

The Timing of Cord Clamping and Oxidative Stress in Term Newborns

AUTHORS: Javier Díaz-Castro, PhD,^{a,b} Jesus Florido, MD,^c Naroa Kajarabille, BD,^{a,b} Maria Garrido-Sánchez, MD,^{c,d} Carmen Padilla, MD,^{c,d} Catalina de Paco, MD,^{c,d} Luis Navarrete, MD,^c and Julio J. Ochoa, PhD^{a,b}

^aDepartment of Physiology, and ^bInstitute of Nutrition and Food Technology “José Mataix Verdú”, University of Granada, Granada, Spain; ^cDepartment of Obstetrics and Gynaecology, School of Medicine, University of Granada, Granada, Spain; and ^dService of Obstetrics and Gynaecology, University Hospital San Cecilio, Granada, Spain

KEY WORDS

cord clamping timing (early or late), term neonate, antioxidant defense, oxidative damage, inflammation

ABBREVIATIONS

CAT—catalase
IL-6—interleukin 6
PGE₂—prostaglandin E₂
ROS—reactive oxygen species
SOD—superoxide dismutase
sTNF-RII—soluble TNF- α receptor II
TAS—total antioxidant status
TNF- α —tumor necrosis factor α

Dr Díaz-Castro drafted the initial manuscript and participated in the analysis; Drs Florido and Kajarabille carried out the initial analyses and reviewed and revised the manuscript; Drs Garrido-Sánchez, Padilla, de Paco, and Navarrete designed the data collection instruments, coordinated and supervised data collection in the Obstetrics and Gynecology Department and Obstetrics and Gynecology Services, and critically reviewed the manuscript; Dr Ochoa conceptualized and designed the study and coordinated and supervised data collection at the Institute of Nutrition and Department of Physiology; and all authors approved the final manuscript as submitted.

www.pediatrics.org/cgi/doi/10.1542/peds.2013-3798

doi:10.1542/peds.2013-3798

Accepted for publication May 21, 2014

Address correspondence to Julio J. Ochoa, PhD, Institute of Nutrition and Food Technology “José Mataix Verdú”, University of Granada, Biomedical Research Centre, Health Sciences Technological Park, Avenida del Conocimiento s/n, Armilla, 18071 Granada, Spain. E-mail: jjoh@ugr.es

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

Copyright © 2014 by the American Academy of Pediatrics

FINANCIAL DISCLOSURE: The authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: No external funding.

POTENTIAL CONFLICT OF INTEREST: The authors have indicated they have no potential conflicts of interest to disclose.



WHAT'S KNOWN ON THIS SUBJECT: Clamping and cutting of the umbilical cord at birth is the most prevalent of all operations; however, the optimal timing of cord clamping is still a controversial issue, with different timings offering advantages and disadvantages.



WHAT THIS STUDY ADDS: Our findings suggest a protective effect of late cord clamping, increasing the antioxidant capacity and decreasing the inflammatory-mediated effects induced during delivery of term neonates.

abstract

BACKGROUND: Clamping and cutting of the umbilical cord is the most prevalent of all operations, but the optimal timing of cord clamping is controversial, with different timings offering advantages and disadvantages. This study, for the first time, compares the influence of early and late cord clamping in correlation with oxidative stress and inflammation signaling. Because cord clamping timing may have a significant influence on placenta-to-infant blood transfer, thereby modifying oxygenation of maternal and fetal tissues, and on the transfer of inflammatory mediators throughout the placenta.

METHODS: Sixty-four pregnant subjects were selected at the Gynecology and Obstetrics Services Department of the Clinico San Cecilio Hospital, Granada, Spain, based on disease-free women who experienced a normal course of pregnancy and a spontaneous, vaginal, single delivery. Half of the subjects had deliveries with early-clamped newborn infants (at 10 s), and the other half had late-clamped deliveries (at 2 min).

RESULTS: Erythrocyte catalase activity was significantly greater in the late-clamped group than in the early-clamped group ($P < .01$ for the umbilical vein and $P < .001$ for the artery). The values for superoxide dismutase, total antioxidant status, and soluble tumor necrosis factor receptor II were all significantly higher in the late-clamped group compared with the early-clamped group ($P < .01$, $P < .001$, and $P < .001$, respectively).

CONCLUSIONS: The results suggest a beneficial effect of late cord clamping, produced by an increase in antioxidant capacity and moderation of the inflammatory-mediated effects induced during delivery of term neonates. *Pediatrics* 2014;134:257–264

Clamping and cutting of the umbilical cord at birth is by far the oldest and most prevalent operation in humans. Although the World Health Organization stated that late cord clamping or not clamping at all represents a more physiologic method of neonatal care,¹ the optimal timing of cord clamping has been a controversial issue for decades, and there is still debate on the issue.² The current obstetric approach in Western medicine is to clamp the cord within the first 10 to 15 seconds after birth.³ However, there has been no sound evidence in favor of this approach in comparison with the age-old practice of clamping the cord between 1 and 3 minutes after birth.⁴ Earlier physiologic studies have shown that, of the total blood volume in combined fetal–placental circulation at full gestation, ~25% to 60% (54–160 mL) is found in placental circulation, and as many as 60% of the fetal red blood cells are found therein.^{5,6}

Early cord clamping at birth has become a routine procedure; however, it means that a part of the blood that would naturally be proportioned to the child remains in the placenta.⁷ This practice may deprive neonates of significant blood volume and could cause short- and long-term problems such as respiratory distress, cerebral palsy, and mental retardation.^{6,8–10} Benefits associated with delayed clamping include improved cardiopulmonary adaptation, a lower frequency of respiratory distress and anemia in infancy, and a longer period of early breastfeeding.^{6,10,11} One of the more widely accepted of these consequences is the increase in placenta-to-infant blood volume transfer, supplying an additional 30 to 75 mg of iron at delivery.¹² The concerns about delayed cord clamping include the possibility of polycythemia, hyperviscosity, hyperbilirubinemia, transient tachypnea of the newborn, delay in resuscitation,

hypothermia, and a possible risk of intraventricular hemorrhage.¹¹

On the other hand, several studies have reported that parturition features a strong oxidative stress for both mother and neonate, implying an increased production of free radicals that must be controlled by their antioxidant systems.^{13,14} Oxygen consumption increases during pregnancy, and therefore mitochondrial respiration also increases, which in turn causes an increase in the formation of reactive oxygen species (ROS).¹⁵ In addition, there are other factors to consider, such as a rapid change from the hypoxic intrauterine to the extrauterine environment, where alveolar P_{O_2} is almost 5 times higher, or the mediation of several physiologic processes involved in the finalization of gestation and delivery, thus promoting the Fenton reaction and leading to the production of highly toxic free radicals.¹³ The neonate is able to cope with this enormous aggression effectively through a perfectly developed antioxidant system and with the indispensable help of the mother's defense system, which is of great importance in the first minutes of life in case the newborn's antioxidant system needs activating. In this sense, the oxidative aggression is diminished in <3 hours.^{13,14} Another important factor to consider during birth, which can contribute to the increase in ROS production, is the evoked inflammation. Parturition has been identified as a source of proinflammatory mediators, such as metabolites of arachidonic acid (prostaglandin E_2 [PGE_2]) and cytokines, including tumor necrosis factor α (TNF- α), and interleukin 6 (IL-6). These mediators are potent stimulators of ROS production. Therefore, a rise in the concentration of cytokines and PGE_2 could be responsible for increased oxidative stress. Furthermore, TNF- α also increases the interaction of electrons

with oxygen, thereby producing superoxide anions.¹⁵

Taking into account the oxidative stress and inflammation induced during delivery, we designed a study that, for the first time, correlates the influence of cord clamping timing with oxidative stress and inflammation signaling. Our study was based on the hypothesis that cord clamping timing may have a significant influence on placenta-to-infant blood transfer (thereby modifying oxygenation of both maternal and fetal tissues), oxygen consumption, and the transfer of inflammatory mediators toward the placenta, parameters directly linked with postdelivery antioxidant status and inflammatory signaling in healthy neonates.

METHODS

Study Design and Subjects

The oxidative status and inflammatory signaling of 64 pregnant subjects were measured in Obstetrics and Gynecology Services of the Clinico San Cecilio Hospital (Granada, Spain) from February to October 2011. Subject inclusion criteria were the following: disease-free, normal course of pregnancy, BMI of 18 to 30 at the start of pregnancy, weight gain of 8 to 12 kg since pregnancy onset, gestational period of 37 to 42 weeks at delivery, single fetus in cephalic presentation, spontaneous vaginal delivery, newborn of appropriate weight for gestational age and with Apgar score ≥ 7 at first and fifth minutes of life, and normal monitoring results. None of the subjects experienced any abnormalities during labor and delivered spontaneously. Clinical parameters were monitored throughout the delivery. Variables such as age, parity, gestational period, and clinical pregnancy outcome were obtained from the mother's medical history. Means of variables were compared

with the Student's *t* test, because this approximation could be applied given the sample size. We assigned 70 full-term pregnant women to 1 of 2 groups upon arrival to the delivery room in our hospital: the early-clamped group, in which the umbilical cord was clamped within 10 s of fetus expulsion, and the late-clamped group, in which the umbilical cord was left at 20 cm below the vaginal introitus and then clamped at 2 min after expulsion. The selection of a 2-min interval was based on current literature³ and the results of a preliminary study where we observed that numerous umbilical arteries had bled after this period. For the first subject, the expectant mother was assigned to a treatment group by coin toss, and for subsequent cases, the subject's order of arrival at the delivery room determined her group (assigned alternately to each group). The sample size was determined the number of normal deliveries at our hospital and laboratory availability. Of the 35 women assigned to the early-clamped group, 2 were excluded from the study, 1 for an Apgar score <7 and the second for inadequate birth weight; therefore, the final early-clamped study group comprised 33 cases. Of the 35 women assigned to the late-clamped group, 4 were excluded from the study, 1 for an Apgar score <7, 2 for operative delivery, and 1 because of anomalies observed during monitoring; therefore, the final late-clamped study group comprised 31 cases. Newborn infants in the late-clamped group were held in their mothers' arms while waiting supine for the cord to be clamped. The maternal–fetal ejection period lasted 45.2 ± 5.5 min for all subjects. Informed consent was obtained from the parents after the nature and purpose of the study had been fully explained to them and they understood it. This study was approved by the University of Granada ethical committee (PI030780).

Blood Sampling

Blood samples were collected from the umbilical vein and arteries immediately after cord clamping. By taking a sample of each blood type, we could assess the substances circulating in the maternal–fetal bidirectional transfer system during delivery. During the examination, the size, shape, consistency, and completeness of the placenta were observed and the absence of pathologic findings recorded. The total processing time was <15 min. Blood was immediately centrifuged at 1750 *g* for 10 min at 4°C in a Beckman GS-6R refrigerated centrifuge (Beckman Instruments, Inc, Fullerton, CA) to separate the plasma and red blood cell pellets. Plasma samples were immediately frozen and stored at –80°C until analysis. Erythrocyte cytosolic and membrane fractions were prepared by differential centrifugation with hypotonic hemolysis and successive differential centrifugations according to the method of Hanahan and Ekholm.¹⁶ The final fractions were aliquoted, snap-frozen in liquid nitrogen, and stored at –80°C until analysis. Cytosolic protein content was measured by the Lowry method.¹⁷

Biochemical Parameters

Total bilirubin was measured in umbilical arterial and venous blood using Spinreact enzymatic kits (Spinreact SA, Girona, Spain), according to the manufacturer's instructions.

Inflammatory Parameters

TNF- α , IL-6, and soluble TNF- α receptor II (sTNF-RII) plasma levels were determined by using Biosource kits (Biosource Europe, Nivelles, Belgium), and PGE₂ was determined using an R&D kit (R&D Systems Europe, Abingdon, UK). The tests for TNF- α , IL-6, and PGE₂ are solid phase enzyme amplified sensitivity immunoassays performed on microtiter plates. When the assays

were complete, samples were read at an appropriate wavelength (450–490 nm) using a BioTek microplate reader (BioTek, Winooski, VT).

The sTNF-RII kit is a solid phase sandwich enzyme-linked immunosorbent assay. The microtiter plate was read at an appropriate wavelength (450 nm) with a BioTek microplate reader.

Oxidative Stress Parameters

Plasma total antioxidant status (TAS) was analyzed with a TAS Randox kit (Randox Laboratories Ltd, Crumlin, UK). Results were expressed in millimoles per liter of Trolox equivalents. The linearity of calibration extends to 2.5 mmol/L of Trolox. The reference range for human blood plasma is given by the manufacturer as 1.30 to 1.77 mmol/L. Duplicate measurements were used to calculate intraassay variability.

Glutathione peroxidase activity was measured according to the method of Flohé and Gunzler,¹⁸ which is based on the instantaneous formation of oxidized glutathione during the glutathione peroxidase catalyzed reaction. The resulting oxidized glutathione is continually reduced by an excess of glutathione reductase and reduced nicotinamide adenine dinucleotide phosphate present in the cuvette. The subsequent oxidation of reduced nicotinamide adenine dinucleotide phosphate was monitored spectrophotometrically (Thermo Spectronic, Rochester, NY) at 340 nm. Cumene hydroperoxide was used as the substrate for the reaction.

Catalase (CAT) activity was determined using a spectrophotometer operating at 240 nm to monitor the H₂O₂ decomposition that results from CAT activity.¹⁹ Activity was calculated from the first-order rate constant *K* (per second).

Superoxide dismutase (SOD) activity was determined according to the

method of Crapo et al.²⁰ The method is based on SOD inhibition of cytochrome *c* reduction, measured spectrophotometrically at 550 nm. One unit of SOD activity is defined as the amount of enzyme needed to produce 50% inhibition of the cytochrome *c* reduction rate.

Plasma hydroperoxide content was determined by using an Oxystat kit (Biomedica Gruppe, Vienna, Austria). Oxystat is a colorimetric assay for the quantitative determination of peroxides in plasma, serum, and other biological fluids. The peroxide concentration is determined by reaction of the biological peroxides and a subsequent color reaction using 3,3',5,5'-tetramethylbenzidine as the substrate. The plate was measured at 450 nm on a BioTek microplate reader.

Erythrocyte membrane hydroperoxide content was estimated according to the method described previously by Ochoa et al,¹⁵ which is based on the rapid peroxide-mediated oxidation of Fe²⁺ to Fe³⁺ in acidic conditions. The latter, in the presence of xylenol orange, forms an Fe³⁺-xylenol orange complex that can be measured spectrophotometrically at 560 nm (Perkin Elmer UV-VIS A-16, Norwalk, CT).

Statistical Analyses

Before any statistical analysis, we checked all variables for normality and homogeneous variance using Kolmogorov-Smirnov and Levene tests, respectively. Categorical variables were compared with χ^2 tests. Data were expressed as the mean \pm SEM. A 2-tailed Student's *t* test was used to determine significant differences. Unpaired Student's *t* tests were used to determine differences between the groups (early versus late clamping), and a paired Student's *t* test was performed to determine differences between blood samples from veins and arteries in each group (vein versus artery). A level of $P < .05$ was

considered to indicate statistical significance. SPSS software version 20.0 (IBM SPSS Statistics, IBM Corporation) was used for data treatment and statistical analysis.

RESULTS

All parents were Spanish Caucasians, and at delivery no differences were found in the age and parity of the mothers, the gestational age, gender, and weight of the newborns, and the maternal-fetal ejection (Table 1); all pregnancies followed a term gestation with cephalic presentation and normal delivery. Bilirubin was also assessed, and the results for early clamping were $31.55 \pm 2.55 \mu\text{mol/L}$ in the umbilical vein and $24.53 \pm 2.03 \mu\text{mol/L}$ in artery. In the late-clamped group the results were $29.42 \pm 1.49 \mu\text{mol/L}$ and $28.82 \pm 1.70 \mu\text{mol/L}$ for vein and artery, respectively. No differences were found between groups; however, they were observed for vein versus artery in the early-clamped group ($P < .001$).

Oxidative stress biomarkers are summarized in Table 2. In our study, erythrocyte CAT activity was significantly higher in the umbilical vein than in the artery for the early cord clamping group ($P < .001$), and we also observed greater activity for the late-clamped group compared with the early-clamped group ($P < .01$ for the umbilical vein and $P < .001$ for the artery). SOD was significantly higher in the late-clamped group than in the early-clamped group ($P < .01$).

In addition, we observed lower levels of plasma hydroperoxides in samples

from umbilical arteries than in those from veins ($P < .001$ in the early-clamped group, $P < .01$ in the late-clamped group). There were also lower levels of membrane erythrocyte hydroperoxides of the umbilical artery in the early-clamped group than in the other group ($P < .01$ for the umbilical vein in early cord clamping and $P < .05$ for the artery and vein in late cord clamping). TAS concentration was greater in the late-clamped group ($P < .001$) than in the early-clamped group. With regard to inflammatory signaling, there was greater expression of IL-6 in the umbilical artery than in the vein in both early and late cord clamping ($P < .001$) (Fig 1A). Moreover, TNF- α (Fig 1B) is lower in the umbilical vein of the early-clamped group than in the artery ($P < .001$), and also differences were found between both groups in the umbilical artery, being lower in the late-clamped group ($P < .05$). It is noteworthy that we observed a remarkable increase in sTNF-RII concentration (Fig 1C) in the late-clamped group compared with the early one ($P < .001$). Finally, no differences were observed in umbilical vein and artery PGE₂ concentrations, whether between or within either of the experimental groups (early-clamped, $871.6 \pm 50.79 \text{ pg/mL}$ and $878.8 \pm 37.94 \text{ pg/mL}$; late-clamped, $862.3 \pm 40.71 \text{ pg/mL}$ and $863.27 \pm 36.74 \text{ pg/mL}$, both measured in the vein and artery, respectively).

DISCUSSION

This study was designed to simultaneously assess the oxidative stress and

TABLE 1 General Characteristics of Mothers and Their Neonates at Delivery

	Early Cord Clamping ($n = 33$)	Late Cord Clamping ($n = 31$)
Age of mother, y	30.8 ± 0.9	29.1 ± 0.8
Parity, primiparous/multiparous	12/21	11/20
Gestational age, wk	38.9 ± 0.4	39.6 ± 0.3
Gender of newborn, male/female	17/16	16/15
Wt of newborn, g	3341.4 ± 85.8	3325.8 ± 79.0
Maternal-fetal ejection, min	46.2 ± 4.8	44.9 ± 5.9

Values are means \pm SEM. No significant differences were found between groups.

TABLE 2 Oxidative Stress Biomarkers in Umbilical Cord Veins and Arteries Taken From Both Groups

	Early Cord Clamping		Late Cord Clamping	
	Umbilical Vein	Umbilical Artery	Umbilical Vein	Umbilical Artery
Plasma parameters				
TAS, nmol/mL	0.903 ± 0.03**	0.962 ± 0.03**	1.038 ± 0.03*	1.192 ± 0.06
Plasma hydroperoxides, nmol/mL	8.021 ± 0.30*	6.752 ± 0.35	7.739 ± 0.32*	6.641 ± 0.44
Erythrocyte parameters				
Erythrocyte cytosol				
CAT, K/sec-mg	0.281 ± 0.02*,**	0.233 ± 0.01**	0.329 ± 0.01*	0.289 ± 0.01
Glutathione peroxidase, U/mg	33.79 ± 1.78	33.86 ± 1.81	31.96 ± 1.12	32.40 ± 1.58
SOD, U/mg	218.7 ± 8.79**	199.35 ± 11.32**	250.1 ± 10.25	243.1 ± 9.30
Membrane hydroperoxides, nmol/mL	26.85 ± 1.48*	21.77 ± 1.38	24.91 ± 1.30	23.45 ± 1.32

Values are means ± SEM. *Mean values differ between vein and artery within the same group, $P < .05$. **Mean values differ between groups for either veins or arteries, $P < .05$.

inflammatory signaling induced by different timings of umbilical cord clamping (early versus late), because there is little information available about this issue in the scientific literature. Approximately half of the subjects delivered early-clamped newborns (at 10 s) and the other half late-clamped babies (at 2 min). Cord blood samples were collected from the umbilical vein and artery, therefore allowing the maternal–fetal bidirectional transfer of substances to be assessed at the time of delivery. One of the most controversial points regarding timing is the concern that delayed clamping can increase the possibility of hyperbilirubinemia and jaundice.²¹ This misgiving is not supported by the current study because no differences in bilirubin levels were observed between early- and late-clamped groups.

The importance of oxidative stress during childbirth and pregnancy is well known, and it originates from several sources, including a higher production of free radicals caused by increased mitochondrial activity, the change from a hypoxic to a hyperoxic environment, and diverse mediators in the childbirth process.^{13,15} These factors are featured in both late and early cord clamping; nevertheless, in late cord clamping

another possible oxidative stress-inducing factor is the concentration of free Fe²⁺. One of the most universally accepted benefits of late cord clamping is the lower incidence of anemia, caused by higher plasma Fe levels.^{6,10} In the perinatal period, especially in preterm babies, low levels of iron carriers can increase plasma free iron, which is a prooxidant factor in both the fetus and newborn.^{22,23} This element increases the necessity to understand how late cord clamping can influence the newborn's antioxidant system.

Cord clamping timing (early versus late) involves diverse changes in antioxidant status. In our study, erythrocyte CAT activity was greater in the umbilical vein than in the artery in the early-clamped group, and we also observed an increase in the late-clamped group compared with the early-clamped group. SOD was significantly higher in the late-clamped group than in the early-clamped group. Sugino et al²⁴ suggest that SOD is steroidally regulated and that decreased activity of this enzyme is implicated in miscarriages, so we can conclude that the higher level recorded in the late-clamped group may have a positive effect on the neonate's future development, increasing their protection against the oxidative stress induced by labor. TAS

was also higher in the late-clamped group, suggesting a protective effect of late cord clamping; because an imbalance between oxidant and antioxidant levels has been noted in preterm infants, that places them at a greater risk of diseases associated with prematurity, whereas term infants appear better adapted to withstand oxidative injury caused by maturation of their antioxidant defense systems.²⁵

There are several causes of the oxidative stress induced during birth. Potential sources of ROS during parturition include the mother, the placenta and the fetus. If the fetus were the main source of ROS, then the umbilical cord arterial ROS levels would be expected to be higher than umbilical venous levels. However, we did observe a lower level of plasma hydroperoxides in umbilical arteries than in umbilical veins. This finding could suggest that the fetus metabolizes, rather than produces, these radicals. Another potential source of free radicals is the mother. Although several studies have found elevated levels of protein or lipid peroxidation products in pregnancies complicated by hypertension or preeclampsia,²⁶ other studies do not support these findings.²⁷ Nevertheless, the effect of maternal oxidative stress on the fetus may be minimal; previous studies have consistently shown a low level of correlation between maternal and cord blood plasma levels of lipid peroxidation products. The remaining potential source of free radicals is the placenta. Some studies have demonstrated lipid peroxidation in the placenta in complicated pregnancies.²⁸ However, Walsh et al²⁹ have shown that in vitro secretion of isoprostane by the placenta is 8 times greater on the maternal side of the placenta than the fetal side.

Erythrocytes have a key role in the evoked oxidative stress of the neonate. The erythrocyte membrane is

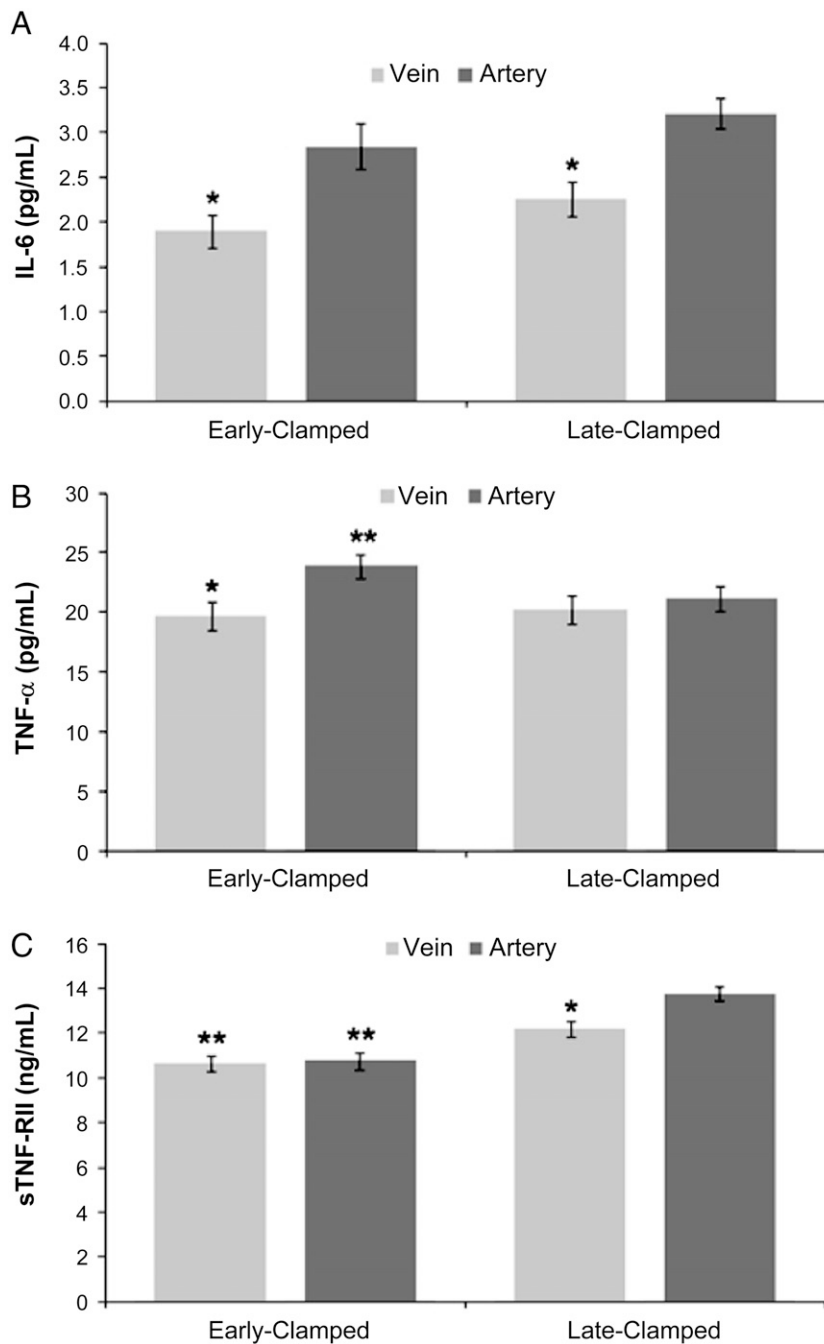


FIGURE 1

(A) IL-6, (B) TNF- α , and (C) sTNF-RII in umbilical cord veins and arteries. Values are means \pm SEM. *Mean values differ between vein and artery within the same group, $P < .05$. **Mean values differ between groups for either veins or arteries, $P < .05$.

particularly sensitive to oxidative damage because of its high polyunsaturated fatty acid content^{13,14}; therefore, it is an important system for evaluating the effect of oxidative stress. Erythrocytes are continuously exposed to ROS injury because of their high cellular oxygen

concentration and heme iron. The autoxidation of hemoglobin, generating superoxide anion radicals, is the main source of ROS in red blood cells.²² In this sense, the lower plasma and membrane erythrocyte hydroperoxide concentrations in umbilical arteries

compared with the veins in both early- and late-clamped groups indicate that the neonate has enough antioxidant capacity to scavenge for and neutralize free radicals produced during the delivery. We think that this result can be directly correlated with the levels of CAT and SOD, which both reduce the oxidative damage caused by ROS that are capable of initiating lipid peroxidation, releasing destructive catalytic enzymes, and damaging cell membranes. In addition, the activity of these enzymes was observed to be greater in the late-clamped group, providing a noteworthy advantage in the erythrocyte. Delivery at all gestational ages has clear biochemical parallels with an inflammatory response, typified by the increased output of prostaglandins, cytokines, and proinflammatory mediators, which can in turn increase the evoked oxidative stress.³⁰ The mechanisms underlying the onset of labor remain unclear. Parturition consists of 5 separate but integrated physiologic events: membrane rupture, cervical dilatation, myometrial contractility, placental separation, and uterine involution. Evidence increasingly supports the roles of prostaglandins and cytokines in each of these events. The role of the proinflammatory cytokines interleukin 1 β , IL-6, interleukin 8, and TNF- α is evident in term and preterm delivery. The uterus is activated by proinflammatory cytokines through stimulation of the expression and production of uterine activation proteins. One of these actions is the stimulation of prostaglandin synthesis. Together these feedforward mechanisms activate the uterus, trigger the production of uterine contractile stimulants, and lead to labor and delivery.^{51–53} A limitation of this study is that the number of patients was small, reflecting limitations in the number of normal deliveries at our hospital, laboratory availability, and budget.

In the current study, for both groups we observed an overexpression of IL-6 in the umbilical artery compared with the vein. Moreover, TNF- α is lower in the umbilical vein of the early-clamped group compared with the late-clamped group. We did not observe any changes in PGE₂ levels in any of the experimental groups, but, surprisingly, we did observe a remarkable increase in sTNF-RII concentration in the late-clamped group compared with early clamping. The onset of labor induces elevated concentrations of IL-6³⁴ and TNF- α .³⁵ Concentrations of TNF- α appear to correlate with the amount of granulocyte infiltration observed in the placenta. Steinborn et al,³⁶ report an increase in IL-6 produced by the placenta after the onset of spontaneous term labor, which they attribute to placental endothelial cell production. TNF-RII overexpression in the late-clamped group reduces the detrimental, proinflammatory effects of TNF in the fetus; by sequestering TNF, the soluble form of TNF-RII limits TNF avail-

ability and binding to TNF receptor I, the receptor subtype that mediates the classic proinflammatory activities of the cytokine.³⁷ In support of this mechanism, a recent TNF-RII shedding study has shown that regulatory T cells inhibit TNF activity.³⁸ TNF-RII signaling exerts neuroprotective and antiinflammatory functions,³⁹ and TNF-RII stimulation has revealed activation of the immunosuppressive interleukin 10 pathway and significantly inhibits the effects of several proinflammatory cytokines.⁴⁰ Therefore, it can be concluded that the increase in sTNF-RII observed in the current study during deliveries that practiced late cord clamping would reduce the inflammatory-mediated effects induced in the neonate.

One of the limitations of the study is the group assignment. When the first subject was assigned at random, subsequent systematic allocation to treatment group, though not pure random assignment, does not introduce a high

bias in our analysis, which has been adjusted for the different variables that could skew the results.

CONCLUSIONS

The increase in antioxidant defenses, such as increased CAT and SOD activities and TAS concentrations, together with the increase in sTNF-RII in the late-clamped group, reveals that delayed cord clamping could have a positive effect in the neonate, increasing the antioxidant capacity and ameliorating the inflammatory-mediated effects induced by the delivery. These findings reveal novel, additional, and relevant information about clamping timing, indicating that late cord clamping could be a beneficial method of neonatal care, influencing antioxidant status and inflammatory signaling in the neonate, leading us to believe that it would be useful to introduce late umbilical cord clamping as a routine procedure in maternal–fetal medicine.

REFERENCES

1. World Health Organization. *Care in Normal Birth: A Practical Guide*. Geneva, Switzerland: WHO Safe Motherhood Technical Working Group; 1996:32–33
2. Zaramella P, Freato F, Quaresima V, et al. Early versus late cord clamping: effects on peripheral blood flow and cardiac function in term infants. *Early Hum Dev*. 2008;84(3):195–200
3. De Paco C, Florido J, Garrido MC, Prados S, Navarrete L. Umbilical cord blood acid–base and gas analysis after early versus delayed cord clamping in neonates at term. *Arch Gynecol Obstet*. 2011;283(5):1011–1014
4. World Health Organization. *Care of the Umbilical Cord: A Review of the Evidence*. Geneva, Switzerland: World Health Organization; 1998:11–12
5. Wardrop CA, Holland BM. The roles and vital importance of placental blood to the newborn infant. *J Perinat Med*. 1995;23(1–2):139–143
6. McDonald SJ, Middleton P, Dowswell T, Morris PS. Effect of timing of umbilical cord clamping of term infants on maternal and neonatal outcomes [Review]. *Cochrane Database Syst Rev*. 2013;7:CD004074
7. Ibrahim HM, Krouskop RW, Lewis DF, Dhanireddy R. Placental transfusion: umbilical cord clamping and preterm infants. *J Perinatol*. 2000;20(6):351–354
8. McDonnell M, Henderson-Smart DJ. Delayed umbilical cord clamping in preterm infants: a feasibility study. *J Paediatr Child Health*. 1997;33(4):308–310
9. Lozoff B, Jimenez E, Wolf AW. Long-term developmental outcome of infants with iron deficiency. *N Engl J Med*. 1991;325(10):687–694
10. Rabe H, Diaz-Rossello JL, Duley L, Dowswell T. Effect of timing of umbilical cord clamping and other strategies to influence placental transfusion at preterm birth on maternal and infant outcomes. *Cochrane Database Syst Rev*. 2012;8:CD003248
11. Mercer JS, Vohr BR, McGrath MM, Padbury JF, Wallach M, Oh W. Delayed cord clamping in very preterm infants reduces the incidence of intraventricular hemorrhage and late-onset sepsis: a randomized, controlled trial. *Pediatrics*. 2006;117(4):1235–1242
12. Blouin B, Penny ME, Maheu-Giroux M, et al. Timing of umbilical cord-clamping and infant anaemia: the role of maternal anaemia. *Paediatr Int Child Health*. 2013;33(2):79–85
13. Ochoa JJ, Ramirez-Tortosa MC, Quiles JL, et al. Oxidative stress in erythrocytes from premature and full-term infants during their first 72 h of life. *Free Radic Res*. 2003;37(3):317–322
14. Ochoa JJ, Contreras-Chova F, Muñoz S, et al. Fluidity and oxidative stress in erythrocytes from very low birth weight infants during their first 7 days of life. *Free Radic Res*. 2007;41(9):1035–1040
15. Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, et al. Inflammation, oxidative stress, and obesity. *Int J Mol Sci*. 2011;12(5):3117–3132
16. Hanahan DJ, Ekholm JE. The preparation of red cell ghosts (membranes). *Methods Enzymol*. 1974;31:168–172
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem*. 1951;193(1):265–275

18. Flohé L, Günzler WA. Assays of glutathione peroxidase. *Methods Enzymol.* 1984;105:114–121
19. Aebi H. Catalase in vitro. *Methods Enzymol.* 1984;105:121–126
20. Crapo JD, McCord JM, Fridovich I. Preparation and assay of superoxide dismutases. *Methods Enzymol.* 1978;53:382–393
21. American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics.* 2004;114(1):297–316
22. Perrone S, Tataranno ML, Stazzoni G, Del Vecchio A, Buonocore G. Oxidative injury in neonatal erythrocytes. *J Matern Fetal Neonatal Med.* 2012;25(suppl 5):104–108
23. Berger HM, Mumby S, Gutteridge JM. Ferrrous ions detected in iron-overloaded cord blood plasma from preterm and term babies: implications for oxidative stress. *Free Radic Res.* 1995;22(6):555–559
24. Sugino N, Nakata M, Kashida S, Karube A, Takiguchi S, Kato H. Decreased superoxide dismutase expression and increased concentrations of lipid peroxide and prostaglandin F(2alpha) in the decidua of failed pregnancy. *Mol Hum Reprod.* 2000;6(7):642–647
25. Buhimschi IA, Buhimschi CS, Pupkin M, Weiner CP. Beneficial impact of term labor: nonenzymatic antioxidant reserve in the human fetus. *Am J Obstet Gynecol.* 2003;189(1):181–188
26. Zusterzeel PL, Mulder TP, Peters WH, Wiseman SA, Steegers EA. Plasma protein carbonyls in nonpregnant, healthy pregnant and pre-eclamptic women. *Free Radic Res.* 2000;33(5):471–476
27. Regan CL, Levine RJ, Baird DD, et al. No evidence for lipid peroxidation in severe preeclampsia. *Am J Obstet Gynecol.* 2001;185(3):572–578
28. Weinberger B, Nisar S, Anwar M, Ostfeld B, Hegyi T. Lipid peroxidation in cord blood and neonatal outcome. *Pediatr Int.* 2006;48(5):479–483
29. Walsh SW, Vaughan JE, Wang Y, Roberts LJ II. Placental isoprostane is significantly increased in preeclampsia. *FASEB J.* 2000;14(10):1289–1296
30. Kelly RW. Inflammatory mediators and parturition. *Rev Reprod.* 1996;1(2):89–96
31. Olson DM. The role of prostaglandins in the initiation of parturition. *Best Pract Res Clin Obstet Gynaecol.* 2003;17(5):717–730
32. Christiaens I, Zaragoza DB, Guilbert L, Robertson SA, Mitchell BF, Olson DM. Inflammatory processes in preterm and term parturition. *J Reprod Immunol.* 2008;79(1):50–57
33. Djuardi Y, Wibowo H, Supali T, et al. Determinants of the relationship between cytokine production in pregnant women and their infants. *PLoS ONE.* 2009;4(11):e7711
34. Gunn L, Hardiman P, Tharmaratnam S, Lowe D, Chard T. Measurement of interleukin-1 alpha and interleukin-6 in pregnancy-associated tissues. *Reprod Fertil Dev.* 1996;8(7):1069–1073
35. Opsjtn SL, Wathen NC, Tingulstad S, et al. Tumor necrosis factor, interleukin-1, and interleukin-6 in normal human pregnancy. *Am J Obstet Gynecol.* 1993;169(2 pt 1):397–404
36. Steinborn A, Kühnert M, Halberstadt E. Immunomodulating cytokines induce term and preterm parturition. *J Perinat Med.* 1996;24(4):381–390
37. Van Zee KJ, Kohno T, Fischer E, Rock CS, Moldawer LL, Lowry SF. Tumor necrosis factor soluble receptors circulate during experimental and clinical inflammation and can protect against excessive tumor necrosis factor alpha in vitro and in vivo. *Proc Natl Acad Sci USA.* 1992;89(11):4845–4849
38. van Mierlo GJ, Scherer HU, Hameetman M, et al. Cutting edge: TNFR-shedding by CD4+CD25+ regulatory T cells inhibits the induction of inflammatory mediators. *J Immunol.* 2008;180(5):2747–2751
39. Serrano J, Alonso D, Encinas JM, et al. Adrenomedullin expression is up-regulated by ischemia-reperfusion in the cerebral cortex of the adult rat. *Neuroscience.* 2002;109(4):717–731
40. Gonzalez P, Burgaya F, Acarin L, Peluffo H, Castellano B, Gonzalez B. Interleukin-10 and interleukin-10 receptor-I are upregulated in glial cells after an excitotoxic injury to the postnatal rat brain. *J Neuropathol Exp Neurol.* 2009;68(4):391–403

The Timing of Cord Clamping and Oxidative Stress in Term Newborns
Javier Díaz-Castro, Jesus Florido, Naroa Kajarabille, Maria Garrido-Sánchez, Carmen Padilla, Catalina de Paco, Luis Navarrete and Julio J. Ochoa
Pediatrics 2014;134;257; originally published online July 14, 2014;
DOI: 10.1542/peds.2013-3798

Updated Information & Services	including high resolution figures, can be found at: /content/134/2/257.full.html
References	This article cites 38 articles, 9 of which can be accessed free at: /content/134/2/257.full.html#ref-list-1
Post-Publication Peer Reviews (P³Rs)	4 P ³ Rs have been posted to this article /cgi/eletters/134/2/257
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): Neonatology /cgi/collection/neonatology_sub Maternal and Fetal Medicine /cgi/collection/maternal_fetal_medicine_sub
Permissions & Licensing	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: /site/misc/Permissions.xhtml
Reprints	Information about ordering reprints can be found online: /site/misc/reprints.xhtml

PEDIATRICS is the official journal of the American Academy of Pediatrics. A monthly publication, it has been published continuously since 1948. PEDIATRICS is owned, published, and trademarked by the American Academy of Pediatrics, 141 Northwest Point Boulevard, Elk Grove Village, Illinois, 60007. Copyright © 2014 by the American Academy of Pediatrics. All rights reserved. Print ISSN: 0031-4005. Online ISSN: 1098-4275.

American Academy of Pediatrics

DEDICATED TO THE HEALTH OF ALL CHILDREN™



PEDIATRICS®

OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

The Timing of Cord Clamping and Oxidative Stress in Term Newborns

Javier Díaz-Castro, Jesus Florido, Naroa Kajarabille, Maria Garrido-Sánchez, Carmen Padilla, Catalina de Paco, Luis Navarrete and Julio J. Ochoa

Pediatrics 2014;134;257; originally published online July 14, 2014;

DOI: 10.1542/peds.2013-3798

The online version of this article, along with updated information and services, is located on the World Wide Web at:

</content/134/2/257.full.html>

PEDIATRICS is the official journal of the American Academy of Pediatrics. A monthly publication, it has been published continuously since 1948. PEDIATRICS is owned, published, and trademarked by the American Academy of Pediatrics, 141 Northwest Point Boulevard, Elk Grove Village, Illinois, 60007. Copyright © 2014 by the American Academy of Pediatrics. All rights reserved. Print ISSN: 0031-4005. Online ISSN: 1098-4275.

American Academy of Pediatrics

DEDICATED TO THE HEALTH OF ALL CHILDREN™

