# Antioxidant Profiles in Full Term and Preterm Neonates

Yuan-Shun Lee, MD; Yi-Hung Chou[1], MD

**Background:** Free radical damage has been recognized to be a common pathogenic mechanism of many neonatal diseases associated with oxygen toxicity.

**Methods:** A set of antioxidant measurements were used to investigate differences in levels between full term and premature infants after birth. Four groups of full term and preterm infants were enrolled, including full term appropriate-for-age (FT-AGA), full term small-for-age (FT-SGA), larger preterm (LPT) and smaller preterm infants (SPT). After birth, seven antioxidants, including superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), nicotinamide-adenine dinucleotide phosphate (NADP) represented by the NADPH ratio (NADPH, the reduced form of NADP)/(NADPNADPH), glucose-6-phosphate dehydrogenase (G6PD), and vitamins A and E were measured.

**Results:** FT-SGA infants had significantly lower levels of GSH, NADPH ratio and vitamin A than the FT-AGA infants but higher CAT, G6PD and vitamin E levels. LPT infants had lower levels of CAT, GSH, NADPH ratio and vitamin A but higher G6PD activity than the FT-AGA infants. SPT infants showed the same pattern of differences in various antioxidants as those of the LPT infants when compared to FT-AGA infants. Vitamin E levels did not statistically differ between SPT and LPT infants. SPT infants had significantly lower levels of GSH and NADPH ratio than the LPT infants.

**Conclusions:** Intrauterine growth retardation and prematurity may influence antioxidant imbalance and free radical damage. In addition, such data for healthy full term and preterm infants may be used as reference data when evaluating antioxidant deficiency in high-risk neonates.

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**Key words: antioxidants, neonates.**

Birth is, in itself, a hyperoxic challenge. Extrauterine aerobic existence requires efficient cellular electron transport systems to produce energy. In concert with energy-producing oxidative metabolism, biochemical defenses protect against oxidation of cellular constituents by oxygen radicals.[1-4]

Free radical damage has been recognized as a common pathogenic mechanism of many neonatal diseases associated with oxygen toxicity.[4-8] It may evolve after hyperoxia or post-ischemic reperfusion. Since there are many interacting complex antioxidant defense systems, their individual activities should be determined first, and subsequently the relationship between diseases and antioxidants can be further investigated. Major antioxidant systems include enzymes (superoxide dismutase, catalase, glutathione peroxidase), vitamins (vitamins A, E and C) and iron-associated antioxidants (ceruloplasmin,

From the Department of Pediatrics, St. Paul's Hospital, Taoyuan; [1]Division of Neonatology, Department of Pediatrics, Chang Gung Children's Hospital, Chang Gung University, Taoyuan, Taiwan.
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Correspondence to: Dr. Yi-Hung Chou, Division of Neonatology, Department of Pediatrics, Chang Gung Children's Hospital, 5, Fushing Street, Gueishan Shiang, Taoyuan, Taiwan 333, R.O.C. Tel.: 886-3-3281200 ext. 8203; Fax: 886-3-3288957; E-mail: yhchou@cgmh.org.tw

apotransferrin).[9-13] The metabolites of lipid peroxidation (the major route of free radical damage) can then be elucidated, including lipid peroxide, malondialdehyde and ethane/pentane in expired air.[14-16]

In this study, we aimed to establish the normal range of antioxidants in sera of healthy full term neonates at birth, including appropriate-for-age (AGA) and small-for-age (SGA) infants, and to investigate the antioxidant profiles of older (33-37 weeks gestation) and younger uncomplicated premature infants (< 33 weeks gestation) at birth.

## METHODS

To investigate the antioxidant status of neonates, we conducted this prospective study from January 2003 to June 2003. Neonates admitted to the normal nursery were consecutively enrolled, except those with the following exclusion criteria: known intrauterine infection, major malformation, abnormal fetal monitoring, need for resuscitation, evidence of perinatal hypoxia or respiratory distress. Those admitted to the neonatal intensive care unit were also enrolled using the same criteria. The neonates were further divided into four groups: Group I consisted of full term, appropriate-for-age infants (FT-AGA, 100 patients with gestational ages of 38-42 weeks); Group II consisted of full term, small-for-age infants (FT-SGA, 30 patients with gestational ages of 38-42 weeks); Group III consisted of larger preterm infants (LPT, 30 patients with gestational ages of 33-36 weeks); and Group IV consisted of smaller preterm infants (SPT, 30 patients with gestational ages of less than 33 weeks). Specimens of venous blood were withdrawn within two hours of birth, after informed consent was obtained from all parents. The project was approved by the Ethics committee of Chang Gung Memorial Hospital. Two milliliters of blood was withdrawn, then immediately centrifuged. The supernatants were liquated into three appendorf tubes. The measurements included the seven antioxidants superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), NADPH ratio (NADPH/NADP-NADPH), G6PD, and vitamins A and E. The analytic methods used have been described previously.[1-3,8-12] The intrarun and between run coefficients of variation (CV) of each analysis were within 5-15%.

To analyze the data, the Student's t test statistical method was used, with the significance level set at $p < 0.05$.

## RESULTS

Among the full term neonates, 79 had normal spontaneous delivery (42 males, 37 females) and 51 were delivered by cesarean section (28 males, 23 females). The characteristics of the four groups of neonates are listed in Table 1. Measurements of the FT-AGA and FT-SGA groups' venous blood are listed in Table 2. Several antioxidants in the FT-SGA group showed significantly lower levels, including GSH, NADPH ratio and vitamin A; in contrast, higher CAT, G6PD and vitamin E levels were observed

**Table 1.** Clinical Characteristics of the Four Groups of Neonates

| Items | FT-AGA (n = 100) | FT-SGA (n = 30) | LPT (n = 30) | SPT (n = 30) |
|---|---|---|---|---|
| Gestational Age | 40.2 ± 1.6 | 38.6 ± 1.4 | 35.2 ± 2.4 | 29.6 ± 2.7 |
| Birth Weight | 3024 ± 275 | 2450 ± 370 | 2080 ± 420 | 1350 ± 390 |
| Gender (M/F) | 53/47 | 17/13 | 16/14 | 13/17 |
| Parity (1st) | 42 | 16 | 18 | 17 |
| NSD/ C-section | 62/38 | 17/13 | 14/16 | 12/18 |
| PROM | 9 | 3 | 5 | 6 |
| PIH | 6 | 4 | 5 | 4 |
| Maternal Smoking | 8 | 3 | 4 | 3 |
| Maternal Diabetes | 5 | 2 | 3 | 2 |
| Resuscitation at Birth | 0 | 0 | 2 | 6 |
| Respiratory Support | 0 | 0 | 2 | 8 |

**Abbreviations:** FT-AGA: full term appropriate-for-age; FT-SGA: full term small-for-age; LPT: larger preterm infants; SPT: smaller preterm infants; M/F: male/female; NSD: normal spontaneous delivery; C-section: cesarean section; PROM: Premature rupture of membrane; PIH: Pregnancy-induced hypertension.

**Table 2.** Antioxidant Profiles of Fullterm Neonates

| Measurement | FT-AGA (n = 100) | FT-SGA (n = 30) | *p* value |
|---|---|---|---|
| SOD (U/mg Hb) | 1.02 ± 0.46 | 1.22 ± 0.58 | 0.68 |
| CAT (U/mg Hb) | 198.9 ± 82.5 | 242.4 ± 73.7 | < 0.05 |
| GSH (nmol/mg Hb) | 9.12 ± 3.65 | 4.76 ± 1.34 | < 0.001 |
| NADPH ratio | 0.62 ± 0.23 | 0.26 ± 0.07 | < 0.001 |
| G6PD activity | 306.4 ± 106.7 | 395.4 ± 42.7 | < 0.001 |
| Vitamin A ((g/dl) | 13.65 ± 0.80 | 12.18 ± 2.37 | < 0.001 |
| Vitamin E (mg/dl) | 0.24 ± 0.10 | 0.31 ± 0.10 | < 0.01 |

**Abbreviations:** FT-AGA: full term appropriate-for-age; FT-SGA: full term small-for-age; SOD: superoxide dismutase; CAT: catalase; GSH: glutathione; NADP: nicotinamide-adenine dinucleotide phosphate; G6PD: Glucose-6-phosphate dehydrogenase.

when compared to the FT-AGA infants.

Among the preterm infants, 26 had normal spontaneous delivery (13 males, 13 females) and 34 were delivered by cesarean section (16 males, 18 females). Two of the larger preterm infants and eight of the smaller preterm infants needed respiratory support by nasal continuous positive pressure or intermittent mandatory ventilation. Antioxidant venous blood measurements for both groups are listed in Table 3. SPT infants showed the same pattern of differences for various antioxidants as those of the LPT infants, when compared to FT-AGA infants. Vitamin E levels did not statistically differ between SPT and LPT infants. As expected, SPT infants had significantly lower levels of GSH and NADPH ratio than the LPT infants.

## DISCUSSION

The results of this study provide antioxidant status for neonates that may have clinical implications. The presence of intrauterine growth retardation in full term neonates caused significant differences in antioxidant measurements, and this has not previously been reported. Older preterm infants had lower levels of several parameters, which fell between the levels for FT-AGA and SPT infants. Younger preterm infants had the lowest levels for most parameters, less than those of FT-AGA and LPT infants, and even those of FT-SGA infants. A set of normal data in uneventful neonates was established, which can be utilized as a reference for Taiwanese neonates. Measurements of various antioxidants can be performed in the laboratory within eight hours in the best conditions, providing timely information for the therapeutic care of neonates.

**Table 3.** Antioxidant Profiles of Preterm Neonates

| Measurement | LPT (n = 30) | SPT (n = 30) | *p* value |
|---|---|---|---|
| SOD (U/mg Hb) | 1.21 ± 0.38 | 1.27 ± 0.72 | 0.68 |
| CAT (U/mg Hb) | 174.5 ± 110.3 | 161.3 ± 75.6 | 0.59 |
| GSH (nmol/mg Hb) | 7.43 ± 2.61 | 4.88 ± 2.76 | < 0.001 |
| NADPH ratio | 0.49 ± 0.28 | 0.32 ± 0.26 | < 0.05 |
| G6PD activity | 412.6 ± 237.6 | 483.4 ± 55.1 | 0.11 |
| Vitamin A (μg/dl) | 12.15 ± 1.37 | 12.81 ± 2.43 | 0.20 |
| Vitamin E (mg/dl) | 0.22 ± 0.06 | 0.24 ± 0.16 | 0.52 |

**Abbreviations:** LPT: larger preterm infants; SPT: smaller preterm infants; SOD: superoxide dismutase; CAT: catalase; GSH: glutathione; NADP: nicotinamide-adenine dinucleotide phosphate; G6PD: Glucose-6-phosphate dehydrogenase.

As reported before, SOD and CAT increase along with gestation in many species, especially those born before the two-thirds point of gestation.[1,2,9,17,18] In this study, a significant difference in CAT was only observed between SPT and FT-AGA infants, and was not seen between LPT and FT-AGA infants. Also lower SOD values were mostly seen in extremely-low-birth-weight infants (< 1000 g). The inadequate difference in gestational ages among the FT-AGA, FT-SGA and LPT infants may have contributed to these results.

GSH and the NADPH ratio are believed to be important indices of the cellular redox state.[6,7,19-21] In this series, both SPT and FT-SGA infants had lower values. Levels of the former group were consistent with other reports,[17,20] although this has not been previously mentioned for FT-SGA infants. This observation may be explained by chronic insufficiency of placental function, which leads to inadequate nutrient delivery or impaired recycling to the fetus.

Patients with G6PD deficiency often have lower antioxidant capacities.[22-24] It has been postulated that total antioxidant activity is a reflection of the integrity of cell membranes; if several indices including G6PD are decreased, then the vulnerability of cells to destruction is increased, i.e. they are more susceptible to oxidant injury. In this report, G6PD levels were higher in FT-SGA and preterm infants than in FT-AGA infants, which may imply a compensatory mechanism in the global defense of cells to combat an adverse environment.

Vitamin A is also known as an important antioxidant in primates.[25,26] It is said to facilitate the re-epithelialization of airways, which may have a crucial role in the treatment of bronchopulmonary dysplasia (BPD). In addition, vitamin A supplementation can partially reverse deficiencies in vitamin A and other antioxidant levels after prolonged oxygen therapy.[27,28] In our series, vitamin A levels were significantly lower in SPT, LPT and FT-SGA infants, in comparison to the FT-AGA group, which may indicate a lower capacity for both lower birth weight and gestational age.

Vitamin E is a potent antioxidant in cell membranes. Some investigators have claimed that vitamin E supplementation may decrease the incidence of retinopathy of prematurity (ROP), while other

reports have revealed that vitamin E may be helpful in the course of IVH and BPD.[29-31] For the studied neonates, vitamin E levels were consistently low at birth in all groups, which may reflect the fact that placental transport was insufficient to maintain an adequate level (> 0.5 mg/dl). Thus, earlier vitamin E supplementation may have benefits for high-risk infants, especially those who cannot be fed orally for the first few weeks.

In analyzing the parameters among different groups, three aspects should be emphasized. First, there was heterogeneity within the groups, which was blunted by using mean measurements; thus high-risk infants may be identified only if the entire panel of antioxidants is measured at the same time. Second, individual antioxidant measurements provide information on only one dimension of cellular defense, and an overall measurement presented by total antioxidant activity or TRAP may be more decisive as to the ultimate defense capacity.[32,33] Third, different gestational ages, birth weights and other inert factors (underlying oxidant stresses) constituted the basis of intergroup differences.

Our study demonstrates that there are marked differences among neonates with different gestational ages and intrauterine growth. Also, we designed a panel of antioxidants that are involved in the oxidative-free radical balance. Further, temporal changes of important antioxidants in the first two weeks that have not mentioned before, were analyzed. This study has practical and obvious implications: it offers clinicians more understanding of the major antioxidant systems, and may help in the refinement of management strategies for nutrition and the intensive care of neonates.

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# 足月及早產新生兒體內之抗氧化物質概況

李淵順 周怡宏[1]

**背 景：** 自由基傷害已經被確認是造成許多與氧氣毒性有關之新生兒疾病的常見病理機轉。這次研究的目的是測量足月兒及早產兒出生後的一些血中抗氧化物質的濃度，藉此研究彼此間是否有差異。

**方 法：** 本研究將所有足月兒及早產兒分成四組：出生體重與出生週數相符 (AGA) 的足月兒，出生體重小於出生週數應有體重 (SGA) 的足月兒，較大的早產兒及較小的早產兒。測量出生後七種血中抗氧化物質的濃度，包括 superoxide dismutase (SOD)，catalase (CAT)，glutathione (GSH)，nicotinamide-adenine dinucleotide phosphate (NADP)（以 NADPH ratio 代表），Glucose-6-phosphate dehydrogenase (G6PD)，維生素 A 及 E。

**結 果：** 在出生體重小於出生週數應有體重的足月兒比起出生體重與出生週數相符的足月兒，有著明顯較低的 GSH，NADPH ratio 及維生素 A 數值，而有著明顯較高的 CAT，G6PD 及維生素E數值。較大的早產兒比起出生體重與出生週數相符的足月兒，有著明顯較低的 CAT，GSH，NADPH ratio 及維生素 A 數值，而有著明顯較高的 G6PD 活性。較小的早產兒比起出生體重與出生週數相符的足月兒的情形與較大的早產兒比起出生體重與出生週數相符的足月兒的情形相同。較小的早產兒比起較大的早產兒，有著明顯較低的GSH 與NADPH ratio 數值。

**結 論：** 子宮內之生長遲滯以及早產可能會影響到抗氧化物質與自由基傷害間的平衡關係。本研究所得到的這些數據，可以用來作爲評估高危險新生兒抗氧化物質缺乏情況的參考依據。

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**關鍵字：**抗氧化物質，新生兒。

桃園聖保祿修女會醫院 小兒科；[1]長庚兒童醫院 台北院區 新生兒科
受文日期：民國94年7月25日；接受刊載：民國94年10月20日
通訊作者：周怡宏醫師，長庚兒童醫院，新生兒科。桃園縣333龜山鄉復興街5號。Tel.: (03)3281200轉8203; Fax: (03)3288957; E-mail: yhchou@cgmh.org.tw