Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 1105–1107, February 1995
Physiology

Sexual dimorphism in the architecture of the lung's gas-exchange region

GLORIA D. MASSARO*, JACOPO P. MORTOLA†, AND DONALD MASSARO*

*Lung Biology Laboratory, Georgetown University School of Medicine, Washington, DC 20007; and †Department of Physiology, McGill University, Montreal, Canada H3G 1Y6

Communicated by Robert E. Forster II, University of Pennsylvania, Philadelphia, PA (received for review March 17, 1994)

ABSTRACT **The lung's only vital function is to provide sufficient gas-exchange surface area (Sa) to meet the organism's needs for oxygen uptake ($\dot{V}O_2$) and carbon dioxide elimination. A direct linear relation between Sa and $\dot{V}O_2$ and an inverse linear relation between the size of the lung's gas-exchange units and the species mass-specific $\dot{V}O_2$ are strongly conserved across species. Within species, Sa increases in response to prolonged (weeks) elevation of $\dot{V}O_2$. We now report sex-dependent deviations from these relationships that seem to anticipate the need for increased gas-exchange capacity engendered in females by the metabolic demands of pregnancy and lactation. We found that although $\dot{V}O_2$ almost doubled in rats during pregnancy and lactation, Sa was the same in age-matched virgin, pregnant, and lactating females. However, at the onset of sexual maturity, virgin female rats and mice had higher mass-specific Sa than males of the same species although mass-specific $\dot{V}O_2$ was identical, within species, in both sexes. In addition, even though mass-specific $\dot{V}O_2$ was identical in males and females, alveoli were 30% and 50% smaller in female rats and mice, respectively, than males of the same species. We suggest the greater mass-specific Sa and smaller alveoli in females in spite of identical mass-specific $\dot{V}O_2$ as males were selected for evolutionarily; they help females meet the metabolic demands of reproduction without adding to the energy demands of these periods a requirement to form additional lung.**

The lung's only known vital function is to provide sufficient gas-exchange surface to meet the organism's needs for oxygen uptake and carbon dioxide elimination. The importance of the relationship between oxygen consumption ($\dot{V}O_2$) and the size of the lung's gas-exchange surface area (Sa) can be appreciated from the strong interspecific conservation of a direct linear relation between $\dot{V}O_2$ and Sa (1, 2). The size of individual gas-exchange units is inversely proportional to the species mass-specific $\dot{V}O_2$; this allows species with a high mass-specific $\dot{V}O_2$ to achieve sufficient Sa to meet the intensity of their metabolic needs without having a lung volume disproportionately large compared to its body mass (1). Interestingly, although the lung has sufficient Sa to sustain a short-term 10-fold increase in $\dot{V}O_2$, as might occur with exercise (3), it responds to smaller but more prolonged (weeks to months) elevations of $\dot{V}O_2$ by increasing its gas-exchange capacity as reflected in its Sa (4–7).

Pregnancy and lactation constitute long periods during which $\dot{V}O_2$ is moderately elevated (8). Because these periods are recurrent, and rapidly so in many species, the high metabolic rate associated with reproduction may represent the "steady-state" condition during much of a female's reproductive life. The intestine adapts to reproduction-associated increased energy needs with elevations of mass and absorptive surface capacity that regress when lactation ends (9). This increased capacity occurs even though the intestine's absorptive capacity in nonpregnant females is at least 2.5-fold greater than is required for normal food intake in the nonpregnant state (9).

We now show that the lung of rats and mice exhibits a different strategy than the intestine, which allows it to maintain, at least in part, its excess capacity over need during the metabolic challenge of reproduction. The lung's gas-exchange Sa does not increase during pregnancy or lactation. Rather, at the onset of sexual maturity in rats and mice, mass-specific Sa is greater in virgin females than in males even though mass-specific $\dot{V}O_2$ is identical in males and nonpregnant females; this difference in gas-exchange Sa is achieved in part by females having smaller alveoli than males.

MATERIALS AND METHODS

Animals. We purchased specific pathogen-free Sprague–Dawley rats from Taconic Farms and CD-1 mice from Charles River Breeding Laboratories. They were maintained on a 12-h light/12-h dark cycle and allowed food and water ad libitum until studied.

Fixation, Tissue Sampling, and Tissue Preparation. Animals were anesthetized with pentobarbital sodium (≈90 $mg \cdot kg^{-1}$, i.p.) and were killed by infusing cold 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) into the trachea at a transpulmonary pressure of 20 cm H_2O. The trachea was ligated, the lungs were removed from the thorax, and fixation was continued for 2 h at 0–4°C. Lung volume was measured by volume displacement. The lung was cut into blocks, and blocks were selected for study by using a systematic sampling technique (10).

Serial sections of lung were cut at ≈0.8-μm thickness. We sectioned three to five blocks per animal, and each group of serial sections was cut to a depth of 150–250 μm. To distinguish between alveoli and alveolar ducts, gas-exchange structures were followed visually through a complete set of prints of serially sectioned lung tissue (11). As previously described (11), the selector method was used to choose alveoli for analysis (12). This method allows the selection of structures based on number rather than size, shape, or orientation. The volume of an alveolus was estimated by the point-sampled intercepts method (11, 13). The volume of an individual alveolus was calculated using the expression

$$v_{est} = \frac{\pi}{3} \overline{l_0^3},$$

where v_{est} is the individual estimated volume and $\overline{l_0^3}$ is the average cubed segment. Mean alveolar volume ($\bar{v}$) was obtained from the arithmetic mean of the individual estimated volumes. The number of alveoli per lung was calculated using the identity $N = V_L \times V_{va}/\bar{v}$, where V_L is lung volume

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Abbreviations: Sa, gas-exchange surface area; $\dot{V}O_2$, oxygen consumption.

Table 1. Body mass and mass-specific Sa in rats

Age, days	Mass-specific Sa, $cm^2 \cdot g^{-1}$		P for Sa	Body mass, g		P for body mass
	Female	Male		Female	Male	
21	21.6 ± 1.0 (3)	23.1 ± 0.7 (3)	NS	45 ± 1 (3)	46 ± 2 (3)	NS
33	15.4 ± 0.6 (3)	15.2 ± 0.4 (3)	NS	108 ± 6 (3)	133 ± 6 (3)	<0.05
45	12.9 ± 0.5 (3)	12.1 ± 1.0 (3)	NS	173 ± 5 (3)	214 ± 11 (3)	<0.025
60	13.4 ± 0.4 (6)	10.9 ± 0.3 (6)	<0.001	212 ± 6 (6)	313 ± 7 (6)	<0.001
95	13.4 ± 0.8 (5)	9.4 ± 0.5 (5)	<0.005	258 ± 3 (5)	392 ± 5 (5)	<0.001

Mean ± SE are given. Numbers in parentheses indicate the number of rats. NS, $P > 0.05$.

(measured by water displacement), V_{va} is the volume density of alveolar air, and $\bar{v}$ is the number-weighted mean alveolar volume. The Sa of the lung's gas-exchange region was determined using point and intersection counting (14, 15).

Oxygen Consumption. $\dot{V}O_2$ was measured by the flow-through method (16), using a chamber composed of an inner transparent Plexiglas container placed inside an outer chamber that served as a water bath to control the temperature of the inner chamber. Air was continuously passed through the chamber at a steady flow of 500–1350 $ml \cdot min^{-1}$ (standard temperature, pressure, dry), depending on the size of the animal, and was controlled by the use of a calibrated flow meter.

RESULTS AND DISCUSSION

We found that at about the onset of sexual maturity (age 60 days), but not before, mass-specific Sa was 23% higher in virgin female rats than in age-matched males (Table 1), although mass-specific $\dot{V}O_2$ was identical in both sexes (Table 2). Female rats achieved a mass-specific Sa that was higher than males by having a larger mass-specific lung volume and, in absolute terms, smaller gas-exchange units (alveoli) than males (Table 2). Females also had 43% more alveoli per kg than male rats (Table 2).

Because mass-specific Sa was higher in females than males at the onset of sexual maturity, we asked if there was an additional pregnancy- or lactation-specific rise in Sa to further accommodate the higher $\dot{V}O_2$ of those conditions. To establish the magnitude of $\dot{V}O_2$ increase and to determine if there was an associated increase in Sa, we measured $\dot{V}O_2$2 and Sa in age-matched virgin, pregnant, and lactating females. Seventy-nine-day-old virgin females ($n = 5$) had a $\dot{V}O_2$ of 4.4 ± 0.2 $ml \cdot min^{-1}$ and mass-specific $\dot{V}O_2$ of 20.2 ± 0.8 $ml \cdot kg^{-1} \cdot min^{-1}$; the values of identical aged 22-day pregnant rats ($n = 5$) were, respectively, 8.6 ± 0.4 and 26.8 ± 1.3. Hence, absolute $\dot{V}O_2$ was 2-fold greater in pregnant than identical aged virgin females. By contrast, the Sa was 3175 ± 206 cm^2 ($n = 3$) in 79-day-old virgin females and 3166 ± 273 cm^2 ($n = 3$) in identical age, 22-day pregnant females. Therefore, a 2-fold rise in metabolic rate was not accompanied by an increase in Sa. Ninety-two-day-old virgin females ($n = 5$) had a $\dot{V}O_2$ of 4.9 ± 0.2 $ml \cdot min^{-1}$ and mass-specific $\dot{V}O_2$ of 20.0 ± 1.0 $ml \cdot kg^{-1} \cdot min^{-1}$; the values for identical aged females ($n = 5$) on the 14th day of lactation (10 pups per litter) were, respectively, 8.4 ± 0.4 and 28.9 ± 1.6. Sa was 3946 cm^2 ($n = 2$) in 92-day-old virgin females and 3396 cm^2 ($n = 2$) in 92-day-old females on the 14th day of lactation (10 pups per litter), indicating that a 1.6-fold higher absolute $\dot{V}O_2$ in lactating dams did not result in a higher Sa.

In contrast to the failure of the pregnancy- and lactation-associated increase in $\dot{V}O_2$ to induce a rise in Sa, other causes of a sustained increase of $\dot{V}O_2$ do result in an elevation of Sa. For example, the administration of thyroid hormone to rats for 8 weeks doubled their $\dot{V}O_2$ and increased Sa 2.7-fold (4). A

Table 2. Body weight, Sa, lung volume, alveolar volume and number, and $\dot{V}O_2$ in 60-day-old female and male rats

Parameter	Female	Male	P
Body weight, g	212 ± 6 (6)	313 ± 7.0 (6)	<0.0001
Lung volume, ml	7.0 ± 0.2 (6)	8.8 ± 0.4 (6)	<0.005
Mass-specific lung volume, $ml \cdot kg^{-1}$	32.9 ± 1.0 (6)	28.1 ± 1.0 (6)	<0.005
Sa, cm^2	2847 ± 123 (6)	3410 ± 97 (6)	<0.005
Mass-specific Sa, $cm^2 \cdot g^{-1}$	13.4 ± 0.4 (6)	10.9 ± 0.3 (6)	<0.001
$\bar{v}$, $\mu m^3 \times 10^{-4}$	7.98 ± 0.5 (6)	10.57 ± 0.4 (6)	<0.025
$N \times 10^{-6}$	36.1 ± 2.2 (6)	41.3 ± 0.3 (6)	NS
$N \times 10^{-6} \cdot kg^{-1}$	180 ± 17 (6)	126 ± 3 (6)	<0.05
$\dot{V}O_2$, $ml \cdot kg^{-1} \cdot min^{-1}$	20.5 ± 0.7 (15)	20.0 ± 0.4 (15)	NS

Mean ± SE are given. Numbers in parentheses indicate the number of rats. NS, $P > 0.05$; $\bar{v}$, average volume of an alveolus; N, the number of alveoli per lung.

Table 3. Sa, alveolar volume and number, and $\dot{V}O_2$ in 60-day-old female and male mice

Parameter	Female	Male	P
Body weight, g	28.5 ± 0.6 (3)	33.8 ± 1.4 (4)	<0.025
Lung volume, ml	0.8 ± 0.1 (3)	1.0 ± 0.0 (4)	<0.025
Mass-specific lung volume, $ml \cdot kg^{-1}$	28.8 ± 2.4 (3)	29.4 ± 1.4 (4)	NS
Sa, cm^2	496 ± 30 (3)	464 ± 9 (4)	NS
Mass-specific Sa, $cm^2 \cdot g^{-1}$	17.6 ± 1.5 (3)	13.8 ± 0.6 (4)	<0.05
$\bar{v}$, $\mu m^3 \times 10^{-4}$	2.41 ± 0.10 (3)	4.86 ± 0.1 (3)	<0.001
$N \times 10^{-6}$	14.8 ± 0.99 (3)	10.0 ± 0.63 (3)	<0.01
$N \times 10^{-6} \cdot kg^{-1}$	528 ± 34 (3)	291 ± 21 (3)	<0.005
$\dot{V}O_2$, $ml \cdot kg^{-1} \cdot min^{-1}$	44.1 ± 2.0 (5)	40.1 ± 2.2 (5)	NS

Mean ± SE are given. Numbers in parentheses indicate the number of mice. NS, $P > 0.05$; $\bar{v}$, average volume of an alveolus; N, number of alveoli per lung.

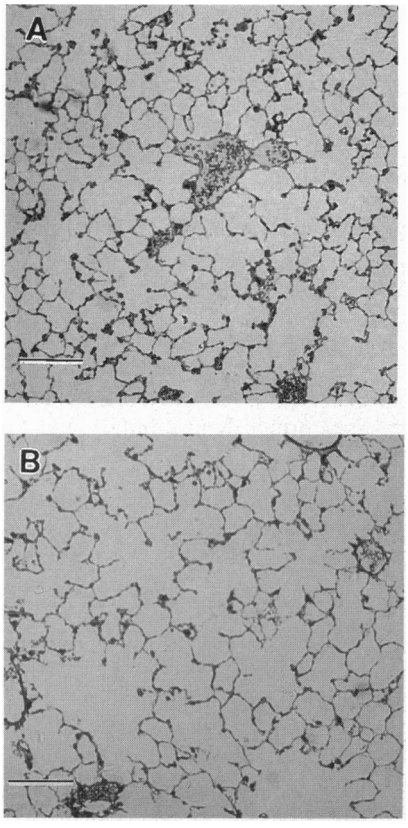

FIG. 1. The terminal gas-exchange units (alveoli) are smaller in lungs of female (*A*) than male (*B*) mice. (Bar = 100 μm.)

26% elevation of $\dot{V}O_2$ produced by administering thyroid hormone to hamsters for 28 days resulted in a 26% rise in Sa (5). Prolonged exposure to low temperature increases $\dot{V}O_2$ and Sa (6).

To determine if the female–male differences in alveolar size and Sa are peculiar to rats, we made similar measurements in 60-day-old virgin mice (Table 3). Females had a mass-specific Sa that was 28% greater than that in males, alveoli that were 50% smaller than those in males, and an absolute number of alveoli that was 48% greater than that in males; mass-specific alveolar number was 80% higher in females than in males, even though $\dot{V}O_2$ was the same in both sexes (Table 3). The intersex difference in alveolar size in mice was so great it could be discerned by microscopical examination of the lung (Fig. 1).

Tenney and Remmers (1), in a seminal report, estimated the dimensions of terminal gas-exchange units. Although they did not use a method that allowed them to distinguish alveolar ducts from alveoli (the actual gas-exchange structure), they presented quite clear evidence that across species the size of terminal gas-exchange units is inversely proportional to the species mass-specific $\dot{V}O_2$. Our data show that in the two species examined, at a time when males and females of the same species have an identical mass-specific $\dot{V}O_2$, females have substantially smaller alveoli than males; in rats females have alveoli that are about 30% smaller than those in males, and in mice females have alveoli that are about 50% smaller than those in males. The fact that the higher weight-specific Sa (23% in female rats and 28% in female mice) does not match the ≈2-fold increase in $\dot{V}O_2$ in pregnant or lactating rats is consistent with the less than complete maintenance of intestinal uptake capacity during increased dietary load (9). However, having smaller alveoli may add to the extra functional capacity of the females' larger mass-specific Sa. This possibility is raised because gas-exchange units are arranged in series along the sequence of alveolar ducts, but alveolar capillaries are perfused in parallel (17). This arrangement may result in the O_2 tension being lower in alveoli at the periphery than in the more central part of the acinar pathway—the degree to which the O_2 tension would be lower in more peripheral alveoli than in more central alveoli would depend on the length of the pathway (17). Thus, having smaller alveoli would diminish the intra-acinous fall in O_2 tension and maintain a higher alveolar-capillary O_2 tension gradient, thereby enhancing O_2 diffusion into the blood.

The absent burden of making additional lung during pregnancy or lactation to meet the metabolic demands of those periods is in accord with the general strategy of spreading out the demands for energy, thereby lessening the magnitude of the needs at a particular time (8).

We thank Ms. Tara Nayak for technical help and Linda Clerch, Ph.D., for reviewing the manuscript. This work was supported by Grants HL37666, HL20366, and HL41610 from the National Institutes of Health, Heart, Lung and Blood Institute.

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