Biologic Role of Fetal Lung Fibroblast Triglycerides as Antioxidants

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ABSTRACT

The pulmonary response to hyperoxia is highly variable, depending on such seemingly disparate biologic factors as gestational age, sex, hormonal milieu, and nutritional status. Descriptively, the magnitude and direction of these biologic differences in response to hyperoxia correlate with the triglyceride content of developing fetal rat lung fibroblasts (FRLFs). Mechanistically, these same factors affect the triglyceride content of FRLFs, *e.g.* d 21 FRLFs contain more triglyceride than d 18 FRLFs; female FRLFs contain more triglyceride than male FRLFs (d 20); dexamethasone increases FRLF triglyceride content, dihydrotestosterone decreases it; nutritionally, exposure of FRLFs to graded amounts of serum triglyceride (0%, 2%, 10%, 20%) results in increased intracellular FRLF triglyceride content. To test the hypothesis that these biologic differences in intracellular triglyceride content may account for differences in the cytoprotection of lung fibroblasts against oxidant injury, fibroblast cultures representing each of these biologic groups were challenged with graded doses of the reactive oxygen species hydrogen peroxide (0.1–1.0 mM for 5 min). The number of surviving cells and their antioxidant status, as measured by lipid peroxidation and glutathione content of the surviving cells, were determined. We found that in response to hydrogen peroxide *1*) d 21 FRLFs were more resistant than d 18 FRLFs; *2*) female FRLFs were more resistant than male FRLFs; *3*) dexamethasone-treated FRLFs were more resistant than dihydrotestosterone-treated fibroblasts; *4*) fibroblasts fed increasing amounts of serum triglycerides were increasingly resistant to hydrogen peroxide; *5*) cell survival in different serum triglyceride- and hormone-treated groups was not related to the antioxidant status as measured by glutathione content. These data are consistent with the hypothesized role of FRLF triglycerides as antioxidants. (*Pediatr Res* 49: 843–849, 2001)

Abbreviations:
FRLF, fetal rat lung fibroblast
H_2O_2, hydrogen peroxide
MDA, malondialdehyde
4HNE, 4-hydroxy-2-(E)-nonenal
GSH, glutathione
DHT, dihydrotestosterone
DEX, dexamethasone
AOE, antioxidant enzyme
DMEM, Dulbecco's minimal essential medium

The pulmonary response to hyperoxia is highly varied, depending on such seemingly disparate biologic factors as gestational age (1), sex (2), hormonal milieu (3), and nutritional status (4). Previous studies of the possible relationship between these biologic variables and classic AOE mechanisms have failed to support a causal relationship (5–7), leaving the biologic nature of the antioxidant protective mechanism unexplained. As an alternative hypothesis, cellular triglycerides have been found to act as antioxidants in a number of cell types, including endothelium (8), epithelium (9), and fibroblasts (10), both *in vivo* (11) and *in vitro* (12).

Several lines of evidence suggest that lipids are cytoprotective against oxygen free radical injury *in vitro* and *in vivo*.

Investigators have suggested that the greater oxygen tolerance of newborn rats and mice, as compared with their adult counterparts, relates, in part, to the greater amount of triglyceride in the lipid fraction of the newborn compared with the adult lung (13). Kehrer and Autor (11) demonstrated that increasing the saturated fatty acid composition of lung triglycerides in adult rats by dietary manipulation produced increased susceptibility to oxygen toxicity. Feeding pregnant rats a high triglyceride diet results in increased triglyceride content in the lungs of their offspring and increased survival and improved clinical and pathologic status after prolonged hyperoxic exposure (14, 15).

The mechanism responsible for this tolerance to hyperoxia has been speculated by Dormandy (16) to relate to the role of neutral lipids as antioxidants, which would confer protection against oxygen and oxygen free radical injury.

Studies from our laboratory have determined that, during fetal lung development, triglyceride uptake and content are

dependent on gestational age (17), sex (18), hormonal milieu (19), and nutritional status *in vitro* (17). Therefore, we hypothesized that the biologic variability in lung antioxidant capacity is dependent on quantitative differences in fetal lung fibroblast triglyceride content at the time of exposure to H_2O_2 as a function of gestational age (18 to 21 d gestation), triglyceride exposure (0–20%), or hormonal treatment (dexamethasone or dihydrotestosterone).

METHODS

Isolation of FRLFs. Five to 10 time-mated rat dams were used per preparation depending on the number of experimental variables to be tested. Isolation of FRLFs was performed according to methods of Smith and Giroud (20) as follows. The fetal lungs were removed into Hanks' balanced salt solution. The Hanks' balanced salt solution was decanted, and 5 vol of 0.05% trypsin was added to the lung preparation. The lungs were dissociated in a 37°C water bath using a Teflon stirring bar to disrupt the tissue mechanically. Once the tissue was dispersed into a unicellular suspension (approximately 20 min), the cells were pelleted at $500 \times g$ for 10 min at room temperature in a 50-mL polystyrene centrifuge tube. The supernatant was decanted, and the pellet was resuspended in DMEM, containing 20% FCS, to yield a mixed cell suspension of approximately 3×10^8 cells, as determined by Coulter particle counter (Beckman-Coulter, Hialeah, FL, U.S.A.). The cell suspension was then added to culture flasks (80 cm²) for 30–60 min to allow for differential adherence of lung fibroblasts. These cells are >95% pure fibroblasts based on vimentin-positive staining.

Treatment of animals and cultured cells with hormones and lipids. These methods have previously been described (17, 19). Briefly, fibroblast cultures were incubated with a rat serum triglyceride fraction at the indicated concentrations on a vol/vol basis (21). All animal experimentation was approved by the Institutional Review Board of the Harbor-UCLA Research and Education Institute.

Cell viability assays. The method used was based on the method of Spitz *et al.* (22) with minor modification. Briefly, fibroblast cultures were treated with H_2O_2 (0.1–1.0 mM) for 5 min at 37°C, during which time there was a linear dose-response relationship between H_2O_2 concentration and cell viability, in an atmosphere of 5%CO_2, balance air. At the end of the incubation, the H_2O_2 was aspirated, and the cultures were washed three times with DMEM; the surviving cells were cultured for 24 h in DMEM/10% FCS. At the end of the recovery period, the surviving cells were removed with 0.1% trypsin, and an aliquot was counted in a Coulter particle counter.

Triglyceride assay. The cellular levels of triglyceride were determined as previously described (17).

Lipid peroxidation assay. Lipid peroxidation products, MDA and 4HNE, were assayed using a colorimetric assay (Calbiochem, La Jolla, CA, U.S.A.). Briefly, after the experimental conditions, cells were lysed by repetitive freezing and thawing in distilled water. Samples were diluted in 20 mM Tris-HCl, pH 7.4. Diluted sample (200 μL) was added to 650 μL of diluted reagent, 10.3 mM *N*-methyl-2-phenylindole in acetonitrile, in a glass test tube. The mixture was vortexed for 3–4 s, and MDA and 4HNE were assayed by adding 150 μL of 15.4 M methanesulfonic acid. The solution was mixed well and incubated at 45°C for 40 min, after which samples were allowed to cool on ice. The samples were centrifuged at 10,000 $\times g$ for 5 min, and light absorbance was measured at 586 nm. Standard curves for MDA and 4HNE were generated as per the manufacturer's protocol, and the concentrations of MDA and 4HNE were calculated based on the extinction coefficients calculated from the standard curves.

GSH assay. Reduced GSH was assayed using a colorimetric assay (Calbiochem). Briefly, after the experimental conditions, cells were lysed and suspended in 500 μL of freshly prepared 5% metaphosphoric acid. Cells were homogenized with a Teflon pestle, and the homogenate was centrifuged at 3000 $\times g$ for 10 min at 4°C. Twenty to three hundred microliters of the resultant homogenate was used for the GSH assay. The total volume was made up to 900 μL with buffer composed of 200 mM potassium phosphate, pH 7.8, containing 0.2 mM diethylene triamine pentaacetic acid and 0.025% Lubrol (Calbiochem). Then 50 μL of a 12 mM solution of 4-chloro-1-methyl-7-fluoromethyl-quinolinium methylsulfate in 0.2 N HCl was added, and the solution was mixed thoroughly. After this, 50 μL of 30% NaOH was added, followed by thorough mixing, and the solution was incubated at 25°C for 10 min in the dark. Final absorbance was measured at 400 nm. Standard curves for GSH were generated according to the manufacturer's protocol, and the concentrations of GSH in the samples were calculated from their absorbance.

Statistical analysis. Student's *t* test or ANOVA for multiple comparisons was used to analyze the experimental data, as indicated.

RESULTS

Bio-dependent differences in fibroblast triglyceride content. We initially analyzed FRLFs for their triglyceride content as a function of *1*) gestational age, *2*) sex, *3*) hormonal exposure, and *4*) nutrition (Fig. 1). The triglyceride content of d 21 FRLFs was 112% higher than the triglyceride content of d 19 FRLFs. A similar difference in triglyceride content was observed when sex-specific FRLFs from d 20 fetal males and females were analyzed (108 ± 32 *versus* 202 ± 26 μg/10^6 cells, respectively). *In vivo* treatment of d 20 fetal rats with DHT (1 mg/kg for 48 h) decreased the triglyceride content of FRLFs by 70%, and DEX (0.25 mg/kg for 24 h) increased it by 30%, resulting in a 2.5-fold difference between these two groups. Treatment with both DHT and DEX resulted in triglyceride content of FRLFs that was the same as for DHT treatment alone, indicating DHT inhibition of the DEX effect on triglyceride uptake by these cells. Furthermore, the triglyceride content of d 19 FRLFs was determined by the serum triglyceride content of the medium in which the cells were cultured (exposure to 0%, 2%, 10%, or 20% serum triglyceride resulted in mean fibroblast triglyceride contents of 97 ± 23, 104 ± 38, 158 ± 40, and 210 ± 44 μg/10^6 cells, respectively).
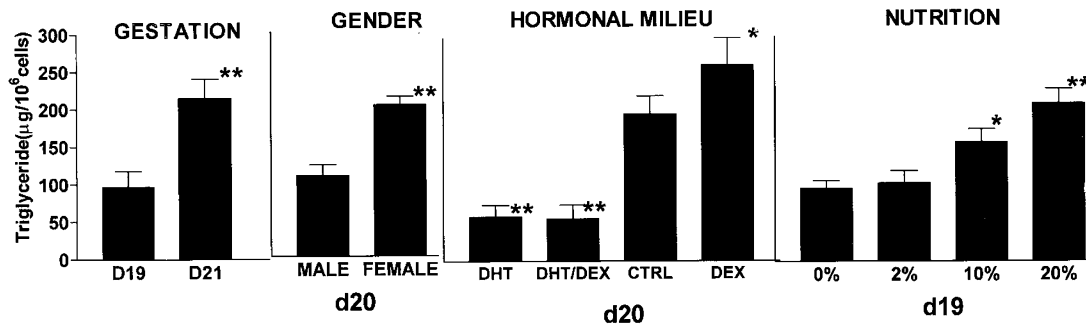
Figure 1. Biovariabilily in FRLF triglyceride content. FRLFs were harvested from the indicated groups and analyzed for their triglyceride content. Groups (from left to right): gestational age, d19 *versus* d21; gender, d20 male and female fetuses; hormonal milieu, d20 pregnant rats treated with DEX (0.25 mg/kg s.c.), DHT (1 mg/kg s.c.), or DHT and DEX (DHT/DEX), or controls (CTRL); nutrition, d19 fibroblasts incubated with 0%, 2%, 10%, or 20% serum triglyceride for 24 h. Each bar represents the mean $\pm$ SD of three to five experiments (n = l2–20). *$p < 0.05$; **$p < 0.01$, d19 *versus* d21; d20 male *versus* female; DHT, DHT/DEX, DEX *versus* CTRL, 2%, 10%, 20% *versus* 0%, respectively, by ANOVA for multiple comparisons.

FRLF viability* versus *gestational age. Having established that there were significant differences in FRLF triglyceride content depending on gestational age (d 19 *versus* d 21), we tested the effect of gestational age on resistance to H_2O_2 injury. As can be seen in Figure 2, there was a significant difference in response to H_2O_2 exposure between d 18 and d 21 FRLFs at the 0.5 and 1.0 mM concentrations (54% *versus* 97%, $p <$ 0.001; 8% *versus* 72%, $p < 0.001$; d 18 *versus* d 21, respectively). Incubation of d 18 FRLFs with serum triglyceride, 20% for 24 h, increased survival of the d 18 cells significantly at both the 0.5 and 1.0 mM H_2O_2 doses (54% *versus* 75%, $p <$ 0.001; 8% *versus* 57%, $p < 0.001$; d 18 *versus* d 18 with serum triglyceride, respectively), approaching the d 21 FRLF survival rate at the 1 mM H_2O_2 exposure level.

FRLF viability* versus *gender. Day 20 FRLFs from male and female fetuses were preincubated with serum triglyceride (0%, 2%, 10%, or 20% for 24 h) and subsequently exposed to 0.5 mM H_2O_2 for 5 min (Fig. 3). Among the male FRLF group there was a significant difference in survival between the 0% *versus* 20% groups (26% *versus* 82%, $p < 0.02$). The viability rates among the female FRLF group were not statistically different, although at the 0%, 2%, and 10% serum triglyceride exposures they were significantly higher than the males (30% *versus* 65%; 48% *versus* 81%; 69% *versus* 93%, male *versus* female, respectively; $p < 0.02$).

FRLF viability* versus *hormonal exposure. FRLFs from d 21 fetuses treated with DHT *in utero* (1 mg/kg/d for 48 h) were markedly sensitized to H_2O_2 exposure at 0.1, 0.5, and 1.0 mM H_2O_2 (52% *versus* 98%, $p < 0.01$; 12% *versus* 96%, $p <$ 0.001; 6% *versus* 71%, $p < 0.001$; DHT *versus* control,
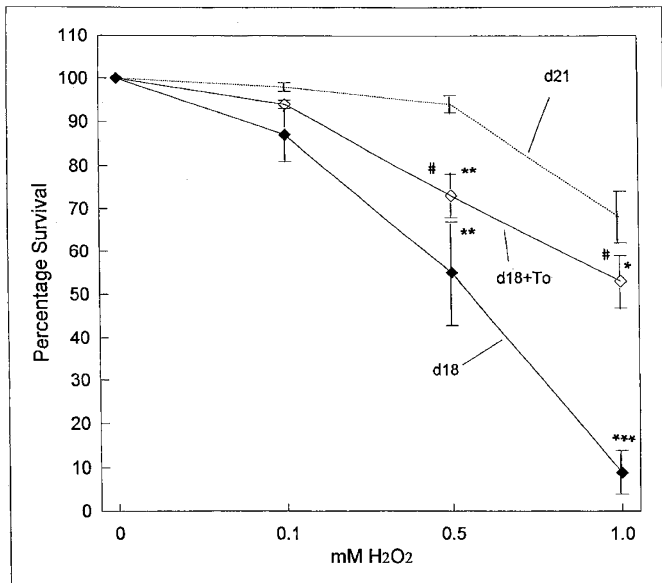

Figure 2. Effect of gestational age on fibroblast resistance to H_2O_2. Confluent cultures of d 18 and d 21 FRLFs were challenged with H_2O_2 for 5 min, and the number of surviving cells was determined 24 h later. d18 + To, d 18 fibroblasts preincubated with 20% serum triglycerides for 24 h. Each value is the mean $\pm$ SD of three experiments ($n = 15$). *$p < 0.05$; **$p < 0.001$; ***p < 0.0001 *versus* d21; and # comparison of d18 and d18 + To groups by ANOVA for multiple comparisons.
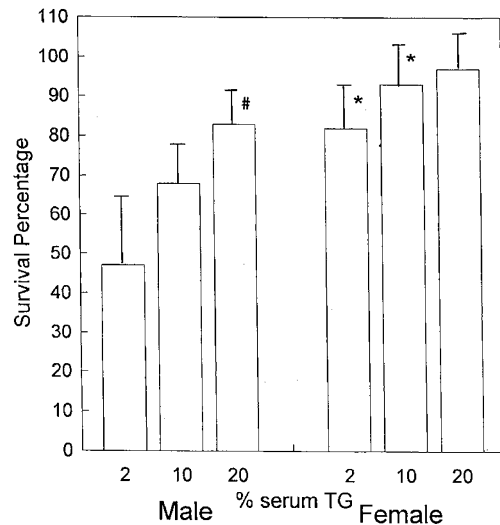

Figure 3. Effect of sex on fibroblast resistance to H_2O_2. Day 20 male and female FRLFs were preincubated with serum triglycerides (TG; 0%, 2%, 10%, 20%) for 24 h and subsequently exposed to 0.5 mM H_2O_2 for 5 min. The number of surviving cells was determined 24 h later. Each bar represents the mean $\pm$ SD of three experiments ($n = 15$). #$p < 0.05$; 20% *versus* 0% male group; *$p < 0.05$, female *versus* male by ANOVA for multiple comparisons.

respectively; Fig. 4). Day 18 survival data are provided for comparison. Conversely, DEX treatment (0.25 mg/kg for 24 h) significantly increased the survival of d 18 FRLFs at the 0.5 and 1.0 mM H_2O_2, exposures (54% *versus* 74%, $p < 0.05$; 8% *versus* 53%, $p < 0.001$; d 18 *versus* d l8 + DEX, respectively; Fig. 5). A 24-h exposure to serum triglyceride (20%) further enhanced the survival of DEX-treated d 18 FRLFs, making them comparable to d 21 control rats.

Direct effect of DEX and DHT on FRLF viability in response to H_2O_2. To directly test the effects of DEX and DHT on FRLF viability, d 19 fibroblasts were treated with DEX (1 $\times$ 10^{-8} M) or DEX + DHT (1 $\times$ 10^{-8} M and 1 $\times$ 10^{-7} M, respectively) for 24 h (Fig. 6). Steroid treatment *per se* had no effect on cell survival. In contrast to this, an additional 15-h exposure to 10% serum triglyceride resulted in a significant difference between control and DEX-treated cells, which was blocked by DHT (control, 58% *versus* DEX, 97%; $p < 0.01$; DEX + DHT, 62%), thus providing evidence that the steroid effect on antioxidant function is triglyceride-dependent.

FRLF viability* versus *nutrition. Day 19 FRLFs were incubated with serum triglycerides (2%, 10%, 20% for 24 h) and exposed to graded doses of H_2O_2 for 5 min. There were significant differences in percent survival between the 2% and 20% serum-exposed groups at the 0.5 and 1.0 mM H_2O_2 exposures (71% *versus* 97%, $p < 0.05$; 29% *versus* 71%, $p < 0.001$, respectively; Fig. 7). The 10% serum-exposed group fell between the 20% and 2% serum-exposed groups, exhibiting a significant difference *versus* the 20% serum-exposed group only at the 1.0 mM H_2O_2 exposure (49% *versus* 69%, $p < 0.01$).

GSH and lipid peroxidation status and FRLF viability. Day 19 fibroblasts were incubated with serum triglycerides (2%, 10%, 20%), DEX (1 $\times$ 10^{-8} M), DHT (1 $\times$ 10^{-7} M), or DEX + DHT (1 $\times$ 10^{-8} M and 1 $\times$ 10^{-7} M, respectively) for 24 h. Fibroblasts were subsequently exposed to H_2O_2 (0.1–1.0 mM/5 min). GSH content and lipid peroxidation status were determined at baseline and in the surviving cells after H_2O_2 expo-

Figure 5. Effect of *in vivo* DEX exposure on fibroblast resistance to H_2O_2. Pregnant rats were treated with DEX (0.25 mg/kg/d for 24 h), and fibroblasts were harvested on d 18 (d18 + DEX). Day 18 fibroblasts were also incubated with serum triglycerides (20%/24 h; d18 + DEX, To). Cells were exposed to H_2O_2 at the doses indicated for 5 min. Day 18 and 21 control data are shown for comparison. Each data point represents the mean $\pm$ SD of three experiments ($n = 15$). *$p < 0.01$; **$p < 0.001$, d18 *versus* d21; #$p < 0.02$; ##$p < 0.05$, d18 + DEX *versus* d21; &$p > 0.05$ d18 + DEX, To *versus* d21, by ANOVA for multiple comparisons.

Figure 6. Effect of *in vitro* DEX and DHT exposure on fibroblast resistance to H_2O_2. Cultured d 19 fibroblasts were treated with DEX (1 $\times$ 10^{-8} M) or DEX + DHT (1 $\times$ 10^{-8} M, 1 $\times$ 10^{-7} M, respectively) for 24 h and subsequently treated with 10% serum triglyceride (TG) for 15 h. The cells were then exposed to 1 mM H_2O_2 for 5 min and assayed for cell survival 24 h later. Each bar represents the mean $\pm$ SD of three experiments ($n = 15$). *$p < 0.01$, DEX *versus* control by *t* test.
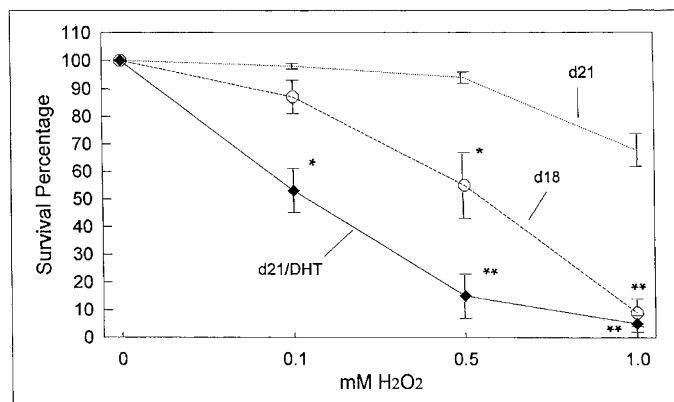

Figure 4. Effect of *in vivo* DHT exposure on fibroblast resistance to H_2O_2. Pregnant rats were treated with DHT (l mg/kg/d for 2 d), and fibroblasts were harvested on d 21 (d2I/DHT). Confluent cultures were exposed to graded doses of H_2O_2 for 5 min, and the number of surviving cells was determined 24 h later. Survival of d 18 fibroblasts is shown for comparison (d18). Each data point represents the mean $\pm$ SD of three experiments ($n = 15$). *$p < 0.01$; **$p < 0.001$ *versus* d21 control by ANOVA for multiple comparisons.
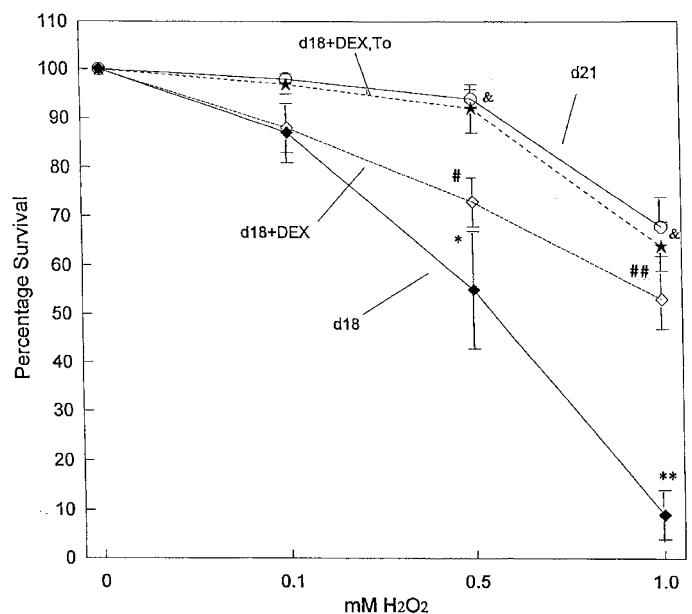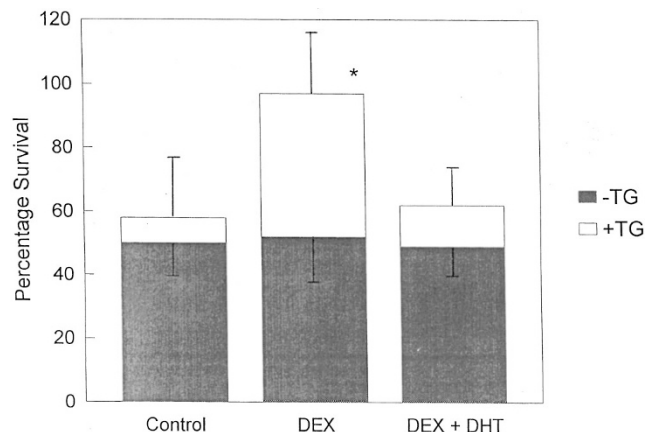
sure. The number of surviving cells was determined, and the GSH content and lipid peroxidation status were corrected for the differences in the number of surviving cells. There were no differences in either GSH content or lipid peroxidation status at baseline in different serum- and hormone-treated groups (Figs. 8 and 9). GSH content significantly decreased after H_2O_2 exposure (at 0.5 and 1 mM, but not at 0.1 mM H_2O_2 exposure) in some serum- (2%) and hormone- (DHT) treated groups. Lipid peroxidation increased significantly in all serum- and
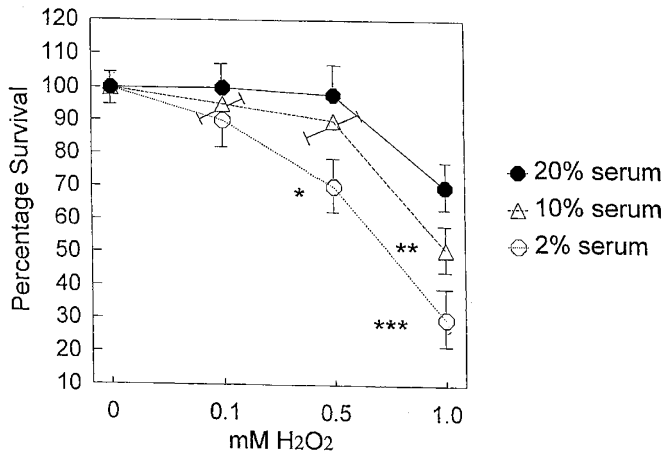
Figure 7. Effect of triglyceride loading on fibroblast survival in response to H_2O_2. Day 19 fibroblasts were incubated with serum triglycerides (2%, 10%, 20%) for 24 h and subsequently exposed to H_2O_2 (0.1–1.0 mM/5 min). The number of surviving cells was determined 24 h later. Each data point represents the mean $\pm$ SD of five experiments ($n = 20$). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ versus 20% serum group by ANOVA for multiple comparisons.

hormone-treated groups in a dose-dependent manner corresponding to the concentration of H_2O_2 exposure. However, there was evidence of increased peroxidation in the 2% serum-supplemented group versus the 10% and 20% supplemented groups. Furthermore, the DEX-treated group showed somewhat decreased lipid peroxidation versus DHT and DEX + DHT-treated groups.

DISCUSSION

In the present series of studies, we have demonstrated that during fetal rat lung development there are significant differences in the triglyceride content of fibroblasts because of gestation, sex, hormonal milieu, and nutrition. More important, these differences in the triglyceride content of the developing lung fibroblast result in significant differences in the viability of these cells on exposure to H_2O_2, which is one of several reactive oxygen species that mediate the biologic effects of hyperoxia (23). These differences were observed in the presence of similar baseline GSH content in different nutritional
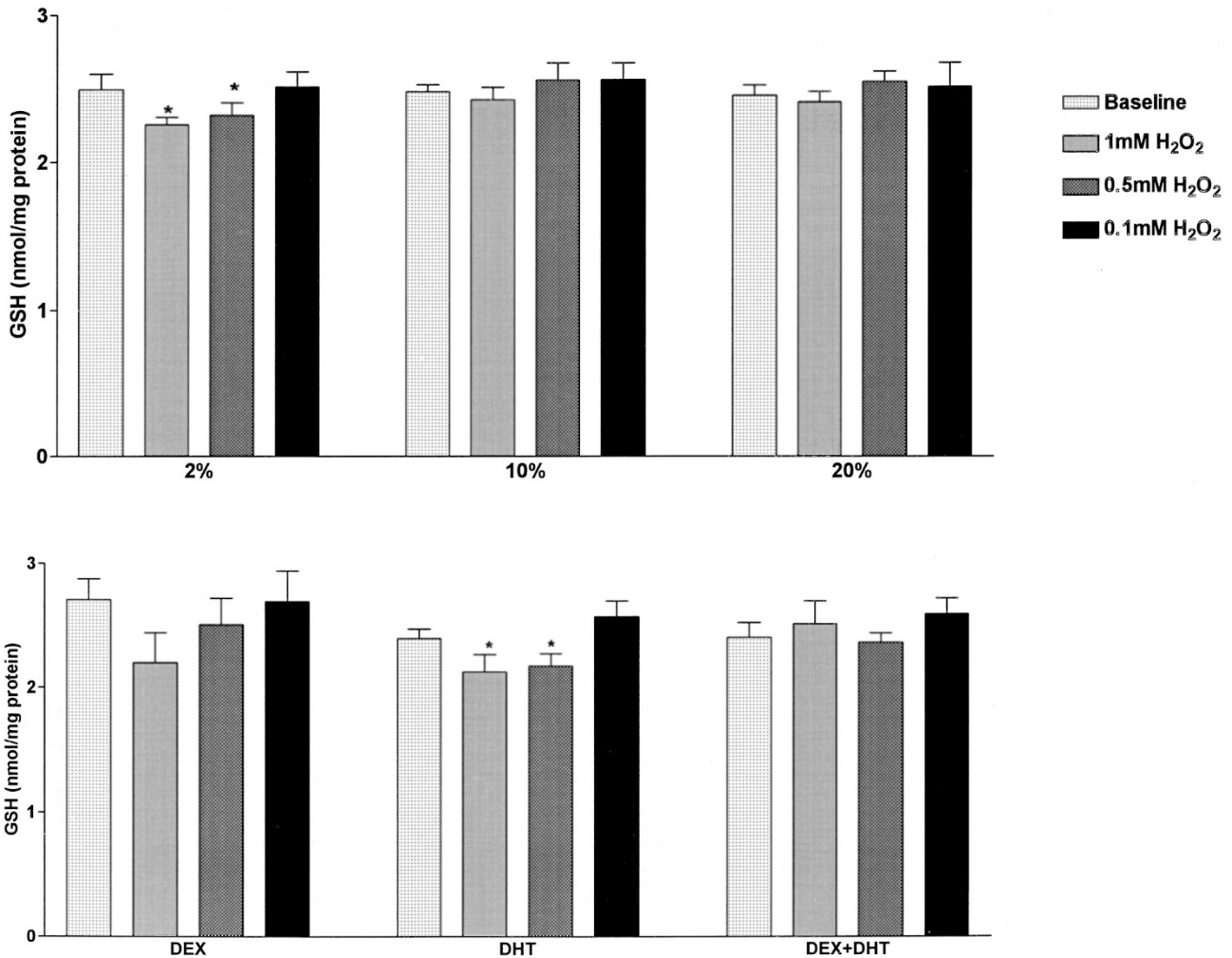

Figure 8. Effect of triglyceride loading and hormonal exposure on reduced GSH status at baseline and after exposure to H_2O_2. Day 19 fibroblasts were incubated with serum triglycerides (2%, 10%, 20%; Top), DEX (1×10^{-8} M), DHT (1×10^{-7} M), or DEX + DHT (1×10^{-8} M, 1×10^{-7} M, respectively; Bottom) for 24 h and subsequently exposed to H_2O_2 (0.1–1.0 mM/5 min). GSH status was determined at baseline and in the surviving cells after H_2O_2 exposure. The number of surviving cells was determined, and the GSH status was corrected for the differences in the number of surviving cells in each group. Each bar represents the mean $\pm$ SD of three experiments ($n = 9$). *$p < 0.05$ versus baseline.
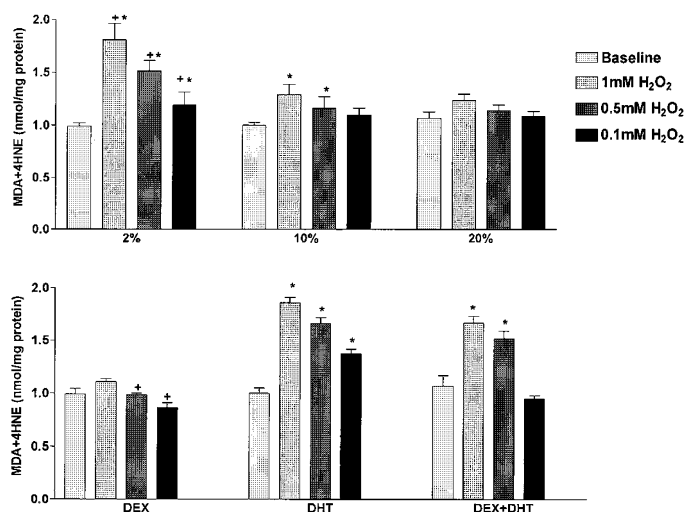
Figure 9. Effect of triglyceride loading and hormonal exposure on lipid peroxidation status at baseline and after exposure to H_2O_2. Day 19 fibroblasts were incubated with serum triglycerides (2%, 10%, 20%; *Top*), DEX (1 × 10^{-8} M), DHT (1 × 10^{-7} M), or DEX + DHT (1 × 10^{-8} M, 1 × 10^{-7} M, respectively; *Bottom*) for 24 h and subsequently exposed to H_2O_2 (0.1–1.0 mM/5 min). Lipid peroxide (MDA + 4HNE) status was determined at baseline and in the surviving cells after H_2O_2 exposure. The number of surviving cells was determined, and the peroxidation status was corrected for the differences in the number of surviving cells in each group. Each bar represents the mean ± SD of three experiments ($n = 9$). *$p < 0.05$ *versus* baseline and +$p < 0.05$ for 2% *versus* 20% by ANOVA for multiple comparisons.

(triglyceride) categories. The lipid-dependence of this relationship is demonstrated by the independent effects of fibroblast lipid stores (owing to either gestational age, sex, lipid exposure, or the hormonal effects of DEX and DHT) on cell viability in response to H_2O_2 exposure. The experimental rationale for using fibroblasts at selected gestational ages (18–21 d) was based on both their endogenous triglyceride stores (17) and their ability to respond to glucocorticoid stimulation (19) and androgen inhibition (18) of triglyceride uptake. Notably, the effects of both stimulatory (DEX) and inhibitory (DHT) steroids on cell viability in response to H_2O_2 were only observed after the cells were incubated with serum triglycerides, suggesting that the cytoprotection was caused by the hormonal effect on triglyceride content of the cells. These hormonal effects may determine the developmental and sex-specific differences in fibroblast triglyceride content inasmuch as glucocorticoids regulate this mechanism developmentally (19) and androgens act as antiglucocorticoids (18, 24, 25), delaying glucocorticoid-dependent fibroblast maturation (25). Thus, hormonal regulation of fibroblast triglyceride metabolism provides a plausible mechanism for the observed biovariability in response to H_2O_2 treatment.

The biologic basis for differences in susceptibility to hyperoxia remains highly controversial. The primary physiologic mechanism by which tissues inactivate oxygen free radicals is dependent on the activities of the AOEs, such as superoxide dismutase, catalase, and the GSH system, which consists of a battery of enzymes (26). In the lung, these enzymes appear during late gestational development (27) and are stimulated by glucocorticoids (28), making them good candidates for a developmentally dependent, hormonally regulated antioxidant

mechanism. However, there are a number of biologic conditions under which there are differences in response to oxygen that cannot be explained on the basis of differences in AOE activities. For example, there is a sex difference in the incidence of bronchopulmonary dysplasia (29), but there is no sex difference in the rate of AOE maturation (7). Similarly, there are age (30) and species (30) differences in susceptibility to oxidant injury that are unaccounted for on the basis of the AOE mechanism (31). Our data further reinforce this assertion because we observed significant differences in cell survival in response to H_2O_2 exposure in the absence of any differences in the baseline GSH status in various serum- and hormone-treated categories. However, inasmuch as we did not measure all of the AOEs of the antioxidant system, including superoxide dismutase and catalase, it is possible that some of the biovariability in response to hyperoxia may be governed by differences in the status of AOEs that were not determined. However, the observation of a lack of any differences in AOE (superoxide dismutase, catalase, and GSH peroxidase) status in the offspring of high- and low-polyunsaturated fatty acid–supplemented rats despite the observed differences in tolerance to hyperoxia (15) concurs with our observations.

In contrast to the lack of correlation between protection against hyperoxia and AOE mechanisms, there does appear to be a good fit with fibroblast triglyceride metabolism and content. For example, we have demonstrated that there is a significant sex difference in the uptake and content of triglyceride in fetal lung fibroblasts, which results in a quantitative difference in the response to H_2O_2 treatment. These data are consistent with the observation by Neriishi and Frank (32) that there is a sex difference in response to hyperoxia by adult rats that is eliminated by castration and reinstated by androgen treatment. Similarly, there are well-documented species differences—neonatal rats, rabbits, and mice are more resistant to hyperoxia than are guinea pigs and hamsters (30). These species differences in response to hyperoxia cannot be accounted for by differences in AOEs (33); however, in studies performed by Kaplan *et al.* (34) on species differences in the lipid content of lung lipofibroblasts it was observed that rat lung lipofibroblasts contain considerably more lipid than do those derived from hamsters. These observations are consistent with our hypothesized role of cellular lipids as cytoprotective agents.

Glucocorticoids have a well-documented effect on protecting the neonatal lung from oxidant injury (29). Steroids have been shown to accelerate maturation of the lung AOE mechanism and reduce oxidant injury (28, 35), consistent with their role in cytoprotection. In the current study, glucocorticoids were shown to also increase the triglyceride content of FRLFs and to decrease the cellular toxicity caused by H_2O_2 injury despite any effect on their glutathione content. However, the effect of DEX on antioxidant activity was only observed after the cells were exposed to triglyceride, further suggesting that the effect of cytoprotection in the steroid-treated group is related to differences in the triglyceride content rather than to the GSH status of these cells. The baseline GSH content of DEX-treated cells and untreated cells was similar, further suggesting that the effect of cytoprotection in the steroid-

treated group is related to differences in the triglyceride content rather than in the GSH status. Similarly, DHT antagonized the DEX effect on cell viability, but only after exposure of the cells to triglyceride. Undoubtedly, the cytoprotection from oxidant injury is a complex process, and we did not explore the underlying specific cellular and molecular mechanisms. From the data presented, however, we conclude that the hormonal effect on FRLFs is not on constitutive AOEs but on the ability of these cells to metabolize triglycerides.

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