Effects of undernutrition on respiratory mechanics and lung parenchyma remodeling

Cristina Márcia Dias, Caroline P. Pássaro, Viviane Ramos Cagido, Marcelo Einicker-Lamas, Jennifer Lowe, Elnara M. Negri, Vera L. Capelozzi, Walter A. Zin, and Patricia R. M. Rocco. Effects of undernutrition on respiratory mechanics and lung parenchyma remodeling. J Appl Physiol 97: 1888–1896, 2004. First published June 11, 2004; doi:10.1152/japplphysiol.00091.2004.—Undernutrition thwarts lung structure and function, but there are disagreements about the behavior of lung mechanics in malnourished animals. To clarify this issue, lung and chest wall mechanical properties were subdivided into their resistive, elastic, and viscoelastic properties in nutritionally deprived (ND) rats and correlated with the data gathered from histology (light and electron microscopy and elastic fiber content), and bronchoalveolar lavage fluid analysis (lipid and protein content). Twenty-four Wistar rats were assigned into two groups. In the control (Ctrl) group the animals received food ad libitum. In the ND group, rats received one-third of their usual daily food consumption until they lost 40% of their initial body weight. Lungs static elastance, viscoelastic and resistive pressures (normalized by functional residual capacity), and chest wall pressures were higher in the ND group than in the Ctrl group. The ND group exhibited patchy atelectasis, areas of emphysema, interstitial edema, and reduced elastic fiber content. The amount of lipid and protein in bronchoalveolar lavage fluid was significantly reduced in the ND group. Electron microscopy showed 1) type II pneumocytes with a reduction in lamellar body content, multimembranous structures, membrane vesicles, granular debris, and structurally aberrant mitochondria; and 2) diaphragm and intercostals with atrophy, disarrangement of the myofibrils, and deposition of collagen type I fibers. In conclusion, undernutrition led to lung and chest wall mechanical changes that were the result from a balance among the following modifications: 1) distorted structure of diaphragm and intercostals, 2) surfactant content reduction, and 3) decrease in elastic fiber content.

diaphragm; surfactant; elastance; elastin

There are some reports describing the effects of prolonged undernutrition on lung structure and function (17, 25, 41, 46). Lungs of nutritionally deprived adult rats show enlargement of terminal air spaces, which is consistent with the morphometric definition of emphysema (15, 21, 45, 46). Additionally, connective tissue modifications are associated with a reduction in lung content of elastin (19, 44, 46). Undernourished patients without lung disease show reduced respiratory muscle strength, maximum voluntary ventilation, and vital capacity (4, 35). Undernutrition causes respiratory muscle weakness, mainly in the diaphragm, with a decrease in strength and endurance (4) partially related to a loss of muscle mass. Weight loss, however, does not seem to be the only mechanism involved in muscle dysfunction. Undernutrition induces a significant decrease in mitochondrial oxygen consumption (31) and reduction in muscle IGF-I expression associated with muscle fiber atrophy (26). The association of respiratory muscle weakness (14, 39) with ventilatory drive reduction and impairment of immunologic function (39) can lead to hypercapnic respiratory failure (14), difficulty in weaning from mechanical ventilation (14, 39), decreased ability to cough, and hence an increased risk of atelectasis and subsequent pneumonia in patients with any type of respiratory disease (39).

These structural changes in lung parenchyma and respiratory muscles could lead to mechanical modifications. However, lung mechanics are not fully understood in malnourished animals. Some authors reported that lung compliance increases in malnutrition (20, 45), whereas others observed that lung compliance decreases (36, 46) or is not altered in malnourished animals after normalization procedures (25, 48). The use of animals of different ages and strains, diverse dietary restrictions, in vivo vs. in vitro studies, and the various methods used to study lung mechanics could explain these controversies. As aforementioned, undernutrition affects both the lung (20, 36, 45) and the chest wall (4, 26, 31), but the partition of respiratory system mechanics into its lung and chest wall components has not been hitherto performed.

Thus the aim of the present study is to test the following hypotheses: 1) both lung and chest wall elastic, resistive, and viscoelastic/inhomogeneous pressures effectively contribute to the mechanical changes found in nutritionally deprived animals; 2) activation and deactivation of respiratory muscles are thwarted by undernutrition; and 3) the mechanical changes are explained by histology (light and electron microscopy and elastic fiber content) and bronchoalveolar lavage fluid (BALF) analysis (lipid and protein content).

MATERIALS AND METHODS

Animals and Nutritional Protocol

The experiments were performed on adult male, normal Wistar rats with an average initial body weight of 180 g. Ten rats were initially placed in individual cages, and their daily food consumption was...
measured over 3 days and found to average 21 g per day. Then, a total of 24 animals were randomly divided into two groups: control and nutritionally deprived. The control group received water ad libitum and food (Purina rat chow, containing 56% carbohydrate, 23% protein, 4.5% fat, 6% fiber, and 10.5% ash and minerals and was enriched with 150 mg/kg antioxidant, 44 μg/kg vitamin B12, 12 mg/kg pyridoxine, 13 mg/kg vitamin B2, 12 mg/kg thiamine, 100 mg/kg calcium pantothenate, 242 mg/kg niacin, 2,800 mg/kg choline, 0.20 mg/kg biotin, 14 mg/kg folic acid, 7 mg/kg vitamin K, 90 μl/kg vitamin E, 4,400 μl/kg vitamin D, 2,800 μl/kg vitamin A, 2 mg/kg cobalt, 0.20 mg/kg selenium, 2 mg/kg iodine, 110 mg/kg manganese, 110 mg/kg zinc, 30 mg/kg copper, and 180 mg/kg iron). Nutritionally deprived animals received water ad libitum and one-third of their estimated daily consumption of food until they lost ~40% of their initial body weight. Animals were housed in microisolator individual cages (filter tops), in a controlled environment, in a specific housing room for rats in Rio de Janeiro throughout the experimental period to reduce the potential spread of infectious agents. All animals received uniform handling and were maintained without physical activities on a 12:12-h light-dark cycle at normal ambient temperature (~27°C). Body weight was measured daily. The average duration of starvation was ~4 wk.

This study was approved by the Ethics Committee of the Carlos Chagas Filho Institute of Biophysics, Health Sciences Center, Federal University of Rio de Janeiro.

All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the U.S. National Academy of Sciences.

Experimental Protocol

The animals of both groups were sedated with diazepam (5 mg ip) and anesthetized with pentobarbital sodium (20 mg/kg ip), and a snugly fitting cannula (1.7 mm ID) was introduced into the trachea. A pneumotachograph (1.5 mm ID, length = 4.2 cm, distance between side ports = 2.1 cm) was connected to the tracheal cannula for the measurements of airflow (V) and changes in lung volume (Vt) (34). The pressure gradient across the pneumotachograph was determined by means of a Validyne MP45–2 differential pressure transducer (Engineering Corp, Northridge, CA). The flow resistance of the equipment (Req), tracheal cannula included, was constant up to flow rates of 26 ml/s and amounted to 0.12 cmH2O·ml−1·s−1. Equipment resistance pressure (Ereq/V) was subtracted from pulmonary resistance pressure so that the present results represent intrinsic values. Tracheal pressure (Ptr) was measured with a Validyne MP-45 differential pressure transducer (Engineering Corp). Changes in esophageal pressure, which reflect chest wall pressure (Pw), were measured with a 30-cm-long water-filled catheter (PE-200) with side holes at the tip connected to a PR23-2D-300 Statham differential pressure transducer (Hato Rey, Puerto Rico). The catheter was passed into the stomach and then slowly returned into the esophagus; its proper positioning was assessed using the “occlusion test” (8). The frequency responses of the pressure measurement systems (Ptr and esophageal pressure) were flat up to 20 Hz, without appreciable phase shift between the signals. All signals were conditioned and amplified in a Beckman type R Dynograph (Schiller Park, IL). Flow and pressure signals were then passed through 8-pole Bessel filters (902LPF, Frequency Devices, Haverhill, MA) with the corner frequency set at 100 Hz, sampled at 200 Hz with a 12-bit analog-to-digital converter (DT2801A, Data Translation, Marlboro, MA), and stored on a personal computer. All data were collected using LABDAT software (RHT-InfoData, Montreal, PQ, Canada).

Decay of Inspiratory Muscle Pressure During Expiration

The determination of the antagonistic pressure exerted by the inspiratory muscles during spontaneous expiration (PmusI) was performed using the Ptr relaxation method (55). Briefly, the airways were occluded at the end of tidal inspirations, and shortly afterward the animals relaxed their respiratory muscles (Breuer-Hering reflex). This was demonstrated by the presence of a plateau on the Ptr tracing. After the airway occlusion at end inspiration, the Ptr represents alveolar pressure (zero flow), and hence the time course of alveolar pressure reflects the decay of the pressure generated by the inspiratory muscles. The onset of decay of PmusI (time = 0) was defined as the point at which Ptr became positive (supra-atmospheric), and hence Ptr was measured at 0.1-s intervals until a plateau was achieved, reflecting relaxation of the inspiratory muscles. The times required for PmusI to decay to 50% (T50), 25% (T25), and 0% (T0) of inspiratory muscle pressure at start of expiration (PmusI0) were also measured. Six to 10 determinations were performed in each rat.

Ventilatory Variables

During spontaneous breathing, the duration of inspiration (T1) and expiration (T2) and the duration of the respiratory cycle (Tt) were measured on the flow signal. Using these variables, the duty cycle (T1/Tt) and the mean inspiratory flow (Vt/T1), inspiratory frequency, and minute ventilation (V̇E) were computed. Six to 10 determinations were performed in each rat.

Shape of the Tracheal Occlusion Pressure Wave

During spontaneous breathing, the airways were occluded at end-expiratory lung volume (Vt) for one breath period to obtain the tracheal occlusion pressure (Ptr(t)), which represents the driving pressure of the system (56). According to previous studies on different species (11, 56), the shape of Ptr(t) wave can be described as a power function of time:

\[-P_{\text{tr}}(t) = a \cdot t^b\]  

where t is time in seconds from the onset of the occluded inspiratory effort, b is a dimensionless index of the shape of the curve, and a is the extrapolated pressure 1 s after the start of the occluded breath, an index of the intensity of the neuromuscular drive. The same approach was used in the present study. Six to 10 determinations were performed in each animal.

Measurement of Respiratory Mechanics

Muscle relaxation was achieved with gallamine triethyliodide (2 mg/kg iv), and artificial ventilation was provided by a constant flow ventilator (Sanay VR15, Universidad de la Republica, Montevideo, Uruguay) with a frequency of 80 breaths/min. During the test breaths, the ventilator was adjusted to generate a 5-s end-inspiratory pause, whereas no pause was used during baseline ventilation. Special care was taken to keep tidal volume (Vt = 1.5 ml) and flow (V = 8 ml/s) constant in all animals to avoid the effects of different flows and volumes (22) and inspiratory duration (50) on the measured variables.

Respiratory mechanics were measured by the end-inflation occlusion method (5, 6). Briefly, after end-inspiratory occlusion, there is an initial fast drop in Ptr (∆Ptr1) from the preocclusion value down to an inflection point (Plrs) followed by a slow pressure decay (∆Ptr2), until a plateau is reached. This plateau corresponds to the elastic recoil pressure of the respiratory system (Pelrs). ∆Ptr1 selectively reflects the pressure used to overcome the combination of airways, pulmonary, and chest wall Newtonian resistances in normal animals (5, 6), whereas no pause was used during baseline ventilation. Special care was taken to keep tidal volume (Vt = 1.5 ml) and flow (V = 8 ml/s) constant in all animals to avoid the effects of different flows and volumes (22) and inspiratory duration (50) on the measured variables.

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the values of \( \Delta P_{rs}, \Delta P_{L}, \) and \( \Delta P_{w}. \) Static elastances (\( E_{\text{stat}} \)) of the respiratory system, lung, and chest wall (\( E_{\text{rs}}, E_{L}, \) and \( E_{w}, \) respectively) were calculated by dividing \( P_{\text{rs}}, P_{L}, \) and \( P_{w}, \) respectively, by \( V_{T}. \) Dynamic elastances (\( E_{\text{dyn}} \)) of the respiratory system, lung, and chest wall (\( E_{\text{dyn,rs}}, E_{\text{dyn,L}}, \) and \( E_{\text{dyn,w}}, \) respectively) were obtained by dividing \( P_{\text{rs}}, P_{L}, \) and \( P_{w}, \) respectively, by \( V_{T}. \) Change in elastance (\( \Delta E \)) was calculated as the difference \( E_{\text{stat}} \) -- \( E_{\text{dyn}} \) yielding the values of \( E_{\text{rs}}, E_{L}, \) and \( E_{w}. \) Respiratory mechanics were measured 10 times in each animal. Lung mechanical parameters were normalized by functional residual capacity (FRC).

The delay between the beginning and the end of the valve closure (10 ms) was allowed for by backextrapolation of the pressure records to the actual time of occlusion, and the corrections in pressure, although very minute, were performed as previously described (22).

All data were analyzed by use of ANADAT data analysis software (RHT-InfoData). The experiments did not last more than 60 min.

**Determination of Lung and Diaphragm Weight, and FRC**

A laparotomy was done immediately after the determination of respiratory mechanics, and heparin (1,000 IU) was intravenously injected. The trachea was clamped 10 min later at end expiration, and the abdominal aorta and vena cava were sectioned, yielding a massive hemorrhage that quickly killed the animals. The chest wall was opened, and the lungs were removed en bloc and weighed. Both hemidiaphragms were also carefully removed and weighed. FRC was determined as previously described (49). Briefly, a jar containing sufficient saline with a surplus weight submerged was placed on a common laboratory scale, which was subsequently adjusted to zero.

The lungs were fixed to a laboratory stand by means of a thread with the surplus weight and completely submerged in the saline. The liquid displaced by the submerged lungs adds correspondingly to the weight on the scale. Because the specific gravity of saline differs no more than 2--3% from 1 g/cm³, the volume of the organ may be expressed directly by the weight gain registered on the scale (49). The ratio of dry to wet lung weights was determined by weighing the lungs before and after drying in an oven.

**Lung Histology and Morphometry**

Morphometric analysis was performed in excised lungs at end expiration. Immediately after the removal of the lungs, the right lung was quick-frozen by immersion in liquid nitrogen and fixed with Carnoy’s solution (ethanol-chloroform-acetic acid, 70:20:10 by volume) at \(-70^\circ\)C for 24 h. Progressively increasing concentrations of ethanol at \(-20^\circ\)C were then substituted for Carnoy’s solution until 100% ethanol was reached. The tissue was maintained at \(-20^\circ\)C for 4 h, warmed to \(4^\circ\)C for 12 h, and then allowed to reach and remain at room temperature for 2 h (37). After fixation, the tissue was embedded in paraffin. Blocks were cut 4 \(\mu\)m thick by a microtome. The slices were stained with hematoxylin-eosin. Two investigators who were unaware of the origin of the material performed the microscopic examination. Morphometric analysis was done with an integrating eyepiece with a coherent system made of a 100-point grid consisting of 50 lines of known length, coupled to a conventional light microscope (Axioplan, Zeiss, Oberkochen, Germany). The volume fraction of collapsed and normal pulmonary areas and the fraction of the lung occupied by large-volume gas-exchanging air spaces (hyperinflated structures with a morphology distinct from that of alveoli and wider than 120 \(\mu\)m) were determined by the point-counting technique (52), made at a magnification of \(\times20\) across 10 random, noncoincident microscopic fields. Briefly, points falling on collapsed or normal pulmonary areas or hyperinflated alveoli were counted and divided by the total number of points in each microscopic field.

Lung tissue distortion was assessed by measuring the mean linear intercept between alveolar walls (\(L_{\text{m}}\)) at a magnification of \(\times200.\) \(L_{\text{m}}\) was determined by counting the number of intercepts between the eyepiece lines and the alveolar septa of each microscopic field.

was expressed as the relation between the line length (1,250 \(\mu\)m) and the total number of intercepts (\(L_{\text{m}} = \Sigma\) line length/number of intercepts).

The amount of polymorpho- and mononuclear cells and pulmonary tissue were determined in each sample by the point-counting technique (52) across 10 random noncoincident microscopic fields at \(\times 1,000\) magnification. Points falling on tissue area and not over air spaces were counted and divided by the total number of points in each microscopic field. Thus data were reported as the fractional area of pulmonary tissue (52).

The slices also underwent a specific staining method (Weigert’s resorcin fuchsin modified with oxidation) to characterize the elastic fibers present in the alveolar septa (53). In each rat 20 different microscopic fields were randomly picked to quantify the amount of elastic fibers by a digital image analysis system and specific software (Bioscan-Optimas 5.1, Bioscan, Edmond, WA) under \(\times200\) magnification. The images were generated by a microscope (Axioplan, Zeiss) connected to a camera (Sony Trinitron CCD, Sony, Tokyo, Japan) and fed into a computer through a frame grabber (Oculus TCX, Coreco, St. Laurent, PQ, Canada) for offline processing. The threshold for fibers of the elastic system was established after the contrast was enhanced until the fibers were easily identified as black bands. The area occupied by fibers was determined by digital densitometric recognition. Bronchi and blood vessels were carefully avoided during the measurements. The area occupied by the elastic fibers in each alveolar septum was divided by the length of that septum to eliminate any bias due to septal edema or alveolar collapse. The results were expressed as the amount of elastic fibers per unit of septal length.

**Transmission Electron Microscopy**

Three slices of \(2 \times 2\) mm were cut from three different segments of the left lung, diaphragm, and intercostals to obtain a stratified random sample. They were fixed with glutaraldehyde 2.5% and phosphate buffer 0.1 M (\(\text{pH} = 7.4\)) for 60 min at \(-4^\circ\)C, rinsed in phosphate buffer, postfixed with 1% osmic tetroxide in the phosphate buffer for 30 min, and rewarshed three times in the phosphate buffer. Finally, the slices were dehydrated in an acetone series and then placed in a mixture of 1:1 acetone:Epon overnight before being embedded in Epon for 6 h. After fixation the material was kept for 48 h at 60°C before undergoing ultramicrotomy for transmission electron microscopy.

**BALF**

Eight other animals (four rats in each group, control and nutritionally deprived) underwent the aforementioned protocol to obtain aliquots of BALF. For this purpose, after \(-4\) wk the rats from both groups were anesthetized [pentobarbital sodium (20 mg/kg ip)], the trachea was cannulated, the lungs were washed eight times with 0.5 ml of sterile saline at 37°C, and the lavage fluid was stored at \(-70^\circ\)C until further processing.

**Lipid extraction and analysis.** One-milliliter aliquots of BALF were used for total lipid extraction, which was performed as previously described (18). Briefly, 5.0 ml of the solvent moisture (CHCl3-MeOH-HCl, 20:10:0.075 vol/vol) were added to the BALF in conic glass tubes and vigorously mixed using a Pasteur pipette. After 10 min on ice, 1.0 ml of 0.6 M HCl was added, and an intense mixing with the Pasteur pipette ensued. The tubes were centrifuged at 600 g for 10 min; the resulting lower phase (containing the lipids) was carefully moved into another glass tube, washed twice with 1.0 ml CHCl3-MeOH-0.6 M HCl (3:48:47 vol/vol), and centrifuged at 600 g for 10 min. The upper phase was removed, and the lower phase was dried under \(N_2.\) Total lipid content was determined gravimetrically. Total lipids were solubilized in a final concentration of 10 mg/ml, and 60 \(\mu\)l were used for the phospholipids separation by unidimensional thin-layer chromatography, as previously described (18). Phospholipids were visualized by exposure of the thin-layer chromatography plate to
iodine vapors, and their identity was clarified by comparison with the standard spots.

Protein determination and immunoblotting. The protein content in the BALF was determined by the Folin-phenol method (30). For Western immunoblotting analysis, 1.0 ml of BALF was centrifuged at 7,000 g for 10 min to remove cells and then at 11,000 g for 40 min. The supernatant was removed, and the pellet, containing surfactant protein-A (SP-A), was resuspended in 100 μl of running buffer. Samples were loaded on the gel, subjected to electrophoresis, and electrophotographed on Nytran. The blot was blocked in 5% milk in PBS with 0.1% Tween 20 (PBST; Fisher Scientific, Pittsburgh, PA) for 1 h at room temperature. Rabbit anti-SP-A antisera (Anti-SP-A, Santa Cruz Research Antibodies, Santa Cruz, CA) was diluted (1:500) in PBST and applied to the blot for 1 h at room temperature. The blot was washed in PBST, and peroxidase-labeled goat anti-rabbit secondary antibody (Santa Cruz Research Antibodies) diluted (1:5,000) in 5% milk in PBST was applied for 30 min at room temperature. The blot was washed in 5% milk in PBST, then in PBST, and finally in PBS. The location of SP-A (monomer molecular mass 29 kDa) was identified by enhanced chemiluminescence (Amersham Biosciences, Uppsala, Sweden), following kit instructions. The densities of the individual bands were compared by densitometry using Scion Image software (Scion, Frederick, MD).

Statistical Analysis

To compare the results from nutritionally deprived and control groups, first, the normality of the data (Kolmogorov-Smirnov test with Lilliefors’ correction) and the homogeneity of variances (Levene test) were tested. Both conditions were satisfied and Student’s t-test was used. All data are reported as means ± SE. The relationships between mechanical parameters and lung morphometry were evaluated by Spearman correlation coefficient. The significance level was always set at 5%. Statistical analysis was done with Sigma Stat 2.0 (Jandel Scientific, San Rafael, CA).

RESULTS

The survival rate in the nutritionally deprived group was 100%, and no rat presented any degree of respiratory distress.

Body, Lung, Diaphragm Weights, and FRC

The initial body weights of the nutritionally deprived and control groups were similar (Table 1). During the ~4-wk period of nutritional deprivation, the body weight of nutritionally deprived rats was reduced to ~60% of its initial weight (Table 1). During this period, the body weight of the control group increased 46% of its initial weight. The diaphragm weight was significantly reduced in the nutritionally deprived rats compared with the control group (Table 1). Wet and dry lung weights were significantly lower in the nutritionally deprived group. Lung weight was reduced to a lesser extent than body weight. The ratio of dry to wet lung weights and FRC were similar in both groups (Table 1).

Decay of Inspiratory Muscle Pressure DuringExpiration

The average values (±SE) of PmusI,0, T50, T25, Tz, Tz/T25, and Tz/T E are listed in Table 2. PmusI,0 was similar in both groups. T50, T25, Tz, and Tz/T E were higher in the nutritionally deprived group than in control group. The shape of inspiratory muscle pressure decay during expiration did not change in nutritionally deprived group, as indicated by the unaltered Tz/T25 index.

Breathing Pattern

Table 3 lists the average values (±SE) of the ventilatory variables in the two groups. Vt, V E, and Vt/Ti were lower in nutritionally deprived rats than in the control group. Ti was longer in nutritionally deprived rats.

Shape of Ptr Wave

Average value of a was significantly lower in nutritionally deprived (15.69 ± 1.21 cmH2O/s) than in control group (20.75 ± 0.85 cmH2O/s), suggesting reduced neuromuscular inspiratory drive or muscle weakness in nutritionally deprived rats. The average values of b were similar in the two groups (0.53 ± 0.05 in control and 0.54 ± 0.04 in nutritionally deprived). The correlation coefficients (r) ranged between 0.91

Table 1. Body, lung, diaphragm, dry-to-wet lung weights, and functional residual capacity in control and nutritionally deprived rats

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ctrl</th>
<th>ND</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight, g</td>
<td>184±4</td>
<td>183±4</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>268±6*</td>
<td>114±3*</td>
</tr>
<tr>
<td>Lung weight, g</td>
<td>1.52±0.05</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>Lung-weight-to-body weight ratio, %</td>
<td>0.62±0.02</td>
<td>0.98±0.05*</td>
</tr>
<tr>
<td>Diaphragm weight, g</td>
<td>0.75±0.05</td>
<td>0.42±0.02*</td>
</tr>
<tr>
<td>Functional residual capacity, ml</td>
<td>1.09±0.09</td>
<td>1.28±0.05*</td>
</tr>
<tr>
<td>Dry/wet weight, %</td>
<td>20.11±0.97</td>
<td>20.74±1.94</td>
</tr>
</tbody>
</table>

Values are means ± SE of 8 rats. In the control (Ctrl) group, the animals received food ad libitum. In the nutritionally deprived (ND) group, rats received one-third of their usual daily food consumption until they lost 40% of their initial body weights. *Significantly different from Ctrl group (P < 0.05).

Table 2. Parameters of inspiratory muscle pressure during expiration in control and nutritionally deprived rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ctrl</th>
<th>ND</th>
</tr>
</thead>
<tbody>
<tr>
<td>PmusI,0, cmH2O</td>
<td>4.91±0.34</td>
<td>3.91±0.39</td>
</tr>
<tr>
<td>T50, s</td>
<td>0.04±0.01</td>
<td>0.07±0.01*</td>
</tr>
<tr>
<td>T25, s</td>
<td>0.06±0.01</td>
<td>0.11±0.01*</td>
</tr>
<tr>
<td>Tz, s</td>
<td>0.15±0.01</td>
<td>0.28±0.03*</td>
</tr>
<tr>
<td>Te, s</td>
<td>0.41±0.03</td>
<td>0.50±0.02*</td>
</tr>
<tr>
<td>T50/T25</td>
<td>50.71±5.16</td>
<td>68.62±3.42</td>
</tr>
<tr>
<td>Tz/T E</td>
<td>14.01±1.45</td>
<td>35.84±1.86*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 8 rats (6–10 determinations in each animal). PmusI,0, inspiratory muscle pressure at start of expiration; T50, T25, Tz, times required for inspiratory muscle pressure to decay to 50, 25, and 0% of PmusI,0, respectively; Ti, inspiratory duration; *Significantly different from Ctrl group (P < 0.05).

Table 3. Breathing pattern parameters in control and nutritionally deprived rats

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ctrl</th>
<th>ND</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vt, ml</td>
<td>1.60±0.06</td>
<td>0.98±0.05*</td>
</tr>
<tr>
<td>Ti, s</td>
<td>0.33±0.01</td>
<td>0.40±0.02*</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>78.72±4.20</td>
<td>68.28±3.24</td>
</tr>
<tr>
<td>VE, ml/s</td>
<td>256.53±7.25</td>
<td>65.92±3.94*</td>
</tr>
<tr>
<td>Vt/Ti</td>
<td>0.78±0.04</td>
<td>0.90±0.04</td>
</tr>
<tr>
<td>Vt/Ti, ml/s</td>
<td>4.93±0.22</td>
<td>2.48±0.18*</td>
</tr>
<tr>
<td>Ti/Ts, s</td>
<td>0.43±0.01</td>
<td>0.45±0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE of 8 rats (6–10 determinations in each animal). Vt, tidal volume; Ti, inspiratory duration; f, respiratory frequency; VE, minute ventilation; Vt/Ti, duty cycle; Vt/Ts, mean inspiratory flow. *Significantly different from Ctrl group (P < 0.05).
and 0.94 in control and 0.90 and 0.96 in nutritionally deprived rats.

Respiratory Mechanics

The mean constant inspiratory flows and volumes did not present significant differences between the two groups (Table 4). Est, L, cmH2O

Histology

Photomicrographs of lung parenchyma from the control and nutritionally deprived animals are shown in Fig. 1. Lung histological changes included hyperinflated alveoli with patchy atelectasis, interstitial edema, and inflammation with increased amount of polymorphonuclear cells in the nutritionally deprived group (Table 5). Some areas of the lung were emphysematous (increased size of air spaces distal to the terminal bronchiole with thinning and partial destruction of septal wall). The Lm was similar in both groups.

The elastic fiber content was reduced in nutritionally deprived rats (0.24 ± 0.04 μm²/μm) compared with control animals (0.39 ± 0.02 μm²/μm) (Fig. 1).

Figure 2 shows the ultramicroscopy of lung parenchyma and diaphragm. Type II pneumocytes of the nutritionally deprived group presented a reduction in lamellar bodies, multilamellated structures, membrane vesicles, and granular debris. Lipoproteinaceous material accumulating in air space macrophages probably represents an abnormal accumulation of the normal constituents of surfactant. Structurally aberrant mitochondria were seen in type II pneumocytes and alveolar macrophages (Fig. 2). Diaphragm and intercostals showed atrophy; disarrangement of the myofibrils causing discontinuity in sarcoplas-
mic reticulum, in the t-tubular system, and consequently in sarcomere; and deposition of collagen type I fibers. In addition, prominent features suggestive of muscle injury were found in mitochondria. The mitochondria underwent enlargement, vesiculation of cristae, formation of intracristal plates, and deposition of granular osmiophilic material in the matrix compartment. Structurally aberrant mitochondria were usually conspicuous among the subsarcolemmal mitochondria and often associated with an overall increase in number. The abnormal mitochondria were irregular with deeply invaginated sarcoplasm and contained aberrant laminar cristae (Fig. 2).

The correlations between mechanical and morphological data considering the control and the nutritionally deprived groups are depicted in Table 6. Est,t and viscoelastic pressure normalized by FRC were correlated with the fraction of area of alveolar collapse and hyperinflation.

**Analysis of BALF**

Nutritionally deprived animals had significantly less lipid and protein in the BALF than control ones as shown in Table 7. The relative amount of SP-A was also decreased in lung tissue from nutritionally deprived rats compared with control group (54%).

**DISCUSSION**

In the present study, both lung and chest wall elastic, resistive, and viscoelastic/inhomogeneous pressures effectively normalized by FRC were correlated with the fraction of area of alveolar collapse and hyperinflation.

![Fig. 2. Electron microscopy of lung parenchyma (A and B) and diaphragm (C and D) in control (A and C) and nutritionally deprived (B and D) animals. M, mitochondria; LB, lamellar bodies; MB, myofibrils; MC, mitochondrial cristae; CF, collagen fiber. Scale bars = 150 nm.](image-url)
contributed to the mechanical changes found in nutritionally deprived animals. Lung mechanical changes were accompanied by a reduction in elastic fiber content and a dysfunction in surfactant production. Ultrastructural modifications of alveoli and intercostals were observed in nutritionally deprived animals leading to chest wall mechanical modifications. Undernutrition also prolonged the activity of inspiratory muscles during expiration, decreased the neuromuscular inspiratory drive, reduced the VT and VE, and increased inspiratory and expiratory times.

In the present study we analyzed the effects of a balanced food restriction (protein calorie diet plus proportional decrement in micronutrients). This kind of malnourishment is rampant in developing countries. Undernutrition lasted 4 wk, which allowed a gradual reduction in body weight with no death. This model of undernutrition has been previously used in other studies to evaluate lung (44) and respiratory muscle function (27). Undernutrition could increase the probability of infection. To avoid this problem, the animals were housed in microisolator cages (filter tops) in a specific room housing rats in Rio de Janeiro, 200 meters from the ocean, where the atmosphere is practically nonpolluted. The use of microisolator cages inhibited a possible spread of infectious agents from one animal cage to another, as well as from investigators to animals and vice versa. All animals were uniformly handled, received water ad libitum, and underwent a 12:12-h light-dark cycle. Thus infection was very unlikely under these conditions. Furthermore, we found no evidence of infection by either light or electron microscopy.

In nutritionally deprived rats, loss of body weight was more intense than lung weight loss, resulting in an increased lung-to-body weight ratio (Table 1). These changes were consistent with previous studies (13, 41) suggesting a lung-protective effect against malnourishment (54). Furthermore, the water content of lung was not altered in nutritionally deprived group, because the wet-to-dry lung weight ratio was similar in both groups. FRC was also similar in the two groups (Table 1), despite the increment in the fraction of collapsed areas in the nutritionally deprived group. This could be attributed to the proportional increment in the amount of hyperinflated alveoli, yielding similar values of mean linear intercept between alveolar walls in nutritionally deprived and control groups. The reduction in diaphragm weight was proportional to the loss of body weight. These data are in accordance with previous reports (10, 27) that demonstrate a proportionate reduction in body and diaphragm weights with similar ratio of diaphragm weight to body weight in nutritionally deprived and control rats.

Undernutrition induces respiratory muscle weakness, particularly in the diaphragm, with a decrease in strength (4). In this line, the activity of the inspiratory muscles was analyzed during inspiration and expiration. The pressure developed at the mouth during complete airway occlusion at functional residual capacity has been extensively used as an index of the neuromuscular output in animals (1) and humans (2). It assesses both the neuronal drive to the respiratory muscles and the effectiveness of inspiratory muscle contraction in producing pressure without being affected by resistance and compliance of the respiratory system. In the present study, undernutrition led to low a values. The reduction in a could be attributed to respiratory muscle dysfunction, contributions related to neural conduction or metabolic rate reduction (39). There are factors that could induce modifications in the shape of the driving pressure wave in nutritionally deprived animals such as damage in muscle fibers, but b values were similar in both groups.

The activity of the inspiratory muscles during expiration opposes rapid emptying of the lungs and has not been previously analyzed in malnourished animals. T30, T25, T2, Tz/Tf, and Tz were higher in nutritionally deprived group compared with control group (Table 2), suggesting a prolongation of the time required for inspiratory muscle relaxation during expiration. The relaxation of respiratory muscles is very important in the regulation of breathing. The diaphragm must return to its optimal muscle length between each inspiration, and adequate diaphragmatic perfusion depends in part on rapid and efficient muscle relaxation, at least during loaded breathing (12). In this context, the mechanisms underlying the delay of inspiratory muscle relaxation in nutritionally deprived animals could be attributed to an increase in the relative contribution of fatigue resistant (type I) fibers to total diaphragma area (27), a selective atrophy of fatigue-sensitive (type II) fibers (3), and alterations in Ca2+ release and sequestration (29). VT, VE, and mean inspiratory flow were lower in nutritionally deprived animals. These data are consistent with previous descriptions (17, 48). The rats of nutritionally deprived group showed higher Ti than control group. The increase in Ti could be a compensatory mechanism determined by changes in respiratory system mechanics (32).

Respiratory system, lungs, and chest wall mechanics have not been previously evaluated in nutritionally deprived animals. The end-inflation occlusion method used for the determination of respiratory mechanics allows the identification of elastic, resistive, and viscoelastic and/or inhomogeneous components. Estt, chest wall elastance, and resistive and viscoelastic/inhomogeneous components. Estt, chest wall elastance, and resistive and viscoelastic/inhomogeneous pressures were higher in nutritionally deprived rats compared with control group (Table 4). However, comparisons of mechanical parameters between different-size

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ctrl</th>
<th>ND</th>
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<tr>
<td>Lipid, μg/ml</td>
<td>1.45±0.09</td>
<td>0.78±0.08*</td>
</tr>
<tr>
<td>Protein, μg/ml</td>
<td>1.96±0.09</td>
<td>0.27±0.08*</td>
</tr>
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Values are means ± SE of 4 animals in the Ctrl and ND groups. *Significantly different from Ctrl group (P < 0.05).
animals often require normalizing procedures (33). Body weight (24, 42, 48), lung weight (15, 42), or lung volumes (17, 25, 43) are among the variables used for normalization. In the present study, EstL values and resistive and viscoelastic/inhomogeneous pressure normalized by FRC were higher in nutritionally deprived animals compared with control ones and were well correlated with the morphometric data (Table 6). Body weight is probably not an adequate variable to normalize lung mechanics in the presence of undernutrition because the loss of body weight is more intense than the loss of lung weight. At any rate, after normalization by lung weight, EstL, and resistive and viscoelastic pressures were significantly lower in nutritionally deprived than in control group and were not well correlated with morphometric data (Table 6).

Prior studies described connective tissue dysfunction in undernourished animals (17, 21, 45, 46). In this context, we observed a significant reduction in the amount of elastic fibers in the alveolar septa and areas of emphysema with elastin gaps in nutritionally deprived animals (Fig. 1). Indeed, these morphological changes could yield a decrease in lung elastance. In this line, Sahebjami and Domino (43) observed decreased lung elastance using a similar model of undernutrition. However, in the present study ultrastructural analysis of lung parenchyma also showed pneumocyte type II changes with reduction in the number of lamellar bodies and aberrant mitochondria (Fig. 2). In contrast, Lin and Lechner (28) reported that the number of type II cells and their content of lamellar bodies were not affected by prenatal starvation. These differences could be ascribed to the moment of dietary restriction, i.e., prenatal vs. adult animals. In our study, the amount of lipid and protein in the surfactant decreased significantly in the nutritionally deprived group (Table 7), probably increasing surface tension, leading to atelectasis and increasing EstL. The discrepancies between our study and Sahebjami and Domino’s (40) could result from 1) comparison of isolated with intact preparations, 2) variability in lung volume, and 3) the diversity of methods used for determining lung compliance. Lung resistive pressure reflects mainly the pressure required to overcome airway resistance. The destruction of elastin fibers in the lung parenchyma decreases elastic recoil. Airflow impairment could result from loss of the normal tethering effect of the pulmonary parenchyma on the airways. Thus the increase in ΔP1 could be attributed to connective tissue dysfunction. The increase in ΔP2,t suggests heterogeneities probably due to collapse of damaged alveoli and overdistention, distortion of patent alveoli, interstitial edema, and inflammation as shown in Fig. 1. Baybutt and colleagues (7) observed that vitamin A deficiency induced emphysema and interstitial pneumonitis. However, an insufficient vitamin A supply on the postnatal development of the lung also leads to focal loss of ciliated cells with keratinizing metaplasia and necrosis of the bronchial mucosa as well as an increase in the amount of secreting cells (9), which were not present in our study. Thus the morphological changes were attributed to protein-caloric restriction rather than vitamin A deficiency. Conversely, we cannot discard that the functional and morphological changes observed in the present model of nutritionally deprived rats is not attributed to other micronutrients deficiencies.

The evaluation of BALF showed reduction in lipid and protein content (Table 7), as well as in the amount of SP-A. SP-A has a role in the structural organization of surfactant, because it is required to maintain the structure and stability of surfactant aggregates and in myelin tubular formation and is also involved in the regulation of surfactant homeostasis (16). Thet and Alvarez (51) showed that the amount of disaturated phosphatidylcholine obtained by lavage decreased by about 20% after 3 days of starvation in adult rats, but there was no difference in elastic recoil between the lungs of control and starved rats. Sakai and colleagues (47) evaluated the content of SP-A in the BALF of rats starved for 2, 4, and 6 days and showed a higher content of SP-A in animals starved for 2 days, but the content of SP-A returned to baseline values after 4 days of starvation. The different dietary restriction could explain the discrepancies with our results. It is possible that the same alteration occurs with other surfactant proteins in the presence of undernutrition, modifying surfactant structure and function. All chest wall mechanical parameters were higher in nutritionally deprived animals (Table 4). These changes were associated with severe ultrastructural alterations in respiratory muscles such as atrophy, disarrangement of the myofibrils, and aberrant mitochondria (Fig. 2). Previous studies show that long-term undernutrition leads to a decrease in fatigue-sensitive fibers (type IIb) and a relative increase in fatigue-resistant fibers (type I, type IIa) of the diaphragm (3, 23, 31, 38). This shift in the type of myosin heavy chain and the decrease in muscle mass are responsible for a decrease in total muscle force (3). Matecki and colleagues (31) showed in vitro that prolonged undernutrition (after ~5 wk) decreases mitochondrial oxygen consumption and that could be involved in the negative effects of undernutrition on respiratory muscle function. Metabolic and structural alterations of respiratory muscle in the presence of undernutrition could be related to the reduction in muscle strength and to the increased chest wall resistive pressure observed in our study. By the same token, chest wall conformation could have been modified, as well as the pattern of change during movement, causing an increase in the mechanical inhomogeneity expressed by the higher ΔP2,w and ΔE,w (Table 4).

In conclusion, undernutrition diminished diaphragmatic muscle mass and respiratory muscle strength that led to reduced muscular output, prolonged the decay of inspiratory muscle pressure during expiration, and increased chest wall resistive and elastic mechanical components. Additionally, surfactant synthesis and extracellular matrix were impaired, increasing EstL and viscoelastic pressure. Thus undernutrition adversely affects lung parenchyma, muscle structure and function, and respiratory mechanics.

ACKNOWLEDGMENTS

We express our gratitude to Antonio Carlos de Souza Quaresma and Alaine Prudente for skillful technical assistance.

GRANTS

This work was supported by The Centers of Excellence Program (PRONEX-MCT and MCT/FAPERJ), The Brazilian Council for Scientific and Technological Development (MCT/CNPq), The Carlos Chagas Filho Rio de Janeiro State Research Supporting Foundation (FAPERJ), The São Paulo State Research Support Foundation (FAPESP), and The Howard Hughes Medical Institute (departmental sharing of Grant no. 55003669).

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J Appl Physiol • VOL 97 • NOVEMBER 2004 • www.jap.org


