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# Antioxidative status and oxidative stress in the fetal circulation at birth: the effects of time of delivery and presence of labor☆

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ABSTRACT

*Background*: There are important physiological changes in the maternal, placental, and fetal compartments during pregnancy and labor. Increased oxidative stress has been demonstrated during labor. Melatonin has been reported to serve as an indirect antioxidant via the stimulation and induction of antioxidant enzymes as superoxide dismutase (SOD) and glutathione peroxidase (Gpx) in several tissues.
*Aim*: To assess whether the melatonin status, presence of labor at the time of birth and the time of delivery influence the extracellular antioxidative enzymes and DNA oxidative stress in newborns.
*Methods*: The extracellular antioxidative status and oxidative stress were analyzed by measuring the concentrations of the SOD3, Gpx3 and 8-hydroxydeoxyguanosine (8-OHdG) in the cord blood of 135 newborns. Newborns delivered during the day and at night and newborns delivered by spontaneous vaginal delivery (labor group) or elective caesarean section delivery (no labor group) were studied.
*Outcome measures*: The concentration of melatonin, SOD3, Gpx3 and 8-OHdG.
*Results*: Independent of the time of delivery, we found significantly higher melatonin, SOD3 and Gpx3 but lower 8-OHdG concentrations in the labor group than in the no labor group. We did not observe a correlation between the concentration of melatonin and SOD3, Gpx3 or 8-OHdG, or a day–night difference in SOD3, Gpx3 or 8-OHdG.
*Conclusion*: Our findings suggest that oxidative stress during labor leads to an elevation of melatonin, SOD3 and Gpx3 in the fetal circulation, protecting the newborn from serious impairment, which is reflected by lower 8-OHdG levels. The melatonin status at the time of birth does not influence the extracellular SOD3 or Gpx3 concentrations.



## 1. Introduction

Oxidative stress represents an imbalance between the generation of free radicals, e.g., reactive oxygen species (ROS) and reactive nitrogen species (RNS), and the ability of the antioxidant defense mechanisms to detoxify these radicals. The antioxidant defense consist of enzymatic antioxidant mechanisms like glutathione peroxidase (Gpx), superoxide dismutase (SOD), and catalase and non-enzymatic antioxidants including ascorbic acid, glutathione, melatonin (MT), and many others. ROS, such as the superoxide radical, the hydroxyl radical and hydrogen peroxide, cause significant damage to macromolecules, including DNA [1]. 8-Hydroxydeoxyguanosine (8-OHdG) results from the oxidative modification of the guanosine nitrogen base in DNA and acts as a marker of DNA oxidative stress [2].

During pregnancy the susceptibility towards oxidative stress increases in the placenta because of increased metabolic activity and reduced scavenging power of antioxidants [3]. An increase in oxidative stress has been demonstrated during labor. Uterine contractions during labor cause intermittent utero-placental hypoperfusion, leading to intervals of recurrent hypoxia and re-oxygenation. This intermittent change in the partial oxygen pressure leads consecutively to an increased production of free radicals [4–6].

The SOD family consists of three antioxidative isoenzymes (SOD1, 2 and 3) that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide [7]. SOD3 (copper and zinc SOD; EC-SOD) is the major SOD isoenzyme in extracellular fluids such as plasma, lymph, and synovial fluid [8]. The Gpx family consists of antioxidative isoenzymes that have the ability to reduce organic hydroperoxides to their

*Abbreviations:* MT, melatonin; SOD, superoxide dismutase; Gpx, glutathione peroxidase; 8-OHdG, 8-hydroxydeoxyguanosine; ROS, reactive oxygen species; RNS, reactive nitrogen species; UA, umbilical artery; UV, umbilical vein; BGA, blood gas analyses; ELISA, enzyme linked immunosorbent assay; BE, base excess; sβ, Standardized Beta.

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corresponding alcohols as well as free hydrogen peroxide to water in the presence of reduced glutathione. Seven isoforms are known to date, and they represent the major cellular defenses against oxidative stress [9]. Gpx3, also called plasma Gpx, is an extracellular enzyme that is secreted into the plasma, milk and lung [10]. MT is a small lipophilic neurohormone that is mainly generated in the pineal gland. The secretion of MT follows a circadian rhythm, with higher levels secreted at night [11]. In addition to its direct antioxidant potential, MT serves as an indirect antioxidant through the induction of several enzymes, such as SOD, Gpx, or catalase, and through the inhibition of nitric oxide synthetase [12,13].

There are important physiological changes in the maternal, placental, fetal, and intraamniotic compartments during pregnancy. The majority of studies on the antioxidative status during labor have focused on maternal and placental parameters. Only a few studies have been performed regarding the antioxidative status of the fetus. However, it remains controversial, whether labor per se influences the antioxidative status in newborns at birth. Some investigators observed an increased oxidative stress associated with either absence [14], or presence of labor [15,16], while others have found no association between oxidative stress and the absence or presence of labor [4,17,18]. We previously demonstrated that the MT concentration (MTc) in umbilical cord blood depends not only on the time of delivery but also on the absence or presence of labor at birth [19]. We found significantly higher MTc in the labor group compared with no labor group at night- and day-time, suggesting a physiological role of MT at the onset of labor. It remains, however, until now unclear, whether the high serum MTc has any additional effect on antioxidant status of fetus at birth. The main purpose of the present study was to evaluate whether the MT status during labor influences the fetal antioxidative status by measuring the SOD3 and Gpx3 concentrations in the newborn cord blood. The second aim was to investigate whether the concentrations of SOD3 and Gpx3 depend on the time of delivery and the absence or presence of labor at the time of birth, which we verified for MT in our previous study [19]. To estimate the level of oxidative stress in the fetus, we measured the 8-hydroxydeoxyguanosine (8-OHdG), a marker of DNA oxidative stress.

## 2. Material and methods

### 2.1. Subjects

In accordance with the Declaration of Helsinki, the study was approved by the Ethics Committee of the University of Bonn. We obtained written informed parental consent for all of the newborns.

The study was carried out in 135 healthy newborns born at the University of Bonn between 2011 and 2012 according to the following criteria: (1) singleton live birth by spontaneous vaginal delivery (labor group) or elective caesarean section delivery (no labor group); (2) gestational age between 34 and 42 weeks; (3) body weight of the newborn greater than 2000 g; (4) Apgar scores of $\geq 6$ at the 1st min and of $\geq 8$ at a postnatal age of 5 min; and (5) an umbilical artery pH $\geq$ 7.1.

We excluded all newborn infants with congenital malformations, inborn errors of metabolism, blood group incompatibility or sepsis. Further exclusion criteria were diabetic mothers, multiple gestations and pregnancies with increased perinatal stress by pregnancy complications (e.g., preeclampsia, inflammatory conditions) and intrapartum complications (e.g., emergency caesarean section, spontaneous vaginal delivery by using forceps or a vacuum device, pathological Doppler, pathological cardiotocography, nuchal cord).

The newborns were divided into four groups according the absence or presence of labor at the time of birth and time of delivery: labor group, no labor group, daytime group (0900-2100) and nighttime group (2100-0900).

### 2.2. Blood collection procedure

One minute after umbilical cord clamping and using a clamped segment of the umbilical cord, we carefully aspirated 2–5 ml fetal blood in non-heparin-containing syringes from the umbilical artery (UA) and umbilical vein (UV). Blood gas analyses (BGA) were performed within 3 min of delivery with a Rapidlab 1245 (Siemens, Eschborn, Germany) blood gas system. Blood samples were centrifuged at 3000 g for 5 min. The plasma was pipetted and frozen at −80 °C until analysis.

### 2.3. Measurement of MT

Plasma MTc was measured using a commercial radioimmunoassay kit (MT direct RIA, IBL-International, Hamburg, Germany). According to the manufacturer's protocol, the intra- and inter-assay coefficients of variation were 3.9–6.9% at a range of 28.8 to 266 pg/ml and 6.2–15.9% at a range of 3.5 to 281 pg/ml. The mean recovery of MT was 102%, and the sensitivity of the assay was 0.9 pg/ml.

### 2.4. Measurement of SOD3

Plasma SOD3 concentration was measured using a commercially available enzyme linked immunosorbent assay (ELISA) kit (Cu/ZnSOD ELISA, IBL-International, Hamburg, Germany). The intra- and inter-assay coefficients of variation were 5.1% and 5.8%, respectively.

### 2.5. Measurement of Gpx3

Gpx3 concentration was assayed using a commercially available ELISA kit (ALPCO Diagnostics, Salem, New Hampshire, USA). The intra- and inter-assay coefficients of variation for Gpx3 were 4.21–9.64% and 1.12–5.04%, respectively.

### 2.6. Measurement of 8-OHdG

To measure the endogenous oxidative DNA damage, we used an ELISA kit (8-OHdG check; IBL-International, Hamburg, Germany). The sensitivity of the assay was 0.5 ng/ml, and the range was 0.5–200 ng/ml.

## 3. Statistical analyses

The statistical analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). The variables were tested for normality using the Kolmogorov–Smirnov test. Because the SOD3-, Gpx3-, 8-OHdG- and MT levels were not normally distributed, we used the two-tailed non-parametric Mann–Whitney U-test for the group comparison. The non-parametric Friedman and Wilcoxon signed-rank tests for paired samples were used for the inferential statistics. Correlation coefficients and stepwise multiple linear regression analyses were used to analyze associations among gestational age, absence or presence of labor at the time of birth, time of delivery, pH, base excess (BE), MTc, SOD3-, Gpx3- and 8-OHdG-levels. For all analyses, p-values of less than 0.05 were considered statistically significant.

## 4. Results

The demographic and clinical characteristics of the newborns enrolled in the study are shown in Table 1.

### 4.1. Time of delivery

The median (Interquartile Range, IQR) gestational age did not significantly differ between the day- and night-time groups (38.1 weeks (37.4–39.0) and 37.9 weeks (37.4–38.9), respectively; $p = 0.612$). Compared with the daytime group, the MTc in the nighttime group was significantly higher in the UA and in the UV ($p < 0.001$). None of the other