THE EFFECTS OF OXYGEN TOXICITY ON THE PREGNANT RAT USING AN APPARATUS DESIGNED TO MEASURE OXYGEN CONSUMPTION METHOD

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Abstract: To evaluate the effects of oxygen toxicity in the pregnant rat at high and low concentrations of oxygen, an oxygen-consumption apparatus was devised to measure the consumption of oxygen continuously over long periods. Oxygen consumption, partial oxygen pressure on the skin (tcPO₂), and lipid peroxide levels in the serum were measured. There was a close correlation between oxygen consumption and body weight of rats that weighed between 150 g and 450 g. Oxygen consumption during the later stages of pregnancy increased by 1.8 ml/day, an increase of 8%. Oxygen consumption by rats during parturition increased markedly for up to 1 h and then reached and remained at a plateau value until the end of delivery. Exposure of pregnant rats to low concentrations of oxygen resulted in a marked depression in oxygen consumption and tcPO₂ during the exposure time. A significant increase in the lipid peroxide level in serum was observed in the mother rat after birth and in the newborn offspring of pregnant rats exposed to 16% oxygen for 3 h.

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**Key words**: Oxygen toxicity, oxygen consumption, pregnant rat, lipid peroxide.

**INTRODUCTION**

Dioxygen participates in the production of high energy compounds in the process of oxidative phosphorylation. The superoxide anion, which can act as an antimicrobial or anticancer agent, is produced by one-electron reduction (Niki, 1987). Furthermore, the hydroxy radical, which is produced by two-electron reduction of dioxygen, is injurious to biological compounds, such as lipids, proteins, and nucleic acid (Steinberg et al., 1983; Pryor, 1976). Lower than normal concentrations of oxygen in the human body can give rise to ischemia of tissues, as a result of hypoxemia, which can induce peroxidative reactions in lipids and proteins (Asada, 1987; Abe et al., 1984). It is wellknown that levels of lipid peroxidation are indicative of the integrity of tissues, and can be closely connected with various disease states (Uchiyama, 1985).

We previously reported a correlation between lipid peroxidation and oxygen consumption of the chick embryo exposed high or low concentration of oxygen (Yoshimura, 1987). We have now measured oxygen consumption by the pregnant rat using a specially developed apparatus. We have evaluated oxygen toxicity at high and low concentrations of oxygen.

**MATERIALS AND METHODS**

*Experimental animals*:

Wistar rats of 3–10 weeks of age, and 10-week-pregnant rats (from the Saitama Experimental Animal Supply Co., Saitama, Japan) were used for these experiments. All rats were maintained in a barrier-sustained animal room at a temperature of 23–25 °C, a relative humidity of 40–60%, and an illumination cycle of 12 h light and 12 h dark.

*Measurement of oxygen consumption*:

Many methods have been reported for measuring oxygen consumption, e.g. a manometric method with a spirometer, gas analysis by use of a Douglas bag (Ikawa, 1977), and a closed cyclic method (Stock, 1975; Heusner et al., 1971; Nagasaka, 1984). We previously devised an oxygen-consumption measuring apparatus, which can measure small amounts of oxygen consumed during gaseous exchanges of fertile eggs (Ohsawa et al. 1985a). A new oxygen-consumption measuring apparatus was devised to measure the amount of oxygen consumed by the rat, as shown in Figure 1. A rat of weight 50–450 g was placed in the 5.60 l chamber in a room with temperature controlled at 23 °C, and 20 g potassium hydroxide was put into the lower part of the chamber to absorb the carbon dioxide produced by exchange of gases by the rat. When the partial pressure of oxygen in the chamber fell as the result of the
Oxygen toxicity on the pregnant rat

Fig. 1. Apparatus for measuring the consumption of oxygen.
1. oxygen cylinder; 2. electromagnetic valve; 3. chamber; 4. CO₂ absorbent (potassium hydroxide); 5. rat; 6. sensor; 7. platinum electrode (0.5 mm); 8. relay; 9. power supply (12 V); 10. resistance; 11. V/F converter; 12. digital counter; 13. D/A converter; 14. recorder

Consumption of oxygen by the rat, the height of a column of mercury in the sensor was increased and allowed to come in contact with the platinum wire. The relay circuit was closed, and oxygen was supplied through the electromagnetic valve from an oxygen cylinder at a flow rate of 60 ml/min, the time during which oxygen was supplied was integrated by the digital counter. The integration values were continuously recorded on a recorder through a D/A converter. One unit corresponded to 0.90 ml of oxygen at 23 °C.

Measurement of lipid peroxides in the serum:
Lipid peroxides in the serum were measured by a fluorescence detection method with 1, 3-diphenyl-2-thiobarbituric acid (DPTBA) (Ohsawa et al., 1985b). To 20 µl of serum, 4 ml of 0.05 M sulfuric acid and 0.5 ml of 10% phosphotungstic acid were added, and the mixture was shaken vigorously. After centrifugation at 3000 rpm for 10 min, the precipitate was added to 0.5 ml of acetate buffer (pH 2.5) and 0.5 ml of 0.12 M DPTBA. The mixture was heated in a water bath at 95 °C for 40 min. The reaction product was extracted with 1-butanol. Twenty µl of the organic layer was taken for analysis by high performance liquid chromatography (HPLC) (Shimadzu LC-5A) with LiChrosorb RP 18 (particle size 10 µm). The mobile phase was acetonitrile/water (1/1) and the flow rate was 0.7 ml/min. The fluorescence intensity of the eluate was monitored at 548 nm with excitation at 525 nm.

Measurement of partial oxygen pressure on the skin:
A Sumitomo Denki Kogyo Oxygen gas monitor, PO-510, was used for measurement of partial oxygen pressure on the skin (tcPO₂) of rats. After the hair on the
abdomen of the rat was shaved over an area of 10 cm², the sensor was stuck to the skin and the tcPo₂ was continuously monitored for a long period.

RESULTS

Effect of body weight on the consumption of oxygen:

Figures 2 and 3 show the relationship between oxygen consumption and body weight of male and female rats. There was a close correlation between oxygen consumption and body weight in the range of 150 g to 450 g for both male and female rats ($r=0.771$, $r=0.818$). However, below body weights of 150 g, there was no correlation between oxygen consumption and body weight.

Oxygen consumption by the pregnant rat:

Figure 4 shows changes in oxygen consumption and body weight of pregnant rats during pregnancy. Body weight increased with increasing gestation time, and decreased from 430 g to 330 g after parturition of newborn rats. Oxygen consumption increased slightly from the start of pregnancy. After the 20th day, a significant increase in oxygen consumption was observed. Figure 5 shows the perinatal consumption of oxygen by a rat. Oxygen consumption increased markedly during the first hour and remained constant for the next two hours. Immediately after parturition oxygen consumption decreased rapidly.

![Graph showing the relationship between oxygen consumption and body weight.](image)

Fig. 2. Relationship between oxygen consumption and body weight of normal male rats. Regression line and correlation coefficient were calculated using the values for the rats of weights above 150 g.
Fig. 3. Relationship between oxygen consumption and body weight of normal female rats. Regression line and correlation coefficient were calculated using the values for the rats of weights above 150 g.

![Graph showing oxygen consumption vs body weight with the equation y = 0.509x + 267.1 and r = 0.818.](image)

Fig. 4. Changes in oxygen consumption and body weights of pregnant rats. Each point represents the mean ± S. D. of data for five animals (●: body weight of control; ○: body weight of pregnant rat; •: oxygen consumption of control; •: oxygen consumption of pregnant rat).
Changes in tcPO₂, oxygen consumption, and lipid peroxide levels in the serum:

Figure 6 shows the changes in tcPO₂ of rats placed in 16% oxygen at 1 atm for 3 h. TcPO₂ before exposure to 16% oxygen was 60 mm Hg. A decrease in the tcPO₂ was observed immediately after exposure, and tcPO₂ fell to 20 mm Hg after 20 min, but recovered to the normal value of tcPO₂ after 40 min. Figure 7 shows changes in oxygen consumption by rats placed for 3 h under 16% or 40% oxygen at the 20th day of pregnancy. At the 40% concentration of oxygen, no significant drop in consumption of oxygen was observed during the 3 h exposure. However, in 16% oxygen, a remarkable decrease in oxygen consumption was observed immediately after exposure, and a 35% decrease in oxygen consumption was observed compared with the control after 1 h.

Table 1 shows changes in lipid peroxide levels in the serum of pregnant, delivered, and newborn rats after exposure of the pregnant, rats to 16% or 40% oxygen for 3 h on the 20th day. A significant difference was observed between the control group and the delivered and pregnant rats exposed to 16% oxygen. The lipid peroxide levels in serum from newborns were higher than those in serum of delivered and pregnant rats. An increase in lipid peroxide levels of the newborns of pregnant rats exposed to either 16% or 40% was observed. In the case of 16% oxygen, the observed increase in lipid peroxide levels of the newborns was statistically significant.

DISCUSSION

For evaluating oxygen toxicity in pregnant rats at high or low concentrations of oxygen, oxygen consumption was measured continuously for a long period with a
Oxygen toxicity on the pregnant rat

Fig. 6. Changes in partial oxygen pressure on the skin of rats placed under 16% oxygen at 1 atm for 3 h at 20th day of pregnancy.
Each point represents the mean ± S. D. of data for five animals.
*: Significantly different from control; P<0.05.

Fig. 7. Changes in oxygen consumption of rats placed for 3 h under 16% or 40% oxygen (1 atm) at 20th day of pregnancy.
Each point represents the mean ± S. D. of data for five animals (●: 16% oxygen; ■: 40% oxygen).
*: Significantly different from control; P<0.05.
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Table 1. Changes in lipid peroxide levels in serum of pregnant and newborn rats after exposure of pregnant rats to 16% or 40% oxygen for 3 h on the 20th day of pregnancy.

<table>
<thead>
<tr>
<th>item</th>
<th>lipid peroxide level (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.85±0.25</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>4.38±0.26</td>
</tr>
<tr>
<td>(10 day)</td>
<td></td>
</tr>
<tr>
<td>Postpartum</td>
<td>4.76±0.28</td>
</tr>
<tr>
<td>Control</td>
<td>4.90±0.26</td>
</tr>
<tr>
<td>40% O₂, 3 h</td>
<td></td>
</tr>
<tr>
<td>16% O₂, 3 h</td>
<td>5.70±0.43*</td>
</tr>
<tr>
<td>Newborn</td>
<td>7.81±0.83</td>
</tr>
<tr>
<td>Control</td>
<td>9.87±0.80</td>
</tr>
<tr>
<td>40% O₂, 3 h</td>
<td></td>
</tr>
<tr>
<td>16% O₂, 3 h</td>
<td>12.53±1.69*</td>
</tr>
</tbody>
</table>

Each value represents the mean±S. D. (n=5).
*: Significantly different from value for each control; P<0.05.

specially devised oxygen-consumption measuring apparatus.

There was no significant difference between oxygen consumption by pregnant rats and by the control group in the initial and intermediate periods of pregnancy. However, an increase in oxygen consumption was observed in the later stages of pregnancy of about 1.8 ml/day. It is to be expected that the basal metabolic rate of the pregnant rat and fetal respiration are elevated in the later stages of pregnancy. The remarkable rise in oxygen consumption after the start of parturition is attributable to not only to the change from fetal respiration to the pulmonary respiration of the newborns, but also to the high demand of oxygen during the delivery period.

Since no significant differences in oxygen consumption and lipid peroxide levels were observed between pregnant rats exposed to 40% oxygen and the control pregnant rats, it appears that a high concentration of oxygen for a short time does not give rise to harmful effect. By contrast, exposure to 16% oxygen for 3 h resulted in a 35% decrease in oxygen consumption, a 67% decrease in tcPo₂ and an increase in lipid peroxide levels. Therefore, a low concentration of oxygen might give rise to ischemia, and induce production of active oxygen moieties such as the superoxide anion and lipid peroxides, which would be followed by serious consequences in the various organs. A significant increase in lipid peroxide levels in newborns was observed after exposure of mothers to a low concentration of oxygen. The low concentration of oxygen may have caused the peroxidation of fetal lipids via ischemia because of an increase in the demand for oxygen during that period of pregnancy.
REFERENCES


