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Melatonin Levels during the First Week of Life and Their Relation with the Antioxidant Response in the Perinatal Period

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Key Words
Melatonin · Premature newborns · Oxidative stress · Antioxidants

Abstract
Aim: Melatonin is a potent free radical scavenger and an indirect antioxidant. Knowledge about the behavior of melatonin secretion in the early neonatal period, which may relate to its properties at a vital stage during very high antioxidant demands, is limited. ***Patients and Methods:*** We studied 35 newborns admitted to the Neonatal Unit with respiratory distress syndrome (RDS) and with no signs of sepsis, severe anemia, hemodynamic compromise or malformation. The gestational age of the newborns was 26–40 weeks (mean value 32.5 weeks) and the weight at birth was 870–4,400 g (mean value 1,800 g). They were classified into two groups: ≤1,500 g or >1,500 g birthweight. In all cases, at 09:00 h on their 1st, 3rd and 7th days of life, serum melatonin was measured by RIA. The clinical history was recorded and treatment and follow-up were performed according to established neonatology practice, and the resultant data recorded. Informed consent from the parents or guardians was obtained in accordance with the Declaration of Helsinki. Statistical analysis was carried out using ANOVA-II (factor I: day of sample; factor II: birthweight). ***Results:*** There were significant increases in melatonin levels with increasing birthweight ($p = 0.017$), but no changes by day of sample. Although in both study groups melatonin levels increased during the first few days this was not statistically significant. ***Conclusions:*** In newborns of low birthweight, we report high melatonin concentrations in the morning and during the first week of life. These increase with maturation, and at full-term were similar to nocturnal levels during the acrophase of pineal gland secretion in toddlers and schoolage children, when pineal gland secretion is maximal and takes place reflecting environmental variations. In the early neonatal period these high levels of melatonin seem to derive from extrapineal sources, which mature to provide antioxidant protection in accordance with other elements of the antioxidant network to compensate for the high levels of oxidative stress that are present in the perinatal period.

Introduction

Classically, the effects of melatonin as a general modulator of human biorhythms were considered to be receptor-mediated, but recently studies have addressed nonreceptor-mediated actions, including its free radical scavenging [1] and indirect antioxidant activities [2]. Among the free radical scavenging activities of melatonin are the upregulation of antioxidant and the downregulation of

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Table 1. Study group characteristics

	≤1,500 g (n = 18)		>1,500 g (n = 17)	
	mean ± SD	range	mean ± SD	range
GA, weeks	30.3 ± 1.9	36–33	33.9 ± 3.5	28–40
Weight, g	1,201 ± 1,891	870–1,490	2,177 ± 549	1,540–4,400
Length, cm	38.0 ± 2.4	35–44.5	45.9 ± 4.4	38–54
HC, cm	27.9 ± 1.8	21–31	31.9 ± 2.1	28–36
Apgar 1'	9	2–10	7	2–9
Apgar 5'	10	7–10	9	5–10

Data are expressed as mean ± SD. HC = Head circumference. For Apgar score the median values are indicated.

pro-oxidant enzymes. This interesting neurohormone presents oncostatic [3], immunomodulatory [4] and anti-ageing properties [1].

A rapidly developing area in biomedicine is that of antioxidative protection, and especially neuroprotection. Although the utility of melatonin in this respect, based on pharmacological doses, remains to be established [5, 6], a remarkable body of evidence exists showing protection in numerous cell culture and in vivo systems [7]. Antioxidant actions can be observed at different levels, including the attenuation of radical formation by anti-excitatory and anti-inflammatory effects. Melatonin efficiently interacts with various reactive oxygen and nitrogen species as well as organic radicals, upregulated antioxidant enzymes (such as glutathione peroxidase and glutathione reductase) and downregulated pro-oxidant enzymes (NO synthases, lipoxygenases) [2].

Knowledge of the antioxidant activity of melatonin in the neonatal period is still very limited, although it is known that melatonin production is activated in newborns in response to stress-inducing stimuli [8, 9].

The more premature the infants, the more intense the oxidative stress, hence it is prematurity rather than the amount of oxygen administered which is the main cause of oxidative stress in very-low-birthweight infants. Oxidative stress is significantly higher in the first week of life of hypoxic newborns than in healthy preterm control infants [10]. During this time oxidative stress also increases significantly in neonates without hypoxia, suggesting that the damage caused by free radicals still occurs in nonhypoxic babies following a normal clinical course [11, 12].

Prior to birth, suitable preparation is required to ensure the successful functioning of the neonatal lung in the relatively oxygen-rich (21%) ex utero environment. The increase in lung activity of superoxide dismutase or glutathione peroxidase several weeks prior to birth is approximately 150–200% and takes place in parallel with a rapid rise in lung surfactant content during the final 10–15% of gestation [13]. The increase in production of reactive oxygen species (oxidative stress) in parallel with the growing maturity of pulmonary antioxidant enzymes (AOE) suggest that increasing enzyme substrate concentrations could be a primary controlling mechanism for increasing lung AOE gene expression in preparation for birth [14]. On the other hand, full-term newborns are capable of inducing greater levels of antioxidants if they experience high oxygen concentrations or conditions associated with oxidative stress [12]. For this reason, full-term newborns are more resistant to oxygen damage than preterm newborns or adults. Free radicals also increase oxidative stress in the preterm newborn through the xanthine-oxidase system which is activated by ischemia-reperfusion episodes [15].

The few reports available have indicated the probable clinical utility of melatonin as an antioxidant in the neonatal period; moreover, in premature newborns with respiratory distress syndrome treatment with the antioxidant melatonin reduces various markers of oxidative stress in the first week of life [16]. In this paper, therefore, we analyze the longitudinal evolution of diurnal melatonin concentrations in preterm newborns of both sexes to determine whether its profile matches possible participation as a physiological antioxidant, as part of the antioxidant network in the early neonatal period.

Subjects and Methods

We studied 35 newborns recruited sequentially between January 2003 and August 2004, presenting the following characteristics:

1. Admitted to the Neonatal Unit with respiratory distress syndrome (RDS).
2. No congenital malformation, anemia, sepsis, severe hemodynamic disorders or metabolic alterations.

Informed consent was obtained from all parents or guardians (none refused) and from the hospital's ethics committee. The gestational age of the newborns was established in accordance with physical and neurological criteria set out by Dubowitz et al. [17]. At the Neonatal Unit, the hospital's regular light-dark schedule (lights on from 08:00 to 21:00 h) was observed, with an ambient illuminance between 300–450 lx when the samples were taken. The general characteristics of the newborns and the obstetric and perinatal information of interest are shown in table 1. The sample comprised 35 newborns, with gestational ages between 26 and 40 weeks (mean value 32.5 weeks), and weighing 870–4,400 g (mean value 1,800 g). The study was structured as follows:

Table 2. Number of patients included in each study group, with different modes of respiratory support

	≤1500 g (n= 18)			>1,500 g (n= 17)		
	admission	3rd day	7th day	admission	3rd day	7th day
FiO_2 ≤40%	5	13	12	9	4	3
FiO_2 >40%	13	5	4	3	3	2
Surfactant treatment	8	–	–	0	–	–
CPAP	10	6	3	5	2	–
Assisted ventilation	6	5	4	4	3	2

The number of patients omitted, to complete the total number of each subgroup, did not receive respiratory support. FiO_2 = Oxygen inspiratory fraction; CPAP= continuous positive airways pressure (H_2O cm).

Table 3. Mean and SD of physiological and arterial acid-base status variables monitored

	≤1,500 g			>1,500 g		
	admission	3rd day	7th day	admission	3rd day	7th day
RR, bpm	57 ± 6	51 ± 3	58 ± 6	51 ± 2	52 ± 3	52 ± 2
HR, bpm	136 ± 7	149 ± 4	145 ± 7	140 ± 3	151 ± 3	146 ± 4
SBP, mm Hg	58 ± 3	64 ± 2	61 ± 4	67 ± 3	69 ± 3	70 ± 3
MAP, mm Hg	35 ± 3	43 ± 2	42 ± 3	47 ± 2	48 ± 2	51 ± 4
FiO_2, %	0.37 ± 0.04	0.29 ± 0.03	0.24 ± 0.02	0.31 ± 0.04	0.25 ± 0.02	0.21 ± 0.0
SO_2, %	95 ± 1	92 ± 4	95 ± 1	93 ± 3	95 ± 2	91 ± 2
PO_2	10 ± 0.7	5.6 ± 0.3	5.6 ± 0.3	6.7 ± 0.5	5.2 ± 0.3	5.2 ± 0.1
(kPa)/(mm Hg)	75 ± 11	42 ± 2	42 ± 2	50 ± 4	39 ± 2	39 ± 1
PCO_2	6.5 ± 0.5	5.7 ± 0.3	5.9 ± 0.3	6.8 ± 0.5	6.3 ± 0.4	6.7 ± 0.3
(kPa)/(mm Hg)	49 ± 4	43 ± 2	44 ± 2	51 ± 4	47 ± 3	50 ± 2
pH	7.32 ± 0.03	7.37 ± 0.01	7.37 ± 0.01	7.28 ± 0.03	7.31 ± 0.02	7.35 ± 0.01
HCO_3^-, mmol/l	24 ± 1	24 ± 1	25 ± 1	24 ± 1	26 ± 2	28 ± 1
BE, mmol/l	–2.4 ± 1.0	–0.8 ± 0.9	0.3 ± 1.4	–2.0 ± 1.3	0.9 ± 1.9	2.4 ± 1.2

RR = Respiratory rate (breaths per minute); HR = heart rate (beats per minute); SBP = systolic blood pressure; MAP = mean arterial pressure; FiO_2 = inspiratory oxygen concentration; SO_2 = oxygen saturation; PO_2 = oxygen partial pressure; PCO_2 = partial pressure of carbon dioxide; HCO_3^- = bicarbonate concentration; BE = bases excess.

1. For the whole sample (n = 35) we studied the evolution of different variables and their relation with melatonin at 09:00 h on the 1st, 3rd and 7th day.
2. We investigated possible differences between newborns with a birthweight of ≤1,500 g or ≥1,500 g. The study times and variables analyzed are the same as above. The sample was divided into two groups (table 1): (a) Group ≤1,500 g, comprising 18 newborns, with gestational ages of 26–33 weeks (mean value 30.3 weeks) and birthweight of 870–1,490 g (mean value 1,219 g). (b) Group >1,500 g, comprising 17 newborns (12 preterms, 5 fullterms) with the following characteristics: gestational ages 30–40 weeks (mean value 35.1 weeks) and birthweight 1,540–4,400 g (mean value 2,490 g).

Following the hospital's clinical protocol, for each of the infants the following procedures were performed (tables 2–4): arterial acid-base balance, hematological analyses and general biochemistry (electrolytes, renal function, hepatic enzymes, C-reactive protein), microbiological cultures when invasive procedures were used and/or when obstetric or perinatal risk factors were present, changing the antibiotic regimen according to the antibiogram received. Bilirubin levels were monitored according to postnatal age, gestational age and weight at birth.

The majority of the newborns weighing ≤1,500 g needed respiratory support by nasal continuous positive airway pressure (CPAP) (n = 10), and 6 infants needed mechanical ventilation (table 2).

Analytical Methods

Serum melatonin was measured by RIA routinely used in our laboratory, using surpluses from the blood samples extracted ac-

Table 4. Mean and SD of the hematological and biochemical variables recorded in each study subgroup

	≤1,500 g			>1,500 g		
	admission	3rd day	7th day	admission	3rd day	7th day
Melatonin, pg/ml	63.2 ± 6.2	71.4 ± 6.8	79.3 ± 6.8	104.2 ± 22.9	96.2 ± 30.0	109.4 ± 24.0
Leukocytes, × μl	10,925 ± 1,724	10,435 ± 1,954	11,775 ± 1,246	17,944 ± 3,278	9,666 ± 879	17,776 ± 6,162
PMN, × μl	2,651 ± 555	4,818 ± 1,447	3,493 ± 796	7,992 ± 2,000	4,391 ± 592	3,158 ± 740
Hct, %	55.5 ± 1.5	49.1 ± 1.9	40.8 ± 1.6	53.1 ± 1.9	49.0 ± 0.7	44.2 ± 1.0
Hb, g/dl	17.7 ± 0.5	18.3 ± 1.9	13.8 ± 0.5	16.7 ± 0.6	16.0 ± 0.3	14.2 ± 0.7
Platelets, × μl	260,722 ± 22,703	311,222 ± 33,341	407,000 ± 37,874	252,382 ± 22,803	270,876 ± 30,135	358,875 ± 39,354
Sodium, mmol/l	136 ± 1	138 ± 1	137 ± 1	135 ± 1	136 ± 1	135 ± 3
Potassium, mmol/l	5.1 ± 0.2	4.6 ± 0.2	4.6 ± 0.2	4.9 ± 0.2	5.0 ± 0.2	4.7 ± 0.2
Calcium	2.3 ± 0.05	2.3 ± 0.05	2.5 ± 0.05	3.6 ± 1.2	2.2 ± 0.05	2.3 ± 0.05
(mmol/l)/(mg/dl)	9.2 ± 0.2	9.4 ± 0.2	9.9 ± 0.2	14.3 ± 4.9	9 ± 0.2	9.3 ± 0.2
Urea (mg/dl)	24.1 ± 1.9	30.4 ± 3.2	27.1 ± 3.7	27.0 ± 2.2	27.0 ± 2.7	21.8 ± 3.2
Creatinine	59.5 ± 25.5	68 ± 0.7	59.5 ± 0.7	68 ± 0.7	68 ± 0.8	59.5 ± 8.5
(μmol/l)/(mg/dl)	0.7 ± 0.3	0.8 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.7 ± 0.1
Glucose	3.21 ± 0.56	4.69 ± 0.27	5.51 ± 0.24	2.7 ± 0.51	4.24 ± 0.34	4.77 ± 0.3
(mmol/l)/(mg/dl)	58.4 ± 10.2	85.3 ± 4.9	100.2 ± 4.4	49.1 ± 9.4	77.1 ± 6.2	86.8 ± 5.5
CRP	3.2 ± 2.4	4.3 ± 0.9	12.4 ± 4.1	3.9 ± 2.3	10.4 ± 3.8	3.3 ± 1.1
(μg/l)/(mg/dl)	0.3 ± 0.2	0.4 ± 0.1	1.2 ± 0.4	0.4 ± 0.2	1.0 ± 0.4	0.3 ± 0.1

PMN = Polymorphonuclear cells; Hct = hematocrit; Hb = hemoglobin; CRP = C-reactive protein.

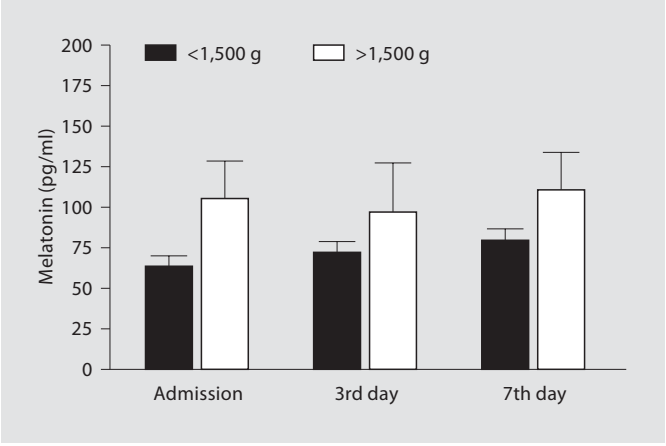

Fig. 1. ANOVA-II analysis of melatonin data, grouped by day of sample (factor I) and weight of newborn (factor II). Factor I: Day of sample: F = 1.76; p = NS. Factor II: Newborn weight: F = 36.8; p = 0.017.

cording to clinical indications at 09:00 h on the 1st, 3rd and 7th day of life. The blood was centrifuged at 3,000 *g* for 10 min, and the serum was separated and frozen at –20°C until assay. Melatonin was measured in duplicate using the LDN kit (Labor Diagnostika Nord GmbH and Co. KG, Nordhorn, Germany: Cat. No. BA 0800), which possesses a range detection for melatonin of 0.75–320 pg/ml, a specificity of 100%, and an intra-assay and inter-assay variation coefficient of less than 10%.

Statistical Methods

The variables analyzed are considered to be typical biological variables, fitting criteria of normality, and so we used an ANOVA-II test to compare two variables with two factors (factor I: day of sample; factor II: weight of the newborns). Statistical analysis was carried out using SPSS 12.05 software.

Results

The changes observed in serum levels of melatonin in the early neonatal period are shown in figure 1. ANOVA analysis revealed significant changes related to increasing weight ($F = 38.6$, $p < 0.017$), while there were no changes dependent on the day of sample. Although in both study groups melatonin levels increased during the first few days this was not statistically significant ($F = 1.76$; p = NS).

In the serum samples taken at 09:00 h the mean (SD) value of melatonin on the first day of life in the group weighing 1,500 g or less was 63.2 (6.2) pg/ml. This value gradually increased to 79.3 (6.8) pg/ml at 7 days (table 4). In the group weighing more than 1,500 g, melatonin values were significantly higher; these also showed a slight increase, from 104.2 (22.9) pg/ml on the first day to 109.4 (24.0) pg/ml at 7 days. In both study groups, the melatonin concentration increased during the first week of life.

Discussion

Previous studies [8] have reported differences between plasma levels of melatonin in the umbilical artery and vein in humans, with higher values in the vein and day-night differences in melatonin (aMT) concentrations, and evidence of the absence of such a circadian rhythm of aMT during the early neonatal period [9]. Consequently, the circadian rhythm of aMT described in fetal circulation and neonates reflects the maternal circadian rhythm of aMT and seems to be due to the transfer of aMT from the maternal to fetal circulation. Thus, the fetal pineal gland, although active, does not secrete aMT during this period in a rhythmic manner. The fetus' own circadian melatonin rhythm appears at the end of the neonatal period and persists thereafter [18].

Intrauterine growth retardation and fetal distress in human infants have been associated with a pronounced reduction in melatonin secretion during the first 3 months of life. Additionally, urinary 6-sulphatoxymelatonin (6SaMT) excretion is impaired in adults who were growth-restricted prenatally or were born after 40 weeks' gestation [19]. The urinary excretion of 6SaMT, which is found in young adults but not in children [20], suggests there is a relation between melatonin production and body size at birth. The mean daily excretion of 6SaMT was significantly lower in response to an apparent life-threatening event (ALTE) than in normal infants and siblings of sudden infant death syndrome victims. The diurnal 6SaMT rhythms in ALTE infants exhibit lower 24-hour mean and amplitude values, whereas the time of peak and nadir excretion rates is similar in all groups. Follow-up, 6–8 weeks later, revealed that 6SaMT excretion increased in all of them, suggesting delayed ontogeny rather than a permanent deficiency of melatonin production in ALTE infants [21].

We have shown previously that neonatal stress (acute fetal distress) increases nocturnal aMT production compared with normal term and preterm neonates [8]. The role of aMT in situations involving stress is well documented [22], and aMT is indeed considered a stress hormone. Together, these findings point to the importance of elucidating the mechanism involved in aMT synthesis and secretion in humans during the first months of life. The increased concentration of aMT reported in a previous study (mean value: 146 pg/ml) [9], compared to the values found in the umbilical artery and vein [8] and to normal values during the first 6 months of life (nighttime mean value: 27.3 pg/ml) [23], corroborates the observations reported here.

We postulate that the high melatonin concentrations we observed, which may remain so during the whole perinatal period, may act as an antioxidant, although our data are not able to confirm or deny this hypothesis. Melatonin is a potent small-molecular-weight antioxidant that often attains especially high levels during physiological ischemia/reperfusion episodes [24] affecting newborns during delivery [8]. Melatonin administration to pregnant rats may prevent ischemia/reperfusion-induced oxidative mitochondrial damage in the premature fetal brain [25]. The high level of melatonin production we report contrasts with the low levels found in the first 3 months of life, when antioxidant needs decline. Therefore, this is not related to the normal endogenous melatonin rhythm, including the gastrointestinal tract [26], since pinealectomy does not totally eliminate circulating levels of this hormone. Additionally, both resting and stimulated human lymphocytes synthesize and release large amounts of melatonin, several times greater than the nocturnal physiological levels in human serum [27]. High melatonin production is found at 09:00 h and consequently does not appear to be suppressed by light. Other reasons for this are the absence of mature circadian rhythms at this stage of life, and the fact that higher melatonin levels may also be present at other times. According to Tan et al. [24], melatonin plays an important role in physiological ischemia/reperfusion, that is, as part of the antioxidant defense system, to protect against the potential oxidative injury induced by physiological ischemia/reperfusion.

In accordance with the above considerations, several indicators of oxidative stress are increased during the perinatal period, and even more so under pathological circumstances [28], which explains the compensatory increased levels of antioxidants. For example, isoprostanes (markers of free radical-catalyzed lipid peroxidation) are higher in the amniotic fluid of pregnancies with fetal growth retardation. Highest values are found in preterm newborns, even higher than in term infants, with a significant inverse correlation between the plasma levels of isoprostanes and gestational age [28]. Other markers, 8-OH-dG (8-hydroxyguanosine) and malondialdehyde (MDA) concentrations in the urine of pregnant women are associated with reduced birthweight in full-term neonates [29].

Melatonin has been reported to reduce oxidative stress in neonates with asphyxia [5], RDS [16] or sepsis [30], and in newborns who have undergone surgical interventions [31]. Serum levels of lipid peroxidation products (MDA and nitrite + nitrate concentrations) in newborns with

asphyxia [5] or sepsis [30] are significantly higher than those in normal infants and, additionally, are significantly reduced by melatonin. Melatonin also improves the clinical outcome of newborns with infection as judged by the measurement of sepsis-related serum markers [32]. In newborns with RDS (grade III-IV) treated with melatonin, the concentrations of interleukin-6, interleukin-8, tumor necrosis factor-α (TNF-α) and nitrite/nitrate levels are significantly lower at 24 h, 72 h and at 7 days than in untreated newborns. Following melatonin administration, nitrite/nitrate levels decrease significantly, whereas they remain high and increase further in the infants with RDS not given melatonin [16]. Melatonin treatment reduces interleukin (IL)-6, IL-8 and TNF-α in tracheobronchial aspirate of mechanically ventilated newborns with RDS and improves the clinical outcome [33]. The newborns not treated with melatonin who developed chronic lung disease (CLD) had much higher levels of these proinflammatory cytokines than infants without CLD [34]. Acute fetal distress may be further complicated by meconium aspiration. Meconium interferes with alveolar macrophage functioning by inducing greater oxidative stress and apoptosis, and by inactivating surfactant [35]. This interference may be counterbalanced by exogenous administration of melatonin [5].

Preterm infants present higher concentrations of hydroperoxides [36], lower levels of α-tocopherol and lower superoxide dismutase and glutathione peroxidase activity, suggesting a strong imbalance between oxidants and antioxidants during their first 72 h of life [37]. Low levels of glutathione are associated with subsequent CLD in preterm infants [38].

Plasma levels of hypoxanthine, xanthine, uric acid, total hydroperoxide and advanced oxidation protein products are higher in hypoxic newborn infants than controls with statistically significant correlations between these factors. The more severe the hypoxia, the higher the lipid and protein damage by free radicals [39]. These markers of oxidative stress are significantly higher in the first week of life in the hypoxic newborn than in preterm control infants. During this time oxidative stress also increases significantly in neonates without hypoxia, suggesting that damage caused by free radicals is less than in hypoxic preterm newborns but that it still occurs in non-hypoxic babies with a normal clinical course [11]. During the last weeks of gestation, the fetus' antioxidant capacity increases in response to the relative hyperoxia of extrauterine life, and melatonin may be one of the antioxidant substances employed in this respect. Moreover, its exogenous administration in very high quantities [5] has been reported to be therapeutic with no side effects being reported.

In summary, the premature infant, and especially its developing brain, is uniquely vulnerable to hypoxic-ischemic injury, with a complex evolution of the injury that affords opportunities for intervention [40]. Melatonin, in reducing free radical production by oxygen, may be an effective neuroprotective treatment for the fetus [41]. The decrease in plasma bilirubin, which is also an antioxidant, coincides with an increase in plasma antioxidant capacity and a decrease in markers of oxidative stress in preterm infants [42].

The hypothesis that an 'oxygen radical disease of neonatology' exists has not been proven, but an increasing body of evidence seems to indicate that free radicals are involved in several disease processes leading to conditions such as CLD, retinopathy of prematurity, necrotizing enterocolitis and periventricular leukomalacia [43]. The more premature the infant, the more intense the oxidative stress, hence it is the prematurity rather than the amount of oxygen administered that causes oxidative stress in very-low-birthweight infants [10]. Thus, a poor clinical outcome is associated with an increase in the concentration of exhaled breath hydrocarbons (ethane, penthane) and malondialdehyde-thiobarbituric acid (MDA-TBA) levels as products of lipid peroxidation initiated by tissue damage (e.g. barotrauma, inflammation and infection) [44].

On the other hand, the probability that melatonin acts as a neuroprotector after birth is reinforced by the observation that low melatonin excretion in the first weeks of life correlates with delayed psychomotor achievements at 3 and 6 months of age [45]. Although melatonin has neuroprotective properties in other models [6, 41, 46], it is possible that the changes in melatonin observed by Tauman et al. [45] may be a consequence of disorders affecting the infant rather than having a pathogenetic role.

We have shown that the concentration of hydroperoxides, as an indicator of levels of oxidative stress, was greater in preterms than at term in the first week of life [36]. Nevertheless, and surprisingly, this concentration was higher in the >1,500 g group than in the very-low-birthweight group [47]. This observation supports our hypothesis about the relation between higher melatonin concentrations in the >1500 g group and greater needs for protection against oxidative injury. The level of hydroperoxides in umbilical cord erythrocytes is greater in preterms delivered by Caesarean section compared to vaginal delivery. From the third hour of life, the evolution and levels of hydrogen peroxide are comparable in both

groups. Conversely, catalase activity is greater in preterms born by spontaneous vaginal delivery, especially in the umbilical cord and at the third hour of life. Birth by Caesarean section induces an imbalance between oxidative stress levels and the compensating enzymatic mechanisms in preterms, which is rapidly compensated in the first hours of life, leading to a similar evolution in both delivery types [47].

As Tan et al. [24] have indicated, in the neonatal period the high melatonin production we have reported is transient, does not appear to be controlled by light, and does not present an endogenous circadian rhythm, which only develops many months later. In newborns of low birthweight, we corroborated high melatonin concentrations in the morning and during the first week of life. These melatonin levels were similar to nocturnal levels during the acrophase of pineal secretion in later vital stages, when the pineal gland is synchronized with light-related environmental stimuli.

In agreement with Tan et al. [24], we hypothesize that melatonin plays a physiologically important role in the perinatal period which extends from the 28th week of gestation to the 1st week of life, as part of the antioxidant defense system, in protecting against potential oxidative injury.

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