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Effects of Maternal Oxygen Supplementation on Fetal Oxygenation and Lipid Peroxidation Following a Single Umbilical Cord Occlusion in Fetal Goats

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Abstract

Maternal oxygen supplementation is commonly performed to improve fetal oxygenation and acid-base balance during fetal asphyxia. The efficiency of this treatment is controversial, which may be associated with the production of oxygen free radicals and lipid peroxidation. However, only a few studies have been performed to evaluate these issues. To clarify them, we investigated the effects of maternal oxygen supplementation on fetal oxygenation and lipid peroxidation following fetal asphyxia in late gestation goats.

We measured fetal blood gases, pH and plasma malondialdehyde (MDA), one of the endproducts of lipid peroxidation, before, during and after fetal asphyxia with and without maternal oxygenation in late gestation goats. Fetal asphyxia was induced by a single total umbilical cord occlusion of 3 minutes' duration, and maternal oxygenation was initiated at 20 min before the cord occlusion and terminated at 20 min after the release of cord occlusion.

Maternal oxygen supplementation resulted in a significant increase in fetal PaO₂ before and after the cord occlusion ($p < 0.05$). During the cord occlusion, the extents of hypoxia and acidemia were not changed by maternal oxygen supplementation. Fetal plasma MDA levels before maternal oxygen supplementation averaged 0.80 ± 0.04 $\mu\text{mol/L}$, significantly increased after the initiation of maternal oxygen supplementation (1.11 ± 0.07 $\mu\text{mol/L}$), and further increased following fetal asphyxia (1.28 ± 0.06 $\mu\text{mol/L}$), and after the release of the cord occlusion (1.58 ± 0.7 $\mu\text{mol/L}$) ($p < 0.05$). These values were significantly higher than those in fetuses without oxygenation. We conclude that maternal oxygen supplementation increased fetal oxygenation but caused a concomitant increase in lipid peroxidation in the fetus.

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Key words: fetus, oxygen supplementation, hypoxia, lipid peroxidation

Introduction

Maternal oxygen supplementation has been performed to restore fetal hypoxia and asphyxia

during labor^{1,2}. However, few data are available on the efficacy of administering oxygen on fetal oxygenation, acid-base balance and physiological status during fetal asphyxia. Previous studies have shown that maternal oxygen supplementation

improves fetal blood gas and acid-base parameters and biophysical profiles during fetal hypoxia^{3,4}. However, other studies have demonstrated that administration of oxygen does not improve fetal oxygenation or acid-base balance⁵. Moreover, oxygen supplementation induces maternal hyperoxia and this may promote the formation of oxygen free radicals and lipid peroxidation^{6,7}. Therefore, it is still not known whether maternal oxygen supplementation improves perinatal outcome or causes harm.

To date, the effects of maternal oxygen supplementation on fetal oxygenation and lipid peroxidation following fetal asphyxia have not been evaluated in late gestation goats.

In the present study, we measured changes in fetal blood gases, pH and circulating malondialdehyde (MDA), one of the endproducts of lipid peroxidation induced by reactive oxygen species before, during and after fetal asphyxia with and without maternal oxygenation in late gestation goats. Then we evaluated the effects of maternal oxygen supplementation on fetal oxygenation and lipid peroxidation following fetal hypoxia in late gestation goats.

Materials and Methods

1. Surgical procedures

Six pregnant mixed-breed goats mated on only a single occasion and of known gestational age (124~134 days, Term = 150 days) were used in the study. Animals were housed in rooms with controlled light cycles. They were fed each morning with alfalfa cubes and had free access to water at all times. Surgery was performed on 6 pregnant mixed-breed goats. After a 24-h fast, the goats were anesthetized with a mixture of 1.5~2.5% halothane and oxygen. Under aseptic conditions the maternal skin and uterus were incised and the fetal head was delivered. Polyvinyl catheters (1.0 mm ID) were inserted into the fetal carotid artery and jugular vein and their tips advanced into the aortic arch and superior vena cava, respectively. An inflatable silicone cuff (In Vivo Metric, model OC 4, Healdsburg, CA, USA) was placed loosely around the umbilical cord near the abdomen. An additional catheter was placed in the amniotic cavity for administration of antibiotics. Then, the fetal body was replaced into the uterus. All catheters were exteriorized through an incision in the maternal

flank and protected in a nylon pouch. The goats were kept in metabolic carts and were allowed to recover at least 4 days before experiments were begun.

Ampicillin was administered to the maternal goats on a daily basis (4 g/day, iv), and given to the fetus via instillation into the amniotic cavity (1 g/day).

2. Experimental protocol

This study was approved by the Ethical Committee of the Hobara Central Hospital, Fukushima, Japan. Experiments were started at 4 days after surgery. Prior to the start of each experiment, samples for measurement of fetal arterial blood gases and pH were taken. Only fetuses with arterial blood gas and pH values in the normal ranges for our laboratory ($\text{PaO}_2 > 17$ mmHg and $\text{pH} > 7.28$) were studied.

The protocol consisted of control, the cord occlusion, and recovery periods with and without maternal oxygen supplementation. Experiments began with the initial control period. After the control period, maternal oxygen supplementation started at 20 min before the cord occlusion and terminated at 20 min after the release of cord occlusion. Maternal oxygen supplementation was done by passing 30 L/min oxygen through a plastic bag over the goat's head. In our preliminary study ($n = 4$), maternal PaO_2 levels were increased approximately from 95 to 190 mmHg using this method.

The umbilical cord was occluded promptly for 3 minutes by inflating the balloon occluder with sterile saline at 20 min after the initiation of maternal oxygen supplementation. The umbilical cord was then released and fetal responses were followed during a 20-min maternal oxygen supplementation after the release of cord occlusion and a 90-min recovery period. This single occlusion/release procedure was chosen because it has been shown to cause fetal asphyxia in fetal sheep⁸, but is not so severe as to cause brain damage⁹. In control experiments, the same protocols were performed with maternal room air supplementation.

During the experiments, fetal arterial blood pressure was measured with a pressure transducer (Cobe laboratories, Lakewood, CO) and heart rate was determined with a cardiometer triggered by the arterial pulse pressure. Fetal arterial pressure and heart rate were transferred to a stripchart recorder (RM-6366, Nihon Koden, Tokyo,

Japan), and values were sampled every 0.01 sec with an NEC computer, with minute averages stored on disk. Fetal blood gases and pH, and plasma malondialdehyde (MDA) levels were measured periodically.

3. Blood sampling

Fetal blood samples were collected from the carotid artery at 30 and 5 min before the cord occlusion, at the end of occlusion and 20, 30, 60 and 90 min after the cord occlusion. The first portion of each fetal blood sample (0.4 mL) was taken into an ice-cold heparinized syringe. Fetal blood gases and pH were measured (Model 148, CIBA-Corning, Medford, MA).

The next sample (2 mL) was taken into an ice-cold heparinized syringe, and immediately transferred into an ice-cold centrifuge tube. Samples were centrifuged at once (1,300 g for 5 min, 4°C). The plasma was collected and stored at -80°C until analysis. Plasma MDA levels were assayed by a high performance liquid chromatographic method as previously described¹⁰. The detection limit was at least 0.01 µmol/L, and the intra- and inter-assay coefficients of variation were 5.2% and 6.6%, respectively.

4. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Student's t test was used to determine significant differences for single comparisons. Analysis of variance was used to determine the significant difference for repeated measurements. If overall significance was observed, then individual group means were compared by the Bonferroni's post hoc multiple comparison test. Differences were considered significant at $p < 0.05$.

Results

1. Changes in fetal heart rate and mean arterial blood pressure

Fig. 1 shows changes in fetal heart rate. In oxygenated fetuses, fetal heart rate averaged 141 ± 3 bpm during the control period, and then significantly decreased to 72 ± 4 bpm during the cord occlusion ($p < 0.05$). After the release of cord occlusion, fetal heart rate increased significantly to 148 ± 5 bpm, and remained significantly elevated until 60 min after the cord occlusion. Changes in mean arterial blood pressure are shown in **Fig. 1**. In oxygenated fetuses,

mean arterial blood pressure averaged 46 ± 2 mmHg during the control period, and then significantly increased to 82 ± 4 mmHg during the cord occlusion ($p < 0.05$). After release of the cord occlusion, mean arterial blood pressure gradually returned to control levels. Mean fetal heart rate and mean arterial blood pressure in oxygenated fetuses were not significantly different from those in fetuses without oxygenation throughout the study.

2. Changes in fetal blood gases and pH

The changes in fetal blood gases and pH during the course of the study are shown in **Fig. 2**. In oxygenated fetuses, fetal PaO₂ averaged 21.1 ± 0.6 mmHg during the control period, and then significantly increased to 27.5 ± 1.1 mmHg after the initiation of maternal oxygen supplementation, which was significantly higher than in fetuses without oxygenation (21.4 ± 0.7 mmHg) ($p < 0.05$). Fetal PaO₂ during maternal oxygen supplementation was significantly higher than in fetuses without oxygenation ($p < 0.05$). During the cord occlusion, the extents of hypoxia and acidemia were not changed by maternal oxygen supplementation. During the recovery period, PaO₂ in oxygenated fetuses was significantly different from that in fetuses without oxygenation. However, there was no significant change in PaCO₂ or pH.

Changes in fetal plasma MDA levels

Changes in fetal plasma MDA levels are shown in **Fig. 3**. In oxygenated fetuses, fetal plasma MDA averaged 0.80 ± 0.04 µmol/L during the control period, and then significantly increased to 1.11 ± 0.07 µmol/L after the initiation of maternal oxygen supplementation. Fetal plasma MDA levels further increased during cord occlusion and after the release of the cord occlusion (1.28 ± 0.06 µmol/L and 1.58 ± 0.07 µmol/L, respectively) ($p < 0.05$), and remained at high levels during the subsequent recovery period. In fetuses without oxygenation, fetal plasma MDA averaged 0.76 ± 0.06 µmol/L during the control period, and increased to 1.09 ± 0.05 µmol/L and 1.28 ± 0.07 µmol/L ($p < 0.05$) during cord occlusion and after the release of the cord occlusion, respectively, and also remained significantly elevated during the recovery period ($p < 0.05$). Mean MDA levels after the initiation of oxygen supplementation and the remainder of the study in oxygenated fetuses were significantly higher than those in fetuses without oxygenation ($p < 0.05$).

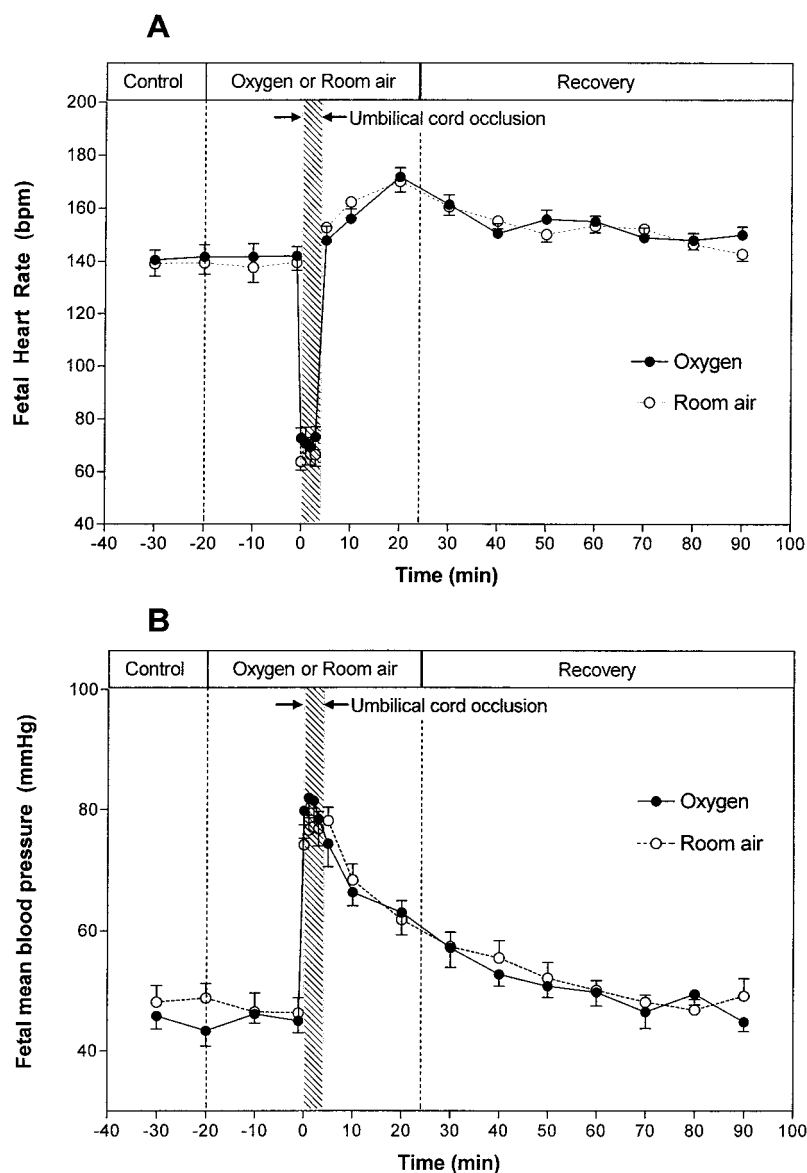


Fig. 1 Time course of fetal heart rate (A) and mean arterial blood pressure (B) in response to maternal oxygenation following cord occlusion in term fetal goats ($n=6$). Data are presented as mean \pm SEM.

Discussion

In the present study, we found that maternal oxygen supplementation resulted in a moderate increase in fetal PaO_2 throughout the study, except during the cord occlusion. Maternal oxygen supplementation was associated with increased MDA, reflecting changes in lipid peroxidation induced by reactive oxygen species, even in fetuses without asphyxia, and further significantly elevated during fetal asphyxia and the recovery period.

These results suggests that maternal oxygen

supplementation increases fetal oxygenation but causes an increase in lipid peroxidation before and after fetal asphyxia, so the clinical advantage of maternal oxygen supplementation is questionable.

In the present study, mean arterial blood pressure increased and fetal heart rate decreased significantly during the cord occlusion in fetuses with and without oxygenation, but the extent of changes in these variables was not affected by oxygen supplementation. These results are in accordance with a previous study in sheep^{7,11}.

In this study, oxygen supplementation increased fetal PaO_2 significantly, but the extent of this

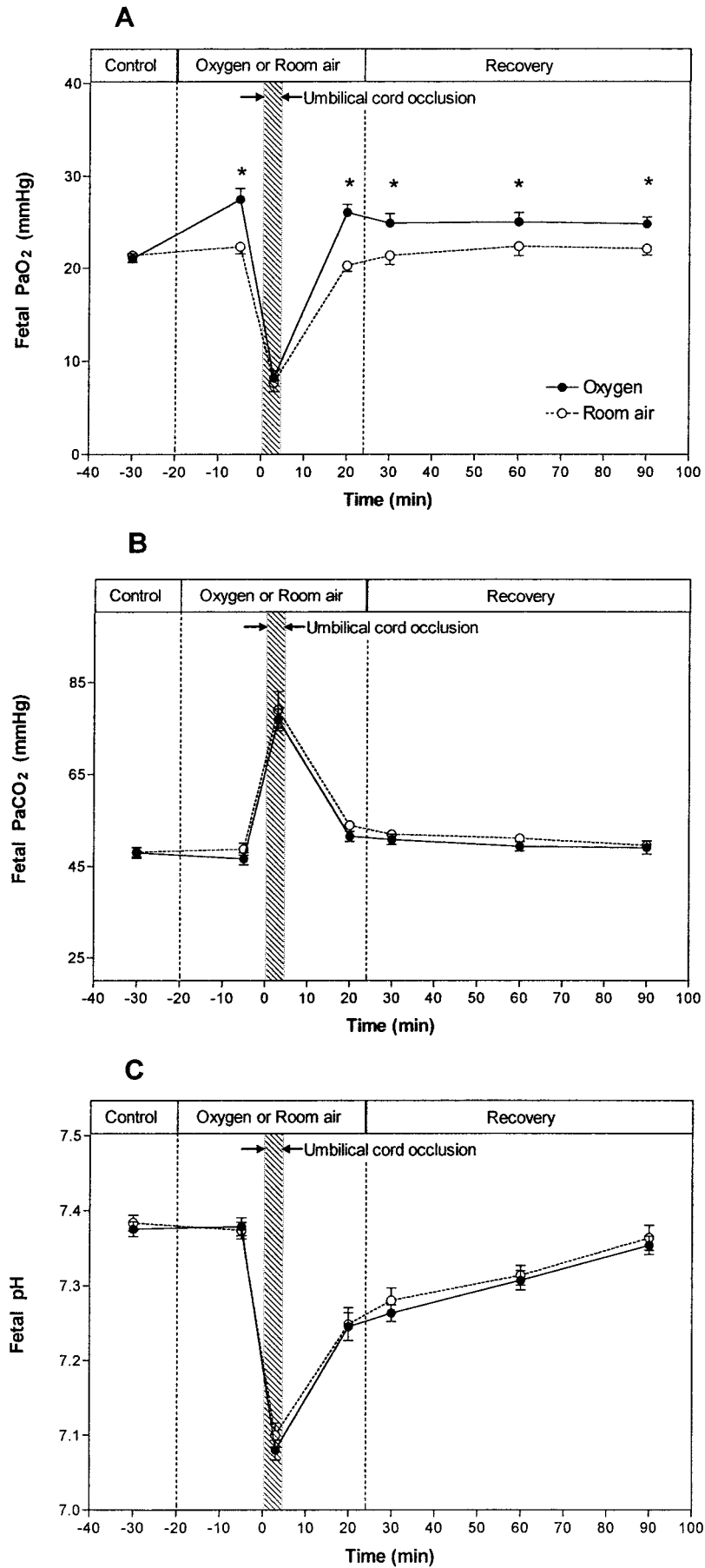


Fig. 2 Time course of fetal PaO₂ (A), PCO₂ (B) and pH (C) in response to maternal oxygenation following cord occlusion in late term goats (n=6). Data are presented as mean ± SEM. *Significantly different from fetuses without oxygenation (p<0.05).

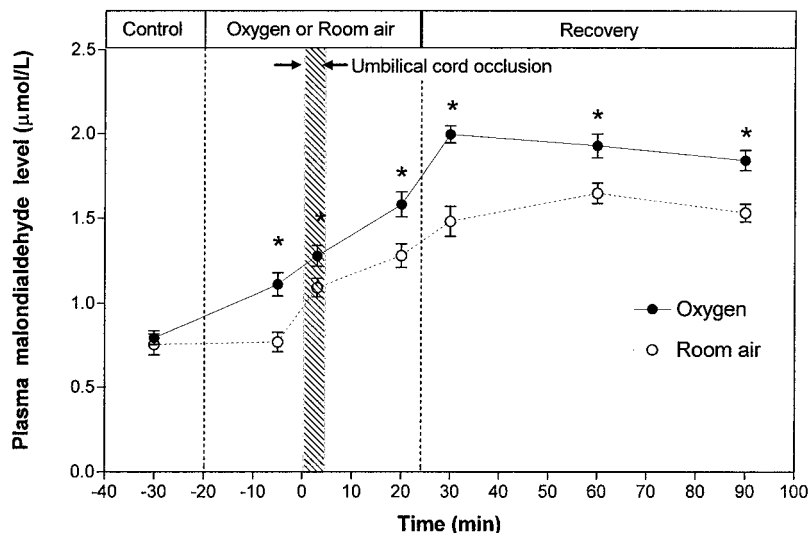


Fig. 3 Time course of fetal malondialdehyde levels in response to maternal oxygenation following cord occlusion in late term goats (n=6).

Data are presented as mean \pm SEM.

*Significantly different from fetuses without oxygenation ($p < 0.05$).

increase was relatively small. During the recovery period, mean PaO₂ in oxygen supplemented fetuses was significantly different from that in fetuses without oxygenation. These results are in accordance with a previous study in sheep⁷. The mechanisms by which PaO₂ remained significantly elevated after the termination of maternal oxygenation may be related to changes in fetal blood flow distribution and reduced oxygen consumption in the fetus.

In this study, fetal MDA levels significantly elevated after maternal oxygen supplementation, and further increased after the cord occlusion and for the remainder of the study. This is the first report regarding fetal plasma MDA response to maternal oxygen supplementation following fetal asphyxia in late gestation goats, so there are no data available to compare with our results.

Pathological generation of free radicals, which induce lipid peroxidation, commonly involves one of several possible pathways^{12,13}. In a previous study, cord plasma MDA levels after maternal oxygen supplementation during elective Caesarian section were already elevated without fetal asphyxia¹⁴, which may be attributed to the production of free radicals by hyperoxia via a pathway involving direct mitochondrial electron transfer, with no concurrent formation of purine metabolites. Therefore, the initial increase in MDA levels after oxygen

supplementation in the present study may be attributed to this mechanism.

In other studies, fetal xanthine levels, which indirectly reflect changes in oxygen free radicals, elevated during fetal asphyxia after maternal oxygenation^{6,7}. In these conditions, free radicals are generated via the pathways involving hypoxic stress and ischemia-reperfusion injury. Hypoxia induces the conversion from xanthine dehydrogenase to xanthine oxidase. After reperfusion, xanthine oxidase catalyses the formation of hydroxyl free radicals from the breakdown of purine metabolites, xanthine and hypoxanthine^{15,16}. Therefore, further increased MDA levels after the cord occlusion and remainder of the study may be attributed to this mechanism. Further study is needed to clarify these mechanisms.

Direct detection of oxygen free radicals is very difficult because of their brief lifespan¹⁷. Therefore, in our study we measured levels of MDA, one of the endproducts of lipid peroxidation induced by reactive oxygen species. Because MDA levels can be influenced by the extent of prostaglandin metabolism as well as by oxygen free radical activity¹⁸, and plasma MDA levels in the peripheral blood do not necessarily reflect, for instance, the more important local environments, such as the fetoplacental unit, care must be taken in interpreting changes in plasma MDA levels.

The relative advantages and disadvantages of

fetal oxygenation during fetal asphyxia are unknown. Some studies have shown benefits^{4,19}. However, maternal hyperoxia may be associated with the formation of oxygen free radicals^{14,20}, which may induce tissue damage in many organs^{21,22}. Further free radicals cause depletion of intrinsic antioxidant systems of the fetus during deliveries with abnormal oxygenation²³. Further study is needed to clarify the relative advantages and disadvantages of maternal oxygen supplementation during fetal asphyxia.

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