

Changes in plasma susceptibility to lipid peroxidation and vitamin C in preterm and full-term neonates

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Abstract

Introduction: This study was designed to compare the plasma lipid peroxidation (LPO) levels in preterm and full-term neonates and their respective mothers, to assess their relationship with the degree of oxidative stress and the levels of vitamin C, an important antioxidant of the body.

Material and methods: The studied groups included 70 neonates, 30 preterm (24–36 weeks of gestation) and 40 full-term (37–42 weeks) neonates. Blood samples were obtained from the cord blood in neonates and from the antecubital vein in their mothers at the time of delivery. Plasma susceptibility to LPO was fluorometrically measured before and after its incubation with 2,2'-azobis-2-amidinopropane hydrochloride (AAPH). Plasma vitamin C level was measured by HPLC.

Results: The basal LPO levels were similar in all groups of patients. After AAPH incubation, however, plasma LPO significantly ($P < 0.0001$) increased in all groups, although maternal plasma (full-term, 6.62 ± 0.14 and preterm, 8.76 ± 0.03 mmol/l) showed higher ($P < 0.001$) levels of LPO than their respective babies (full-term, 5.11 ± 0.03 and preterm, 7.74 ± 0.15 mmol/l). AAPH-induced LPO was higher in both maternal and preterm neonates' plasma than in full-term ones ($P < 0.001$). Vitamin C levels were similar in maternal plasma of both groups, but preterm neonates showed higher levels than full-term ones (171.65 ± 9.38 vs. 118.25 ± 2.75 mmol/l respectively, $P < 0.001$).

Conclusions: The results suggest that the preterm group was more prone to LPO than the full-term group, whereas vitamin C was not correlated with the degree of oxidative stress.

Key words: oxidative stress, antioxidants, newborns.

Introduction

Free radicals formed in plasma are scavenged from the circulation by hydrophilic antioxidants, but when plasma levels of free radicals exceed the protective capacity of these antioxidants, lipoproteins and other macromolecules may be affected [1, 2]. Vitamin C is an aqueous-phase antioxidant that participates in the antioxidative defence against oxidative damage [3]. A number of investigations have been carried out to clarify the significance of lipid peroxidation (LPO) as either a marker or a causative factor for the development of oxidative stress [4–7]. However, the relationships between the pro-oxidant and antioxidative factors present

in whole plasma, the balance of which could be essential in determining the susceptibility of lipoprotein to peroxidation, is still unclear.

During the neonatal period, plasma lipoproteins are frequently exposed to oxidative stress due to conditions such as respiratory distress, infection or haemorrhage [7]. Under normal pregnancy, LPO concentrations increase with gestational age, perhaps due to the production of lipid peroxides in the placenta [8]. The production of reactive oxygen species (ROS) also increases due to a respiratory burst of neutrophils [9]. The organism reacts against this physiological rise in LPO by increasing the antioxidative defence, including vitamins C and E and the glutathione system [10, 11]. Normally, this response is enough to prevent oxidative damage. Labour is another situation leading to ROS increase that should be neutralized by the organism. But the formation of lipid peroxides may cause severe injury [12], especially in prematures, whose antioxidative defence systems are immature [13].

However, information regarding the plasma antioxidative status during the neonatal period is limited, especially in preterm neonates. Therefore, the present study was designed to evaluate the presence of LPO in plasma from preterm neonates as a marker for oxidative stress, and its relationship with plasma levels of vitamin C, an antioxidant present in both maternal and neonatal circulation [14].

Material and methods

Patients

A total of 140 subjects, 70 neonates and their respective mothers, were studied in the Granada

University Hospital. Informed consent was obtained in all cases upon admission to the hospital for the mothers and from the hospital's Ethical Committee of the Granada University Hospital, according to the 1983 revised Helsinki Declaration of 1975. The clinical data, and the somatometric and analytical data were recorded (Table I). The study included two groups of mother-infant pairs: a) a full-term group, including 30 normal neonates, and b) a preterm group, comprising 40 premature neonates. All were non-smokers and non-diabetics who were not currently on vitamins, with no evidence of recent infection based on maternal or pregnancy history.

Following delivery, 2 ml of cord blood was collected by unclamping the cord towards the placenta and allowing the blood to flow freely into heparinized tubes. At the same time, another 2 ml of blood was obtained from the maternal antecubital vein. All of these samples were collected within 30 min following delivery. After centrifugation of blood specimens, plasma aliquots were frozen at -80°C until assays were performed. All samples were processed within 60 min of delivery.

LPO measurement

Plasma LPO ($\mu\text{mol/l}$) was measured in the absence (basal) and presence (AAPH-induced) of 50 nM of 2,2'-azobis-2-amidinopropane hydrochloride (AAPH), a free-radical initiator that yields peroxy radicals at a constant rate [15].

Vitamin C measurement

The determination of plasma vitamin C ($\mu\text{mol/l}$) was performed by high-performance liquid chromatography (HPLC) with electrochemical detection. Briefly, an aliquot of 100 μl of plasma was extracted with an equal volume of 5% meta-phosphoric acid containing 1 mmol/l of diethylenetriaminepentaacetic acid, and then centrifuged [16, 17]. Twenty microlitres of the supernatant was mixed with 74 μl of the mobile phase (40 mmol/l sodium acetate, 0.54 mmol/l EDTA- Na_2 , 1.5 mmol/l dodecyl triethylammonium phosphate, 7.5% methanol, pH 4.75), and 6 μl of 2.58 mol/l potassium phosphate, yielding a pH of 9.8.

Table I. Main characteristics of the studied groups

Variable	Full-term neonates	Preterm neonates
Number of cases	30	40
Gestational age [weeks]	37-42	24-36
Birth weight [g]	2508-4670	560-4103
Caesarean	3	10
Vaginal delivery	27	20

Table II. Plasma levels of lipoperoxidation and ascorbic acid in the studied groups

	Full-term group		Preterm group	
	newborn	mother	newborn	mother
LPO Basal	2.23 \pm 0.07	2.17 \pm 0.07	2.21 \pm 0.07	2.23 \pm 0.06
LPO AAPH	5.11 \pm 0.03	6.62 \pm 0.14**	7.7 \pm 0.15#	8.76 \pm 0.03*##
Ascorbic acid ($\mu\text{mol/l}$)	118.25 \pm 2.75	60.12 \pm 1.65**	171.65 \pm 9.38##	59.33 \pm 2.58**

Data are expressed as mean \pm SEM. LPO and ascorbic acid are expressed in $\mu\text{mol/l}$

* $P < 0.01$ and ** $P < 0.001$, mother vs. newborn within each group, # $P < 0.01$ and ## $P < 0.001$, mother vs. mother or newborn vs. newborn between groups

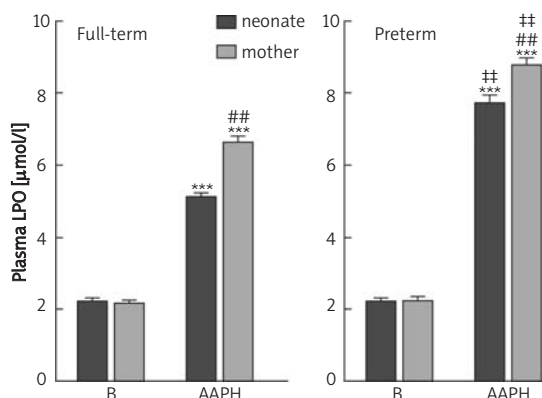


Figure 1. Basal and AAPH-induced LPO levels in plasma from full-term (left) and preterm (right) groups. No significant differences in basal LPO levels were observed between full-term and preterm groups. AAPH induced a significant increase in LPO, mainly in the preterm group
 *** $P < 0.0001$ vs. basal, ## $P < 0.001$ vs. neonates, ** $P < 0.001$ vs. full-term

Statistical analysis

Data are expressed as the means \pm SEM. A one-way ANOVA followed by Student's t test was used to compare the differences between groups. A P value less than 0.05 was considered statistically significant.

Results

Figure 1 shows the levels of LPO in full-term and preterm groups. In the full-term group (left), basal plasma LPO levels were similar in cord ($2.23 \pm 0.07 \mu\text{mol/l}$) and maternal ($2.17 \pm 0.07 \mu\text{mol/l}$) plasma. After incubating the plasma with AAPH, LPO levels significantly ($P < 0.0001$) increased in cord ($5.11 \pm 0.03 \mu\text{mol/l}$) and maternal ($6.62 \pm 0.14 \mu\text{mol/l}$) plasma, the latter showing higher levels than the former ($P < 0.001$). Figure 1 (right) shows the LPO levels in the preterm group. Basal LPO levels were similar in cord ($2.21 \pm 0.07 \mu\text{mol/l}$) and maternal ($2.23 \pm 0.06 \mu\text{mol/l}$) plasma, and they were also comparable to those found in the full-term group. Plasma AAPH incubation significantly ($P < 0.0001$) increased the levels of LPO in cord ($7.7 \pm 0.15 \mu\text{mol/l}$) and maternal ($8.76 \pm 0.03 \mu\text{mol/l}$) plasma, the latter being significantly higher than the former ($P < 0.001$). The induction of plasma LPO by AAPH was significantly greater in the preterm than in the full-term group ($P < 0.001$).

Plasma levels of vitamin C are shown in Figure 2. In both groups, i.e. full-term and preterm groups, maternal plasma shows comparable levels of vitamin C (60.12 ± 1.65 and $59.33 \pm 2.58 \mu\text{mol/l}$, respectively), and they were lower than those found in neonates. Cord plasma from preterm neonates showed higher levels of vitamin C than full-term

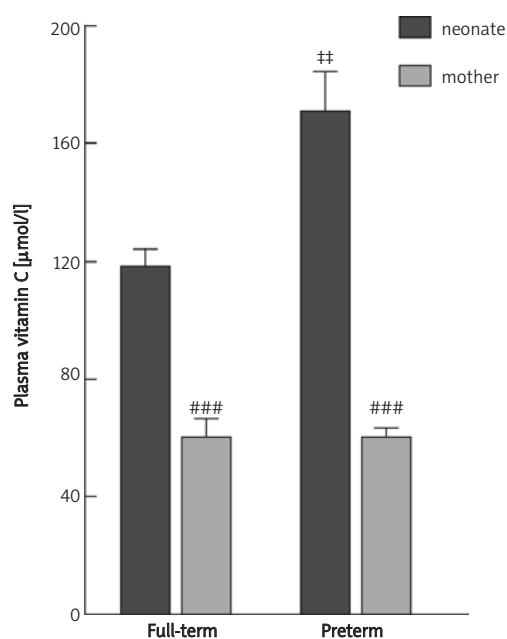


Figure 2. Vitamin C levels in plasma from full-term and preterm groups. No significant differences in maternal plasma levels of vitamin C were found. Plasma from neonates, mainly in the preterm group, show higher vitamin C concentration than maternal plasma
 ### $P < 0.0001$ vs. neonates, ** $P < 0.001$ vs. full-term

(171.65 ± 9.38 vs. $118.25 \pm 2.75 \mu\text{mol/l}$, respectively, $P < 0.001$).

Discussion

Plasma LPO levels are a valuable index reflecting the oxidative status in the body. The relationship between LPO and oxidative stress at birth has been studied following different methodologies with diverse results. Some authors quantified ethane plus pentane, which reflects LPO produced in the whole organism, in the expired air of low-weight neonates [18-20]. Maximal amounts of expired ethane and pentane were significantly higher in neonates with poor outcomes than in normals, suggesting the participation of oxygen radicals in the pathogenesis of the former. A correlation between elevated levels of an index of lipid peroxidation such as malondialdehyde-thiobarbituric acid and poor outcomes was also found [21]. In this case, elevated plasma malondialdehyde-thiobarbituric acid levels were correlated with adverse respiratory (chronic lung disease, bronchopulmonary dysplasia) and ophthalmological (retinopathy of prematurity) events. Measuring TBARS in neonates with birth weight ranging from 830 to 3,700 g, it was found that the serum dose required for maximal inhibition of autoxidation (D_{max}) was inversely related to birth weight [13]. In contrast, other authors did not find any correlation between total plasma antioxidant

activity and adverse neonatal outcomes including chronic lung disease, intraventricular haemorrhage, retinopathy of prematurity or death [22].

We report here two important findings: a) the existence of comparable LPO levels in maternal and neonatal plasma in both groups when LPO was directly measured in these samples, and b) when susceptibility to LPO was measured, after incubating plasma with AAPH, maternal plasma, mainly in the preterm group, was more prone to LPO than the respective neonate plasma samples. Levels of vitamin C in maternal plasma were, however, unrelated to its redox status because they were similar in preterm and full-term groups. The changes in LPO showed here agree with data elsewhere reported obtained after measuring LPO with different methodologies [23-25]. Other authors reported that plasma antioxidant activity and LPO were higher in term cord blood than in maternal plasma [26]. It was suggested that these findings would be related to plasma α -tocopherol depletion since α -tocopherol depletion in adult plasma increased LPO susceptibility [6]. Although plasma α -tocopherol depletion is unlikely to occur under physiological conditions in neonates, it was recently shown that vitamin E levels start decreasing soon after delivery and they reach the lowest levels 36 h later [14]. Vitamin E reduction was correlated with the decrease in LPO within 24 h post-partum [11]. During pregnancy, vitamin E is one of the most important antioxidants protecting against LPO, and the reduction of oxidative stress that takes place after delivery may explain the decline of vitamin E levels [14].

Vitamin C is important because besides contributing to the antioxidant defence against ROS [14], it regenerates vitamin E by reducing α -tocopherol radicals [27]. It was reported that vitamin C levels were lower in women during delivery than at caesarean, which was interpreted as the effect of ROS generation by repetitive ischaemia-reperfusion of uterine tissue after each contraction [28]. Other authors, however, did not find changes in vitamin C and E levels in the postpartum period [14]. Because in this study the first sample was taken 6 h after delivery, a possible effect of labour might have disappeared at the time of analysis. Our results show higher levels of vitamin C in preterm neonates compared to full-term ones. Since in our case the samples were obtained within 60 min after delivery, they must reflect changes at the time of labour.

The endogenous antioxidant system should be different in pre- and full-term neonates. The thiobarbituric acid assay showed that adult plasma displays higher protection against copper-induced LPO than plasma from neonates [24]. Besides, plasma from preterm newborns presented higher protection against peroxidation than plasma from term neonates. Our data suggest the existence of

lower defence against oxidative stress in premature than in full-term newborns, since the former show higher susceptibility expressed as exogenous LPO levels. Although the reason for the high levels of vitamin C in preterms reported here is unknown, they may reflect the elevated oxidative stress in these neonates. Thus, plasma antioxidant activity seems to be sufficient to handle redox balance in full-term newborns but not in preterm neonates. This interpretation agrees with reports showing that the degree of oxidative stress outweighs the antioxidant defence mechanisms, which is especially true in premature neonates [29].

In conclusion, measurement of susceptibility to LPO in AAPH-treated plasma enabled us to detect significant differences in the maternal and neonate plasma redox status, and suggests that at least some of the contradictory data in the literature related to LPO levels in neonates may be related to the method of LPO measurement employed [30]. The information obtained reflects the level of free radicals produced by the labour process [31]. These changes may affect the fetus, and the acid-base balance deviation [32]. It was recently found that birth weight and length were greatest when the levels of vitamins C and E were high [33]. These findings reflect the importance of an antioxidant nutrition balance for pregnant women. If antioxidants can prevent adverse birth outcome, the risk will be reduced by the use of an antioxidant-rich diet or supplementation. Thus, measuring vitamins C and E and plasma susceptibility to LPO at the time of delivery, which will allow the redox conditions at birth to be known, would be of primary importance, because antioxidant nutrition at this time can prevent future health problems [33].

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