Transient ligation of umbilical vessels elevates placental tissue oxygen index (TOI) values measured by near-infrared spectroscopy (NIRS) in clawn miniature pig animal model

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Summary

We recently found a significant elevation in placental tissue oxygen index (TOI) values in cases of fetal growth restriction using near-infrared spectroscopy (NIRS), indicating high oxygenation in the placental tissue. We hypothesized that insufficient fetoumbilical blood flow is causatively associated with high oxygenation levels in placental tissue. We transiently (for 15 sec) ligated the whole umbilicus, umbilical arteries, or veins of pregnant Clawn miniature pigs (102-113 days of gestation) and assessed the changes in TOI values of the placenta and fetus. The ligation significantly increased placental TOI values ($p < 0.01$, respectively), but concomitantly decreased fetal TOI values ($p < 0.01$, respectively), suggesting a decline in oxygen inflow from the maternal to fetal circulation in the placental tissue to be causative of the elevated placental TOI values. These observations suggest the promising clinical use of placental TOI values measured noninvasively by the transabdominal application of NIRS to assess the fetoplacental circulation.

Key words: Placenta; Pregnancy; Near-infrared spectroscopy (NIRS); Umbilical.

Introduction

The placenta is a critical organ for fetal development as it transports oxygen and nutrients from maternal to fetal blood. Therefore, it is plausible that a chronic and/or acute malfunction of fetomaternal transportation in the placenta leads to a deterioration in fetal well-being, such as fetal growth restriction and/or hypoxia. Indeed, both fetal growth restriction [1] and hypoxic-ischemic encephalopathy [2] are closely associated with the mortality as well as morbidity of newborns and important clinical issues even in modern medicine. However, no gold standard for the intrauterine diagnosis of fetal hypoxia has been established, despite recent improvements in neonatal medical care, partly because of the methodological complexity in clarifying the physiology and pathophysiology of changes in placental tissue oxygenation. Maternal and fetal blood circulate separately throughout the structure of the placenta, which makes it difficult to assess overall placental tissue oxygenation by direct measurement with a pulse oximeter, an electrode [3], tissue or blood sampled by needle biopsy etc.

Jobsis first reported a technique for the noninvasive monitoring of tissue oxygenation in intact organs, near-infrared spectroscopy (NIRS) [4]. NIRS has since been utilized in various clinical fields all over the world [5, 6]. Recently, we transabdominally applied NIRS to the human placenta, successfully evaluated concentrations of oxyhemoglobin (HbO_2) and hemoglobin (Hb) [7], and proposed a placental tissue oxygen index (TOI), calculated according to the formula [HbO_2/HbO_2 + Hb] x 100 (expressed as a percentage) [8], as an index for the assessment of placental oxygenation [8, 9]. Subsequently, we observed a significant elevation of placental TOI values in cases of fetal intrauterine growth restriction (IUGR) [8, 9], especially those complicated by chorangiosis, a distinct pathological change of the placenta [10]. The TOI values positively correlated with tissue oxygenation in a wide range of organs, such as the brain [11, 12], muscle [13], and liver [14, 15] in both humans and animal models. Therefore, our recent observations indicate the co-existence of paradoxically high levels of oxygenation in the placental tissues, because some cases of IUGR [16], and especially those with placental chorangiosis [17, 18] are potentially associated with long-standing, rather low-grade, fetal hypoxia.

In the placenta, maternal blood, mainly from uterine arteries, supplies oxygen to and receives carbon dioxide from fetal blood. Studies with both human subjects [19] [20] and animal models [21, 22] have suggested that insufficient maternal blood flow in the uterine arteries was causatively associated with fetal hypoxia. However, as not only maternal, but also fetal blood circulates in the placental tissues, it is possible that fetoplacental circulation affects placental tissue oxygenation, contributing to an exquisite regulatory system to maintain appropriate oxygenation in the fetoplacental compartment.

In the present study, we hypothesized that insufficient fetoumbilical blood flow reduces the amount of oxygen transported from the maternal to fetal circulation in the placenta, thereby contributing to a high level of oxygena-

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Fig. 1A

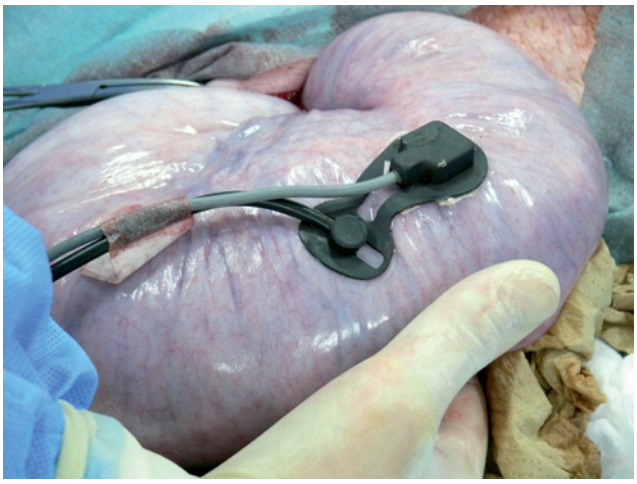

Fig. 1B

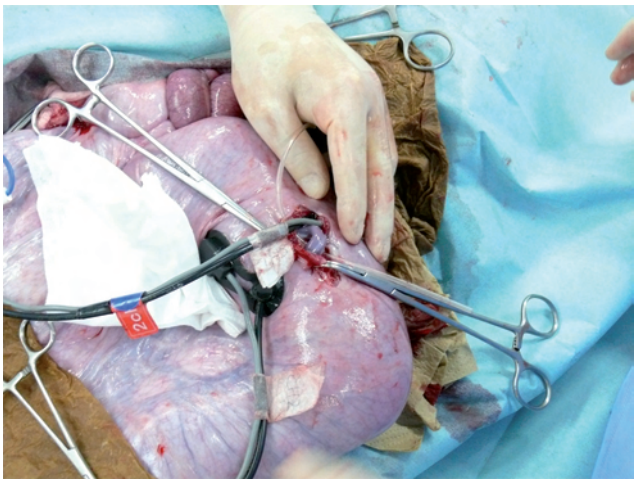

Fig. 1C

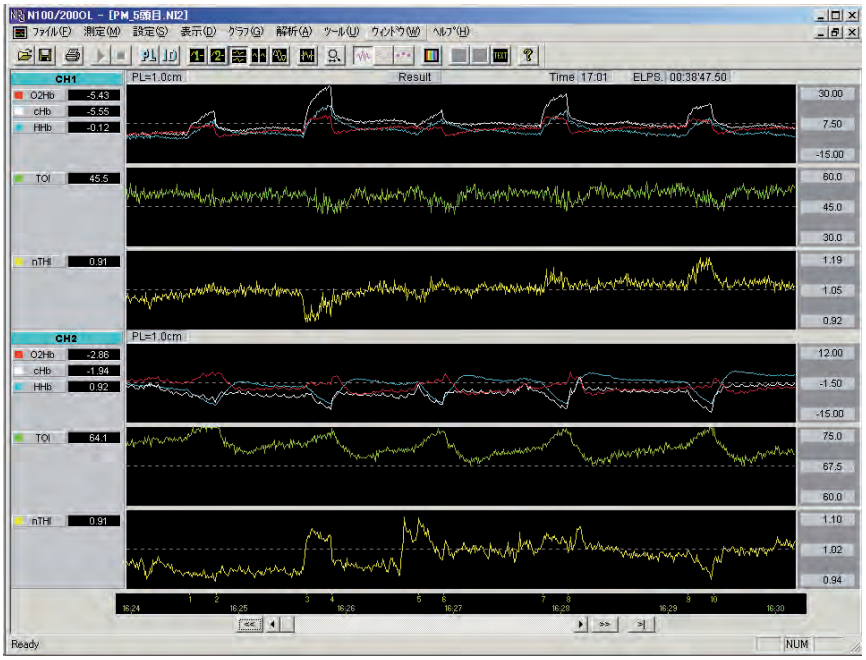

Figure 1. — Experimental procedures using pregnant miniature pigs
Placental TOI values were measured by attaching the NIRS probe to the surface of the uterine wall just outside the placental attachment (A), where a thin layer of aluminum was inserted between the placenta and fetus to prevent near-infrared rays from reaching the fetal circulation. Fetal TOI values were measured by placing the probe directly on the fetus (B). The changes in oxyhemoglobin (red lines), deoxyhemoglobin (blue lines), and total hemoglobin (white lines) levels, and TOI values (green lines) were continuously recorded in both the fetus (upper panel) and placenta (lower panel) during repeated ligation of the umbilical arteries (Figure 1C). White arrows indicate the time of ligation (Figure 1C). Closed circles and vertical error bars represent means and ± SD of TOI values, respectively.

tion in placental tissues, as indicated by the elevated TOI values in our recent studies. To test the hypothesis, we temporarily obstructed the umbilical vessels of pregnant Clawn miniature pigs and assessed the changes in placental tissue oxygenation by measuring TOI values using NIRS. We found that acute obstruction significantly increased placental tissue oxygenation, which is to our knowledge the first evidence supporting the concept that the feto-umbilical circulation is causatively linked with the regulation of placental tissue oxygenation, in addition to the changes in maternal blood supply.

Materials and Methods

Pregnant miniature pigs as an animal model

All animal experiments were carried out at Kobe Medical Device Development Center (Kobe, Japan) with the permission of the institutional animal experiment committee. A total of six pregnant Clawn miniature pigs at 109, 112, 113, 102, 108, and 105 days of gestation (39.6-48.4 kg; term: 114 ± 3 days of gestation) were anesthetized with an intramuscular injection of 15 mg/kg of ketamine (Daiichi Sankyo Co., Ltd., Tokyo, Japan), 10 mg/kg of xylazine (Bayer Medical Ltd., Tokyo, Japan), and 0.1 mg/kg of atropine (Tanabe Co., Ltd., Osaka, Japan) followed by ventilation with 100% oxygen gas mixed with 2-3 % isoflurane (Merck Japan, Tokyo, Japan). In each animal, a longitudinal incision was made in the abdomen, and six to eight fetuses (155 to 676 g) were used for the experiments. Maternal blood oxygen saturation was continually monitored transcutaneously using a DS-7141 Patient Monitor (Fukuda Denshi, Co., Ltd., Tokyo, Japan).

Assessment of oxygenation by NIRS

Assessment of tissue oxygenation was carried out as reported for human placenta [8]. In brief, spectral changes in near-infrared rays of four different wavelengths (775, 825, 850, and 905 nm), reflecting changes in oxyhemoglobin (HbO2) and deoxyhemoglobin (Hb) concentrations in placental tissue, were

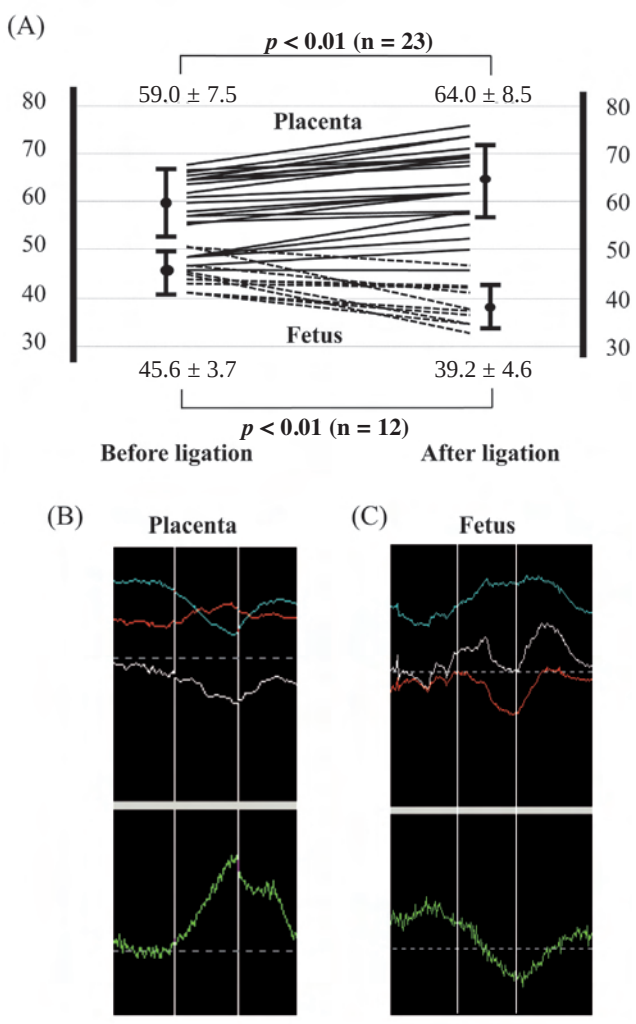

Figure 2. — Changes in placental and fetal TOI values after transient ligation of the whole umbilical cord (A). Representative changes in oxyhemoglobin (red lines), deoxyhemoglobin (blue lines), and total hemoglobin (white lines) levels, and TOI values (green lines) of the placenta (B) and fetus (C) during transient ligation of the whole umbilical cord. Left and right white vertical lines indicate the start and end of ligation, respectively, separated by 15 seconds, when changes in TOI values were assessed (B,C). Closed circles and vertical error bars represent means and ± SD of TOI values, respectively.

monitored using an NIRO200 spectrophotometer (Hamamatsu Photonics, Hamamatsu Japan). TOI values were calculated according to the formula [HbO_2/HbO_2 + Hb] x 100 (expressed as percentages) [8].

Measurement of placental and fetal TOI values

A small longitudinal incision was made in the lateral side of the uterus with an electric scalpel at the fetal compartment. Placental TOI values were measured by attaching the NIRS probe to the surface of the uterine wall just outside the placental attachment (Figure 1A), while a thin layer of aluminum was inserted between the placenta and fetus to prevent near-infrared rays from reaching the fetal circulation. Fetal TOI values were measured by placing the NIRS probe directly on the fetus (Figure 1B).

Table 1. — *Representative patterns of changes in oxyhemoglobin, deoxyhemoglobin, and total hemoglobin underlie the increased placental and decreased fetal TOI values. Horizontal arrows indicate unchanged values. Up and down arrows indicate increased and decreased values, respectively. Two and only one arrows indicate large and small changes.*

		Ligation of whole umbilical cord (Figure 2)	Ligation of umbilical artery (Figure 3)	Ligation of umbilical vein (Figure 4)
Placenta	Oxyhemoglobin	↑	↑	↑↑
	Deoxyhemoglobin	↓↓	↓↓	→
	Total hemoglobin	→	↓↓	↑↑
	TOI values	↑↑	↑	↑
Fetus	Oxyhemoglobin	↓↓	↑	↓↓
	Deoxyhemoglobin	↑↑	↑↑	↑↑
	Total hemoglobin	→	↑↑	↓↓
	TOI values	↓↓	↓	↓↓

The whole umbilical cord, umbilical arteries or veins were ligated for 15 sec using silk string, and the changes in placental and fetal TOI values were continuously recorded (Figure 1C). TOI values of the placenta and fetus at the end of the ligation were compared to those just before the ligation.

Statistical analysis

Values are expressed as the means ± SD. Significant differences were assessed using the Wilcoxon signed rank test for comparison of paired TOI values just before the ligation with those at the end of the ligation (15 sec); p values less than 0.05 were regarded as significant.

Results

Changes in TOI values with the ligation of the whole umbilicus

Acute ligation of the whole umbilicus significantly increased the placental TOI values (59.0 ± 7.5 [SD]% vs 64.0 ± 8.5%, n = 23 ligations/12 fetuses, $p < 0.01$), but simultaneously decreased fetal TOI values (45.6 ± 3.7% vs 39.2 ± 4.6%, n = 12 fetuses, $p < 0.01$) (Figure 2A).

A small increase in oxyhemoglobin, decrease in deoxyhemoglobin, and unchanged total hemoglobin resulted in a significant increase in placental TOI values (Figure 2B; Table 1). A decrease in oxyhemoglobin, increase in deoxyhemoglobin, and unchanged hemoglobin resulted in a significant decrease in fetal TOI values (Figure 2C; Table 1). There were no significant changes in oxygen saturation in the dams (data not shown).

Changes in TOI values with the ligation of umbilical arteries

Acute ligation of umbilical arteries significantly increased the placental TOI values (62.0 ± 3.4% vs 64.9 ± 4.9%, n = 14 fetuses, $p < 0.01$), but simultaneously decreased fetal TOI values (47.7 ± 3.9% vs 45.1 ± 2.9%, n = 20 ligations/14 fetuses, $p < 0.05$) (Figure 3A).

A small increase in oxyhemoglobin, decrease in deoxyhemoglobin, and decrease in total hemoglobin resulted in

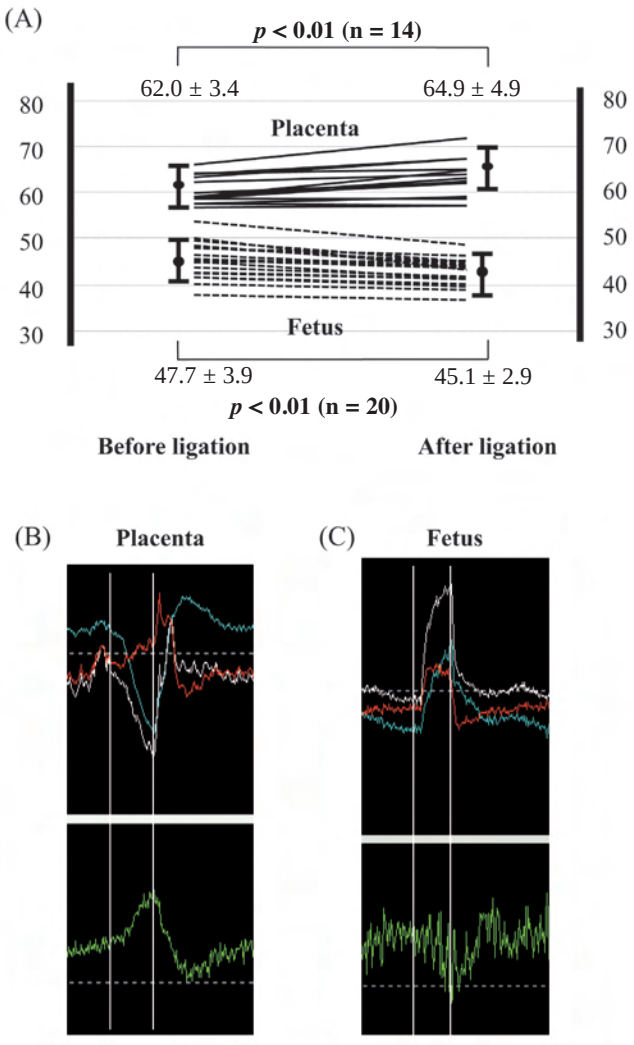

Figure 3. — Changes in placental and fetal TOI values after transient ligation of the umbilical arteries (A).
Representative changes in oxyhemoglobin (red lines), deoxyhemoglobin (blue lines), and total hemoglobin (white lines) levels, and TOI values (green lines) in the placenta (B) and fetus (C) during transient ligation of the umbilical artery. Left and right white vertical lines indicate the start and end of ligation, respectively, separated by 15 seconds, when changes in TOI values were assessed (B, C). Closed circles and vertical error bars represent means and ± SD of TOI values, respectively.

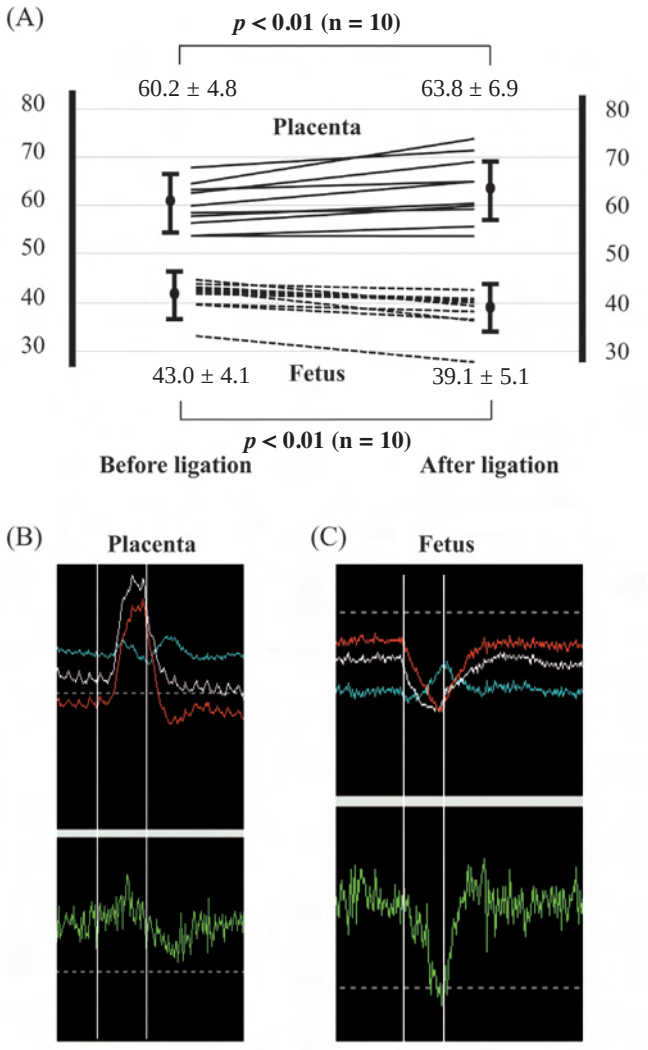

Figure 4. — Changes in the placental and fetal TOI values after transient ligation of the umbilical veins (A).
Representative changes in oxyhemoglobin (red lines), deoxyhemoglobin (blue lines), and total hemoglobin (white lines) levels, and TOI values (green lines) in the placenta (B) and fetus (C) during transient ligation of the umbilical veins. Left and right white vertical lines indicate the start and end of ligation, respectively, separated by 15 seconds, when changes in TOI values were assessed (B, C). Closed circles and vertical error bars represent means and ± SD of TOI values, respectively.

a significant increase in placental TOI values (Figure 3B; Table 1). A small increase in oxyhemoglobin, large increase in deoxyhemoglobin, and large increase in total hemoglobin resulted in a small but significant decrease in fetal TOI values (Figure 3C; Table 1). There were no significant changes in the oxygen saturation in the dams (data not shown).

Changes in TOI values by transient ligation of umbilical veins

Acute ligation of umbilical veins significantly increased the placental TOI values (60.2 ± 4.8% vs 63.8 ± 6.9%, n = 10 fetuses, $p < 0.01$), but simultaneously decreased fetal TOI values (43.0 ± 4.1% vs 39.1 ± 5.1%, n = 10 fetuses, $p < 0.05$) (Figure 4A).

An increase in oxyhemoglobin, stable deoxyhemoglobin, and increase in total hemoglobin level resulted in a small but significant increase in placental TOI values (Figure 4B; Table 1). A decrease in oxyhemoglobin, increase in deoxyhemoglobin, decrease in total hemoglobin resulted in a significant decrease in fetal TOI values (Figure 4C; Table 1). There were no significant changes in oxygen saturation in the dams (data not shown).

Discussion

In the present study, we showed that acute ligation of the whole umbilicus significantly decreased fetal TOI values and concomitantly increased placental TOI values (Figure 2; Table 1). Since the TOI values positively correlated with tissue oxygenation in various kinds of research models [11-15], these data indicate the co-existence of low and high levels of oxygenation in the fetus and placenta, respectively. It is reasonable that complete blockage of both the inflow and outflow of fetal blood decreased fetal oxygenation, as shown by the reduction in fetal TOI values, which will decrease the oxygen inflow from maternal blood in the placental tissues. Therefore, the synchronized elevation in placental TOI values strongly supports our hypothesis that a decline of oxygen inflow from the maternal to fetal circulation in the placental tissue is one of the factors responsible for the elevated placental TOI values. Indeed, the local coordinated changes to oxyhemoglobin and deoxyhemoglobin levels underlie the increase in placental TOI values (Table 1). The stable maternal oxygen saturation (data not shown) suggested the high placental oxygenation to be caused independently of the general maternal status of oxygenation. Acute ligation of uterine arteries significantly decreased both placental and fetal TOI values (data not shown), supporting the hypothesis that placental TOI values properly represent placental oxygenation in this animal model.

Acute ligation of the umbilical arteries (Figure 3; Table 1) or veins (Figure 4; Table 1) caused a similar increase and decrease in placental and fetal TOI values, suggesting that a decrease in the inflow or outflow of fetal blood to the placenta reduces oxygen absorption from the maternal blood, contributing to the elevation of placental TOI values. Therefore, disturbances of fetal circulation, the flow either to or from the placenta, immediately raised the placental TOI values, which also supports our hypothesis that a deterioration of fetal circulation is causatively associated with the augmentation of placental TOI values.

Irradiating near-infrared rays pass through the entire placenta; therefore, maternal as well as fetal blood could affect placental TOI values. In the human placenta, we recently demonstrated that placental TOI values mainly represent the oxygenation of maternal blood [10]. Although porcine studies have provided excellent information concerning the physiology and pathophysiology of the placenta [23-26], the porcine placenta is "epitheliochorial" and not exactly the same as the "hemochorial" human placenta [27]. Therefore, the contribution of fetal blood to placental TOI values in this animal model is unclear. However, the paradoxical increase in TOI values with the obstruction of the fetoumbilical circulation itself suggests that they too mainly represent the oxygenation of maternal blood in the placental tissues, although no information on the real oxygen saturation of maternal blood in the porcine placenta has been published to our knowledge.

Nylund *et al*. injected a radio-isotope in pregnant women before delivery and demonstrated a 50% or more reduction in uteroplacental blood flow in cases of fetal growth restriction [28]. Macara *et al*. reported that structural analysis of placental terminal villi of growth-restricted fetuses suggested a decreased oxygen transfer from the intervillous space to the fetal circulation [29]. Paradi *et al*. reported that uterine venous oxygen saturation of growth-restricted fetuses was significantly higher than that of appropriate growth for gestational age [30]. Kingdom and Kaufmann proposed a hypothetical concept of 'postplacental hypoxia' as one of origins of fetal hypoxia, when the placental villi is exposed to a higher oxygen tension than under normal circumstances [31]. The findings of the present animal study supports the concept despite the morphological difference between human and porcine placenta [27].

Hypoxia is one of the most ominous fetal conditions, proceeding to fetal demise. The present study suggests the promising use of placental TOI values obtained by the transabdominal application of NIRS to assess the insufficiency of the fetoplacental circulation, as a cause or result of fetal hypoxia. Clinical studies are now under way.

Some caution is required, because fetal IUGR and chorangiosis, as we previously observed in humans, are based on rather long-lasting, chronic changes. In the present study, we examined the acute termination of fetal circulation to the placenta in Clawn miniature pigs. Moreover, the study was carried out by using animals between 102 and 113 days of gestation (114 days full term) due to institutional availability of this animal. These experimental conditions do not exactly mimic chronic changes of fetal IUGR in humans. Therefore, our next aim is to clarify the effect of chronic deterioration of fetal circulation on placental oxygenation.

In conclusion acute occlusion of the whole umbilicus or umbilical vessels significantly increased placental TOI values measured using NIRS in pregnant Clawn miniature pigs. This is the first evidence that a disturbance of the fetal circulation at least partly regulates placental tissue oxygenation.

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