic Chamber; Lansing, MI). A Personal Data RAM (MIE, model PDR-1000AN, Bedford, MA) made real-time estimates of the aerosol mass concentration in the exposure chamber. The estimates were used within a computer-controlled feedback system, which regulated the diluent air entering the chamber in order to achieve the desired concentration. This ensured that the animals were exposed to a constant aerosol mass concentration. The aerosol size distribution was determined with an

APS aerodynamic particle analyzer (TSI, model 3300, Amherst, MA). A typical distribution indicated a mass median diameter of 1 μm with a geometric standard deviation of 1.2. Gravimetric samples were collected from the exposure chamber by drawing the aerosol through a filter (Gelman 37-mm PVC filter, Ann Arbor, MI) at a rate of 1 L/min. The filters were extracted for 6 h with pyrogen-free water, and a modified *Limulus* amebocyte lysate (LAL) assay (BioWhittaker, Walkersville, MD) was used to determine the total assayable endotoxin on the filter samples ($7.5 \times 10^4$ EU/$m^3$). The control rats were exposed to HEPA-filtered air in a whole-body inhalation exposure system that was similar to that used for LPS exposures. The inhalation exposures lasted 3 h, and the animals were studied at 18 h postexposure. This timing was chosen based on previous work showing that LPS inhalation can result in pulmonary inflammatory responses in rats using this protocol (Huffman et al., 1997).

### Collection of Blood and Bronchoalveolar Lavage Fluid and Cell Samples

The rats were first anesthetized with sodium pentobarbital (65 mg, ip; Butler, Columbus, OH). Blood was collected from the abdominal vein into a syringe and placed in a glass tube without anticoagulant. The left renal artery was then cut. A tracheal cannula was inserted and an initial bronchoalveolar lavage was performed with 6 ml of cold $Ca^{2+}$/$Mg^{2+}$-free phosphate-buffered saline (PBS; 145 m*M* NaCl, 5 m*M* KCl, 9.4 m*M* $Na_2HPO_4$, 1.9 m*M* $NaH_2PO_4$, and 5.5 m*M* dextrose, pH 7.4). This lavage solution was introduced into and withdrawn from the lungs for a total of three times. The total return of the initial lavage averaged 4 ml/rat. Subsequent bronchoalveolar lavages were performed with eight ml of PBS each until a total volume of 80 ml lavage fluid was collected. The initial and subsequent lavage samples were then centrifuged (500 × g, 5 min, 4°C). The supernatants from the initial lavage were processed for analyses of lactate dehydrogenase (LDH) activities and albumin levels. The cell pellets from the initial and subsequent lavages were then combined and resuspended in 5 ml PBS. The samples were centrifuged to pellet the cells and the supernatants were aspirated to waste. This wash procedure was performed a total of three times. Following the final wash, the cells were resuspended in 3 ml HEPES-buffered medium (145 m*M* NaCl, 5 m*M* KCl, 10 m*M* HEPES, 1 m*M* $CaCl_2$, and 5.5 m*M* dextrose, pH 7.4).

### Determination of Bronchoalveolar Lavage Cell Counts, Cell Profiles, and Cell Viabilities

Total counts of phagocytes (alveolar macrophages and neutrophils) in the bronchoalveolar lavage cell suspensions were determined using an electronic cell counter equipped with a cell-sizing attachment (Coulter Multisizer II, Coulter Electronics, Hialeah, FL). Portions of the harvested cells ($1 \times 10^5$ phagocytes/0.1 ml) were then deposited on slides using a cytocentrifuge (Shandon Scientific, London) and stained with a Wright stain

(Volu-Sol, Henderson, NV). The percentages of alveolar macrophages and leukocytes present on the slides were determined using light microscopy. Greater than 99% of these cells were alveolar macrophages or neutrophils. Viability of the phagocytic cell population for each rat was assessed using a trypan blue exclusion test (Phillips, 1973). This involved mixing an aliquot of cells with a trypan blue solution (Sigma Chemical Co., St. Louis, MO) for 4 min before the addition of a buffered formalin solution to fix the cells. The percentages of viable phagocytes were then determined by placing aliquots of the treated cells in a hemocytometer and scoring 100 cells for the absence (viable cells) or presence (dead cells) of blue staining. Alterations in thyroid hormone status did not affect the percentages of viable phagocytes from rats exposed to air (group mean ranges from 87–90%) or from rats exposed to LPS (group mean ranges from 92–94%).

### Analysis of Circulating $T_4$ Levels

Blood samples collected from the rats were centrifuged (1010 × g, 10 min, 4°C). The serum was separated from the blood and stored at –20°C prior to analysis. Serum $T_4$ levels were measured using a commercially available radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA). The results are expressed as micrograms per deciliter.

### Analyses of LDH Activities and Albumin Levels in Bronchoalveolar Lavage Fluid Samples

LDH activities in supernatants from initial bronchoalveolar lavage fluid samples were analyzed using the Roche Reagent for LDH using a COBAS FARA II chemistry system (Roche Diagnostic Systems, Nutley, NJ). The results are expressed as units per liter. Albumin levels in supernatants from initial bronchoalveolar lavage fluid samples were measured using a dye-binding assay procedure (Sigma Diagnostic Procedure 631; Sigma Chemical Co., St. Louis, MO) on a COBAS FARA II chemistry system (Roche Diagnostic Systems, Nutley, NJ). The results are expressed as milligrams per milliliter.

### Measurement of Nitric Oxide Production by Bronchoalveolar Lavage Cells

The production of nitric oxide by bronchoalveolar lavage cells was assessed by measuring media nitrate and nitrite levels following in vitro culture of the cells. Bronchoalveolar lavage cells were suspended in culture medium (X-VIVO 15, BioWhittaker, Walkersville, MD) containing penicillin (100 U/ml; Sigma Chemical Co., St. Louis, MO) and streptomycin (100 U/ml; Sigma Chemical Co., St. Louis, MO). The culture medium used is a defined medium that does not contain serum and does not contain thyroid hormones. The cells were then placed into wells of 24-well tissue culture plates (Costar Corp., Cambridge, MA). Each well contained $5 \times 10^5$ viable phagocytes per 0.5 ml culture medium. Nitric oxide pro-

duction was measured under basal conditions or following the addition of LPS (20 ng/ml; from *Escherichia coli* serotype 055:B5; Difco Laboratories Inc., Detroit, MI). The concentration of LPS was chosen on the basis of preliminary experiments and is a concentration that does not stimulate maximal nitric oxide formation by lung phagocytes from untreated rats under the studied conditions. The cells were incubated for 18–20 h at 37°C in an incubator with a humidified atmosphere (relative humidity 90%) of 95% air–5% $CO_2$. After the incubation period, the tissue culture plates were centrifuged (500 × g, 10 min, 4°C). The cell-free supernatants were stored at –20°C prior to analysis. All samples were first incubated with a nitrate reductase enzyme generated by *E. coli* according to the protocol outlined by Bartholomew (1984). Total nitrite levels in media samples were then determined using a spectrophotometric assay based upon the Greiss reaction (Green et al., 1982). The amount of nitrate and nitrite in the samples was calculated from a standard curve that was constructed from sodium nitrite standards diluted in culture media. Conversion of nitrate to nitrite was checked in each assay with sodium nitrate standards. The results are expressed as micromolar.

### Statistical Analyses

Two-way analyses of variance were performed. Following any overall significant analysis of variance result, differences were assessed using specific comparison procedures (SAS Institute, Inc., 1985). For data concerning the percentages of alveolar macrophages or neutrophils in bronchoalveolar lavage samples, the distribution assumption for ordinary analysis of variance was not met. These data were analyzed using a rank transform analysis of variance (Conover & Iman, 1981). Significance was set at $p \leq .05$.

## RESULTS

### Circulating $T_4$ Levels and Body Weights

The effects of thyroidectomy or thyroxine treatment on serum $T_4$ levels were determined, and the results are shown in Figure 1. Circulating $T_4$ levels in control rats were similar to those that have been reported previously (Coiro et al., 1979; Huffman et al., 1992). As expected, thyroidectomy was associated with a reduction in circulating $T_4$ levels. $T_4$ levels in thyroidectomized rats were reduced to levels at or below the detection limit of the assay (0.5 µg/dl). In contrast, serum $T_4$ levels were raised approximately sixfold in rats receiving thyroxine treatment with respect to control levels. Circulating $T_4$ levels in control, thyroidectomized, or thyroxine-treated rats were not significantly altered by the inhalation of LPS. These results indicate that the animal treatments were associated with the pre-

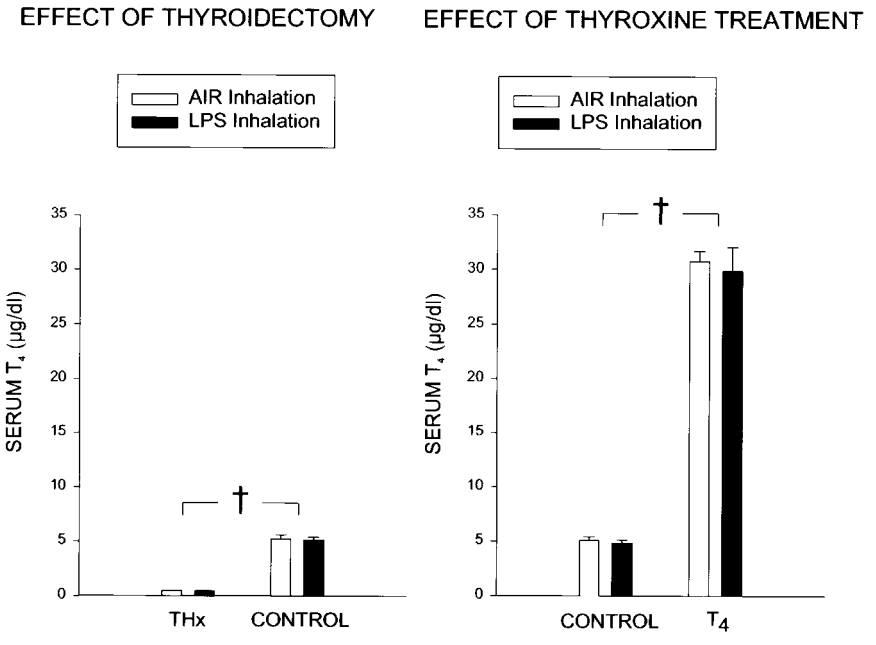


**FIGURE 1.** Effects of alterations in thyroid status on circulating $T_4$ levels following exposure of rats to air or LPS. Animals were thyroidectomized (THx; control = sham operated) or treated with thyroxine ($T_4$; control = vehicle injected) and then exposed to air or LPS by inhalation as described in Methods. Blood samples were collected 18 h after the inhalation exposures and the serum separated for analyses. Serum $T_4$ levels were then measured by radioimmunoassay. Values are the means ± SE for 7–8 determinations in each group. Dagger indicates significant at $p \leq .05$ vs. control.

dicted alterations in thyroid status and that the inhalation of LPS did not markedly alter circulating $T_4$ levels.

Body weights for the groups of animals are shown in Table 1. Body weights for both the thyroidectomized and thyroxine-treated rats were lower than those of the respective control groups of rats. Decreases in body weight of 21–22% occurred in thyroidectomized rats, whereas 5–7% decreases in body weight were noted following thyroxine treatment. The effects of thyroidectomy or thyroxine treatment on rat body weights are similar to those which have been reported previously (Coiro et al., 1979; Redding et al., 1972). Inhalation exposure to LPS had no significant effect on body weight.

### LDH Activities and Albumin Levels in Bronchoalveolar Lavage Fluid Samples

The effects of thyroidectomy or thyroxine treatment on LDH activities in bronchoalveolar lavage fluid samples are shown in Figure 2. LDH is an intracellular enzyme, and its presence in bronchoalveolar lavage fluid is an indicator of lung cell damage. LDH activities in bronchoalveolar lavage

**TABLE 1.** Effects of Alterations in Thyroid Status on Body Weights Following Exposure of Rats to Air or LPS

| Treatment | Body weight (g) | |
|---|---|---|
| | Air inhalation | LPS inhalation |
| Thyroidectomy | | |
| Control | 294 ± 3 (8) | 294 ± 4 (8) |
| THx | 228 ± 2[a] (8) | 232 ± 6[a] (8) |
| Thyroxine | | |
| Control | 294 ± 7 (8) | 290 ± 7 (8) |
| $T_4$ | 280 ± 5[a] (8) | 269 ± 5[a] (8) |

*Note.* Animals were thyroidectomized (THx; control = sham operated) or treated with thyroxine ($T_4$; control = vehicle injected) and then exposed to air or LPS by inhalation as described in Methods. Body weights were measured 18 h after the inhalation exposures. Values are the means ± SE. Numbers of rats are indicated in parentheses.

[a]Significant at $p \leq .05$ vs. control.

fluid samples from rats exposed to air alone were altered by changes in thyroid status. The amounts of LDH were significantly reduced in samples from thyroidectomized rats and increased in samples from thyroxine-treated animals relative to control. Exposure of the animals to LPS led to significant increases in bronchoalveolar lavage fluid LDH activity over that observed in air-exposed rats in all groups. Furthermore, alterations in thyroid status of the LPS-exposed animals produced changes similar to those seen in the air-exposed group; that is, there was less LDH in samples from thyroidectomized animals and more LDH in samples from thyroxine-treated rats relative to control following exposure to LPS.

Albumin levels in bronchoalveolar lavage fluid samples are presented in Table 2. Albumin is normally confined to the intravascular space. However, albumin levels in bronchoalveolar lavage fluid samples increase when the alveolar–capillary barrier is disrupted. The amounts of albumin were significantly reduced in bronchoalveolar lavage fluid samples from thyroidectomized rats and increased in samples from thyroxine–treated animals relative to control following exposure to air only. Exposure of the animals to LPS caused a significant increase in bronchoalveolar lavage fluid albumin levels relative to air exposure. In the LPS-exposed rats, there was less albumin in bronchoalveolar lavage fluid samples from thyroidectomized animals and more albumin in samples from thyroxine-treated rats relative to control.

### Bronchoalveolar Lavage Cell Numbers and Profiles

The effects of thyroidectomy or thyroxine treatment on the total number of phagocytes, that is, alveolar macrophages and neutrophils, that were harvested by bronchoalveolar lavage are shown in Figure 3. The absolute number of phagocytes recovered by bronchoalveolar lavage was altered by thyroid status alone. Thyroidectomy was associated with a reduction, while thyroxine treatment was associated with an increase in the total number of lung phagocytes harvested from rats exposed to air alone. Exposure of animals to LPS caused a significant increase in the number of lung phagocytes relative to the air-exposed group. Alterations in thyroid status produced changes in the LPS-exposed groups that were similar to those obtained in the air-exposed groups.

The percentages of neutrophils in lung phagocytic cell populations are shown in Table 3. In all air-exposed animals, the percentages of neu-

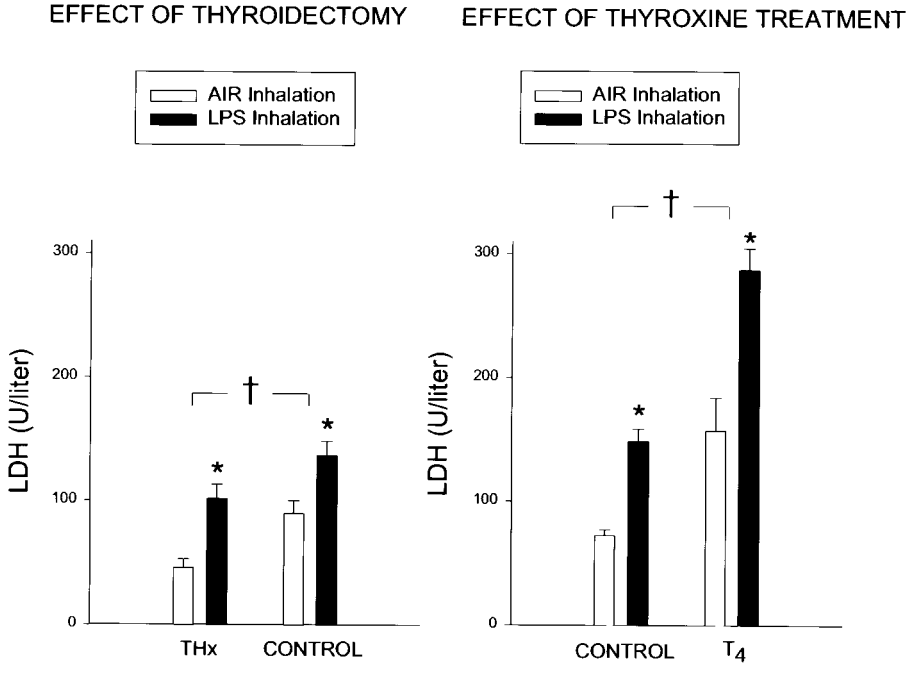


**FIGURE 2.** Effects of alterations in thyroid status on LDH activity in bronchoalveolar lavage fluid following exposure of rats to air or LPS. Animals were thyroidectomized (Thx; control = sham operated) or treated with thyroxine ($T_4$; control = vehicle injected) and then exposed to air or LPS by inhalation as described in Methods. Bronchoalveolar lavage fluid samples were obtained 18 h after the inhalation exposures. LDH activities in initial acellular bronchoalveolar lavage fluid samples were analyzed using commercially available reagents. Values are the means ± SE for 4–8 determinations in each group. Asterisk indicates significant at $p \leq .05$ vs. air inhalation. Dagger indicates significant at $p \leq .05$ vs. control.

**TABLE 2.** Effects of Alterations in Thyroid Status on Albumin Levels in Bronchoalveolar Lavage Fluid Following Exposure of Rats to Air or LPS

| | Albumin (mg/ml) | |
|---|---|---|
| Treatment | Air inhalation | LPS inhalation |
| Thyroidectomy | | |
| Control | 0.48 ± 0.05 (8) | 0.58 ± 0.04[b] (8) |
| THx | 0.32 ± 0.03[a] (8) | 0.41 ± 0.03[a,b] (8) |
| Thyroxine | | |
| Control | 0.31 ± 0.04 (8) | 0.44 ± 0.06 (8) |
| $T_4$ | 1.59 ± 0.42[a] (8) | 0.74 ± 0.14[a] (8) |

*Note.* Animals were thyroidectomized (THx; control = sham operated) or treated with thyroxine ($T_4$; control = vehicle injected) and then exposed to air or LPS by inhalation as described in Methods. Bronchoalveolar lavage fluid samples were obtained 18 h after the inhalation exposures. Albumin levels in initial acellular bronchoalveolar lavage fluid samples were analyzed using commercially available reagents. Values are the means ± SE. Numbers of rats are indicated in parentheses.

[a]Significant at $p \leq .05$ vs. control.

[b]Significant at $p \leq .05$ vs. air inhalation.

trophils in lung phagocytic cell populations were low, and alveolar macrophages were the predominant phagocyte harvested by bronchoalveolar lavage. In control or thyroidectomized rats that were exposed to air, greater than 98% of harvested phagocytes were alveolar macrophages. Thyroxine treatment was associated with the appearance of some neutrophils in the bronchoalveolar lavage harvested from air-exposed rats. In these animals, neutrophils accounted for 9 ± 4% of harvested cells. Following exposure to LPS, there was a marked increase in the percentage of neutrophils in bronchoalveolar cells harvested from control, thyroidectomized, or thyroxine-treated rats (Table 3). In these animals, the percentages of neutrophils in harvested bronchoalveolar cells averaged 45–50% and were not affected by thyroid status.

### Effects of Alterations in Thyroid Status on the Production of Nitric Oxide by Lung Phagocytes

The ability of lung phagocytes to produce nitric oxide was determined by measuring media nitrate and nitrite levels following in vitro culture of the cells. Nitric oxide production was evaluated under basal conditions or following in vitro stimulation with LPS. In these studies, equal numbers of phagocytes were cultured from all rats. The effects of thyroidectomy or thyroxine treatment on basal nitric oxide production are shown in Figure 4. The production of nitric oxide by lung phagocytes

was altered by thyroid status. Thyroidectomy was associated with a reduction, while thyroxine treatment was associated with an increase, in the amount of nitric oxide produced by lung phagocytes. In these experiments, the inhalation of LPS resulted overall in an increase in the amount of nitric oxide generated in vitro under basal conditions by lung phagocytes from the thyroxine-treated and the respective control group of rats. The production of nitric oxide following in vitro stimulation with LPS is shown in Figure 5. Under these conditions, there was an increase in the amount of nitric oxide produced by lung phagocytes that had been harvested from rats exposed to LPS inhalations relative to that produced by lung phagocytes harvested from rats exposed to air inhalations. Thyroid hormone status appeared to modulate the production of nitric oxide by lung phagocytes following in vitro LPS stimulation in that thyroidectomy was associated with decreases, while thyroxine treatment was associated with increases, in the overall amount of nitric oxide produced.

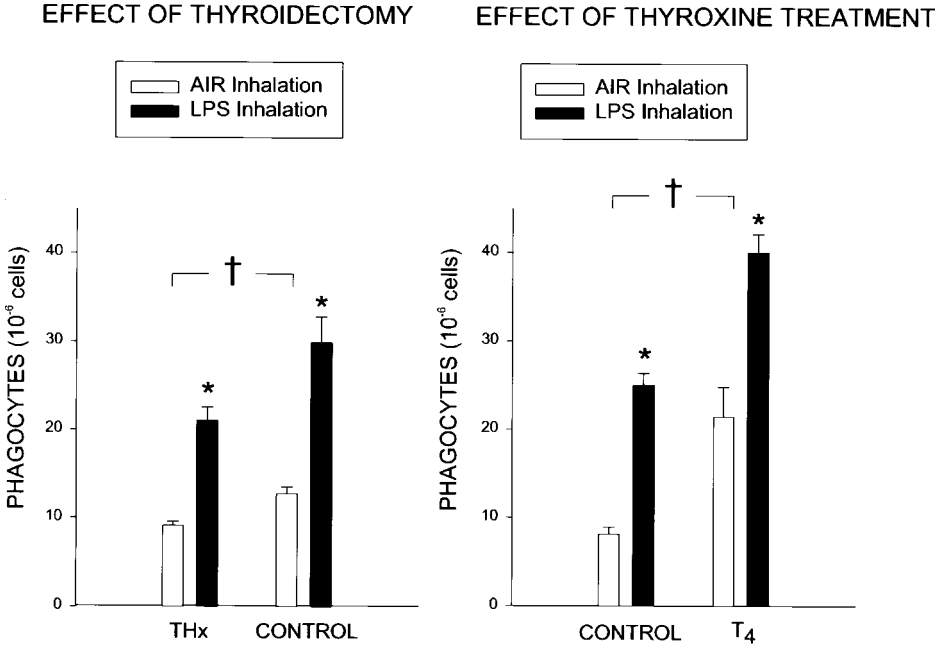


**FIGURE 3.** Effects of alterations in thyroid status on the number of total phagocytes harvested by bronchoalveolar lavage following exposure of rats to air or LPS. Animals were thyroidectomized (Thx; control = sham operated) or treated with thyroxine ($T_4$; control = vehicle injected) and then exposed to air or LPS by inhalation as described in Methods. Lung phagocytes were harvested by bronchoalveolar lavage 18 h after the inhalation exposures. The numbers of total phagocytes, that is, alveolar macrophages and neutrophils, that were harvested by bronchoalveolar lavage were determined as described in Methods. Values are the means ± SE for 8 determinations in each group. Asterisk indicates significant at $p \leq .05$ vs. air inhalation. Dagger indicates significant at $p \leq .05$ vs. control.

**TABLE 3.** Effects of Alterations in Thyroid Status on the Percentages of Neutrophils in Lung Phagocytic Cell Populations Following Exposure of Rats to Air or LPS

| | Neutrophils (%) | |
|---|---|---|
| Treatment | Air inhalation | LPS inhalation |
| Thyroidectomy | | |
| Control | 0.1 ± 0.1 (8) | 49.9 ± 1.5[b] (8) |
| Thx | 0.5 ± 0.5 (8) | 50.1 ± 4.2[b] (8) |
| Thyroxine | | |
| Control | 1.1 ± 0.4 (8) | 45.5 ± 4.5[b] (8) |
| $T_4$ | 9.0 ± 3.6[a] (8) | 46.0 ± 4.0[b] (8) |

*Note.* Animals were thyroidectomized (THx; control = sham operated) or treated with thyroxine ($T_4$; control = vehicle injected) and then exposed to air or LPS by inhalation as described in Methods. Lung phagocytes were harvested by bronchoalveolar lavage 18 h after the inhalation exposures. Portions of harvested cells were deposited on slides using a cytocentrifuge and stained for differential analyses of phagocytic cell populations. The percentages of neutrophils were then determined using light microscopy. Values are the means ± SE. Numbers of rats are indicated in parentheses.

[a]Significant at $p \leq .05$ vs. control/air inhalation.

[b]Significant at $p \leq .05$ vs. air inhalation.

## DISCUSSION

To our knowledge, this is the first report to demonstrate that alterations in thyroid status alone can affect lung cell damage and the functional state of the alveolar–capillary barrier in mature lungs. Although the specific factors that might contribute to these effects have not been defined, it is known that the lung is a target tissue for thyroid hormones. Thyroid hormone receptors can be detected in both fetal and adult lung tissue (Lindenberg et al., 1978; Morishige & Guernsey, 1978; Perez-Castillo et al., 1985). In addition, thyroid hormones have been shown to modulate some aspects of lung metabolism. For instance, hyperthyroidism is associated with increases in oxygen consumption by lung slices from adult rats, whereas oxygen consumption by lung slices is decreased in a hypothyroid state (Joyner et al., 1976). Alterations in thyroid hormone-linked changes in oxygen consumption may affect the baseline generation of reactive species in the lung, since when pulmonary oxygen consumption is increased, there is an elevation in the production of oxygen radicals (Freeman & Crapo, 1981). In fact, it is known that a thyroid hormone-linked rise in oxygen consumption can be associated with increases in the generation of free radicals and oxidative damage in the liver (Fernandez et al., 1985). If elevated thyroid

hormone levels are associated with these collective pro-oxidant effects at the lung, then enhanced damage of lung tissue and compromise of the alveolar–capillary barrier might result.

Thyroid hormone status also appears to affect the number of lung phagocytes that are present in bronchoalveolar regions of the lung. Hyperthyroidism is associated with an increase in the number of phagocytes that can be harvested from the lung by bronchoalveolar lavage, whereas a hypothyroid condition is associated with a decrease in the number of harvested phagocytes. The overall metabolic state of the lung may be a contributing factor to this observation. It is known that under normal conditions, alveolar macrophages are the predominant phagocytic cell type in the lung, and that one role of these cells is to scavenge particulates and remove debris (Crystal, 1991). As indicated earlier, thyroid hormone status appears to modulate the overall amount of lung cell damage and therefore could impact the amount of cellular debris in bronchoalveolar areas. Over time, this might affect the absolute number of alveolar macrophages

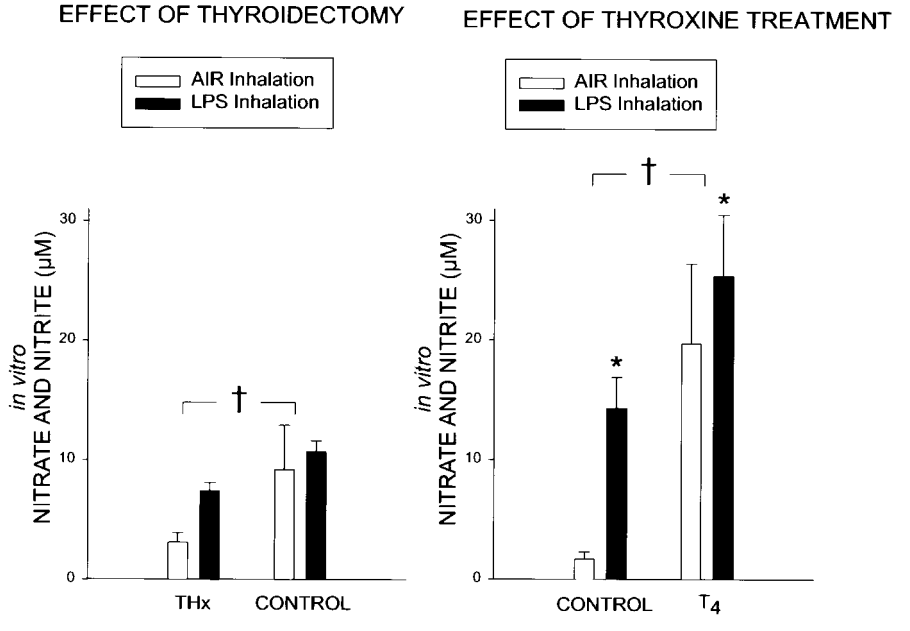


**FIGURE 4.** Effects of alterations in thyroid status on basal nitric oxide production by lung phagocytes following exposure of rats to air or LPS. Animals were thyroidectomized (Thx; control = sham operated) or treated with thyroxine ($T_4$; control = vehicle injected) and then exposed to air or LPS by inhalation as described in Methods. Lung phagocytes were harvested by bronchoalveolar lavage 18 h after the inhalation exposures. Lung phagocytes ($5 \times 10^5$ cells/0.5 ml) were then incubated in culture medium (X-VIVO) for 18–20 h at 37°C in 95% air–5% $CO_2$ (relative humidity 90%). After the incubation period, nitrate and nitrite in supernatants were measured as described in Methods. There were 7–8 determinations in each group. Asterisk indicates significant at $p \leq .05$ vs. air inhalation. Dagger indicates significant at $p \leq .05$ vs. control.

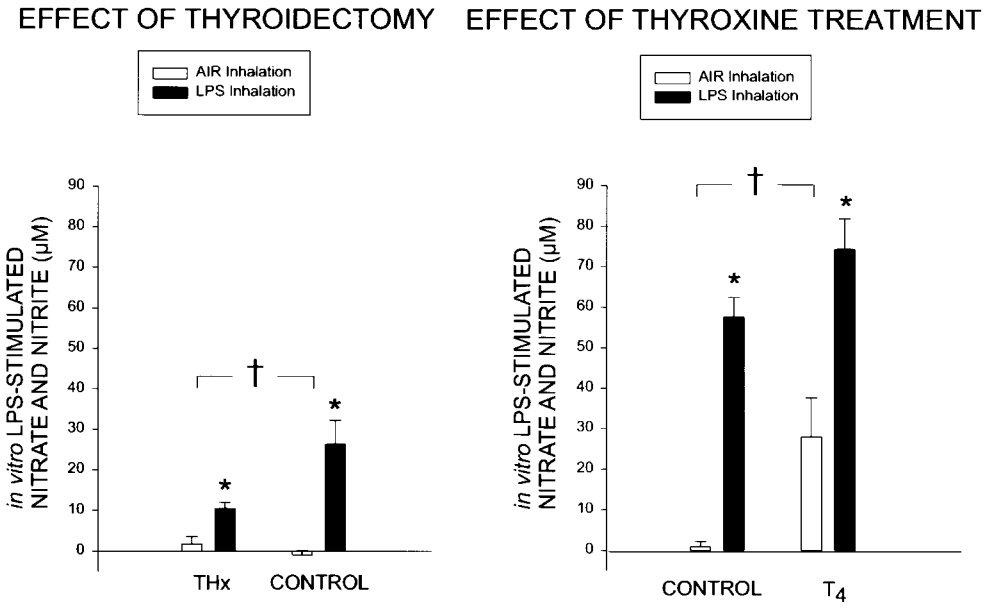


**FIGURE 5.** Effects of alterations in thyroid status on nitric oxide production by lung phagocytes following in vitro stimulation with LPS following exposure of rats to air or LPS. Animals were thyroidectomized (Thx; control = sham operated) or treated with thyroxine ($T_4$; control = vehicle injected) and then exposed to air or LPS by inhalation as described in Methods. Lung phagocytes were harvested by bronchoalveolar lavage 18 h after the inhalation exposures. Lung phagocytes ($5 \times 10^5$ cells/0.5 ml) were then incubated in culture medium (X-VIVO) containing LPS (20 ng/ml) and maintained for 18–20 h at 37°C in 95% air–5% $CO_2$ (relative humidity 90%). After the incubation period, nitrate and nitrite in supernatants were measured as described in Methods. The difference between LPS-stimulated and basal nitrate and nitrite levels (Figure 4) was calculated for each rat. Values are the means ± SE of the difference values. There were 7–8 determinations in each group. Asterisk indicates significant at $p \leq .05$ vs. air inhalation. Dagger indicates significant at $p \leq .05$ vs. control.

that are present in bronchoalveolar regions of the lung and be associated with an increase in alveolar macrophage cell number in hyperthyroidism and a decrease in a hypothyroid state. In addition, hyperthyroidism per se was associated with a small rise in the proportion of neutrophils recovered by bronchoalveolar lavage. This phenomenon may also be related to the overall degree of lung tissue damage associated with a hyperthyroid state, since lung damage is often associated with a recruitment of neutrophils into the lung (Parsons et al., 1991).

Another focus of the present study was to evaluate whether alterations in thyroid hormone status were associated with changes in nitric oxide production by lung phagocytes. Our observations suggest that alterations in thyroid hormone status can affect the ability of lung phagocytes to produce nitric oxide. Hyperthyroidism was associated with an increase in the amount of nitric oxide produced by cultured cells, whereas nitric oxide production was decreased in hypothyroidism. As far as we know, this is

the first report that thyroid hormone status can modulate nitric oxide production by lung phagocytes. Our results complement those of Fernandez and colleagues (1997), who observed that hyperthyroidism was associated with an increase in nitric oxide production by the liver and that Kupffer cells, which are resident hepatic phagocytes, appeared to contribute in part to this effect. In our study, thyroid status was modulated in vivo, and it is possible that alterations in thyroid hormone levels could have affected the ability of lung phagocytes to produce nitric oxide by direct or indirect mechanisms. It has been reported that detectable levels of thyroid hormones are present in alveolar macrophages obtained from normal rats and that there is uptake of thyroxine into nuclear fractions of human alveolar macrophages (Liu et al., 1989; Nishizawa et al., 1998). In addition, in vitro stimulation of human alveolar macrophages with thyroxine has been reported to result in increased superoxide anion production (Kanazawa et al., 1992; Nishizawa et al., 1998). These findings suggest that alveolar macrophages may be specific targets of thyroid hormones.

The effects of alterations in thyroid status on lung responses to inhalation exposures to LPS were also investigated in the present study. The inhalation of LPS was associated with lung inflammatory reactions in control, hyperthyroid, and hypothyroid rats. Evidence for this includes increases in lung cell damage, disruption of the alveolar–capillary barrier, and a rise in the number of neutrophils in alveolar regions. The absolute magnitudes of these pulmonary reactions were greater in a hyperthyroid state and relatively less in a hypothyroid condition. It has previously been reported that the capacity of hyperthyroid rats to respond to inflammatory stimuli at some tissue sites is impaired, and that this effect is, in part, related to an increased secretion of adrenal corticosteroids (Cury & Garcia-Leme, 1984; Rittenhouse & Redei, 1997). In those studies, inflammatory responses were elicited by intracutaneous injections of histamine and serotonin, injections of carrageenin into the hind paw, or intraperitoneal injections of streptococcal cell wall preparations. Our observations suggest that this is not the case for lung inflammatory responses following acute inhalation exposures to LPS. Our results provide some pathophysiological basis for the observation that hyperthyroid states are associated with increased mortality following acute systemic exposure to gram-negative agents (Martin & Bullard, 1969; Melby & Spink, 1959). The factors that might cause enhanced responses of hyperthyroid animals to the adverse effects of gram-negative agents have not been identified. It has been postulated that conditions that promote oxidation may increase susceptibility to endotoxin (Stark & Jackson, 1990). Our results suggest that elevated thyroid hormone levels are associated with pro-oxidant pulmonary effects, and this phenomenon may underlie the apparent increase in the inflammatory effects of inhaled LPS in the lungs of hyperthyroid rats.

The treatments used in the present study to alter thyroid hormone status were similar to those used by others (Coiro, et al., 1979; Redding et

al., 1972) and, as expected, produced quite marked changes in circulating thyroid hormone levels. For instance, the dose of exogenous thyroxine used produced sixfold increases in circulating thyroxine concentrations above those in control rats. It should be noted that further studies will be necessary to define the pulmonary effects of more modest alterations in thyroid hormone levels. Nevertheless, this study does show that some of the factors involved in lung inflammation and injury can be modulated by thyroid status.

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