Analysis of the Antioxidant Activity in Human Milk, day vs. night

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ABSTRACT

Human milk has many advantages for the development of the breast-fed baby, among vitamins A, C and E, which are essential as an antioxidant defense. In newborn infants, oxidative stress is important due to the immaturity of the antioxidant defense mechanisms and the digestive system. It is well known that the components of breast milk are not constant throughout the 24 hour period. This variation can depend on the mother's diet during this period. The aim of the study was to analyze the antioxidant capacity of the human milk throughout the 24 hour period, and to study the antioxidant changes between day and night period. The levels of Trolox Equivalent were statistically significant ($P<0,01$), in the milk samples collected at 18:00h and 21:00h compared to the samples collected at 24:00h. We observed an increase in the antioxidant capacity in the samples of the night period compared to the samples of the diurnal period, probably as consequence of the immunological activity of the mother and the amount of vitamins and proteins in the mothers' diet during the day.

Key words: human milk, antioxidant, chronobiology.

ABBREVIATIONS

TEAC : Trolox equivalent antioxidant capacity.
WHO : World Health Organization.
UNICEF : United Nations Children's Fund.

INTRODUCTION

A joint declaration by the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) stated that, breast milk is the optimal food for infants and can never be equaled by artificial substitutes. It covers all the child's physiological and nutritional needs during the first 4–6 months of life (WHO, 2003).

It is well known the advantages of the human milk for the development of the breastfed baby, among vitamins A, C and E, which are essential as an antioxidant defense. In newborn infants, oxidative stress is important due to the immaturity of the antioxidant defense mechanisms and the digestive system. The purpose of the antioxidative defense is to inactivate reactive oxygen particles (Tsopmo et al., 2009).

Human milk antioxidant capacity value represents a complex mixture of numerous compounds with antioxidant activities functioning by different chemical reactions (Friel et al., 2002; Kitts et al. 2003; Silvestre et al. 2008), which collectively culminate in a stable food source for the breastfed infant.

Vitamins E and C, retinol and b-carotene, lactoferrin and glutathione, and antioxidant enzymes including catalase, superoxide dismutase and glutathione peroxidase are all present in human milk (Friel et al., 2002; Shoji et al., 2004; Kasapovic et. al., 2005), and are known to have specific antioxidant roles against lipid peroxidation.

Earlier studies reported that breast milk is not constant throughout the 24 hour period (Cubero et al., 2005; Sánchez et al., 2009). This variation can depend on the mother's diet during this period (Sánchez et al., 2008).

The aim of the study was to analyze the antioxidant capacity of the human milk throughout the of 24 hour period, the antioxidant changes between the day and the night.

MATERIALS AND METHODS

Subjects

We recruited 7 healthy mothers from the region of Extremadura (Spain), in the Service of Neonatology (Hospital "Perpetuo Socorro", S.E.S.).

The subjects were considered healthy on the basis of their breast-feeding success, a physical examination, and a follow-up. All subjects were informed about the research project and gave written consent.

During the study, the subjects took no drugs that would disturb the levels of vitamins. The Ethical Investigation Committee of University of Extremadura approved the study.

Samples

Milk samples were collected from 7 breastfeeding women between 1-5 days postpartum (colostral milk) in polystyrene tubes before each feed over 24h (Hanna et al., 2004), during March to July and stored frozen at 80°C until analysis in duplicate.

Antioxidant analysis

We used an adapted technique from Arnao et al. (2001). The antioxidant capacity was determined by the improved spectrophotometric method TEAC (Trolox equivalent antioxidant capacity). This method calculates the percentage inhibition of the cation radical ABTS- + by Trolox, a water soluble analogue of alpha-tocopherol which is the standard antioxidant. The samples analysis was performed on a microplate

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reader TECAN M200. The wavelength device was 730nm.

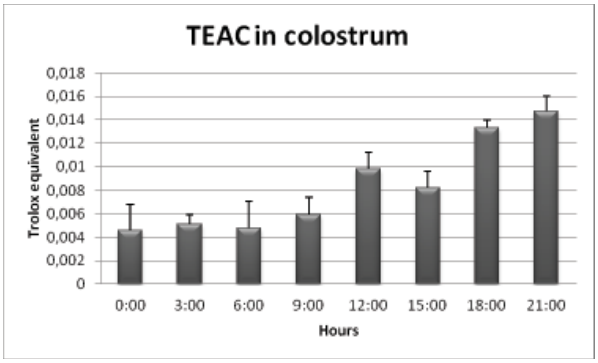
Statistical analysis

For the statistical analysis it was used: descriptive statistics (mean ± standard deviation) and inferential test Non-parametric Kruskal-Wallis. A value of $P<0.01$ was considered to be significant.

RESULTS

We found variation in the antioxidant activity between the night and diurnal samples of human milk. The levels of Trolox Equivalent are statistically significant ($P<0,01$) in the samples collected at 18:00h and 21:00h opposite to the samples of milk collected at 24:00h (figure 1).

DISCUSSION AND CONCLUSION



*p< 0,01 respect to 0:00h.

Figure 1. Trolox equivalent antioxidant capacity (TEAC) in colostrum human milk (24h period).

Studies show human milk can suppress oxidative stress and oxidative DNA damage in newborn infants more effectively than infant formula and indicate that human milk contains a unique defense mechanism, which is not available in commercial infant formulas or in bovine milk (Shoji et al., 2004).

The influence of human milk on oxidative stress intensity in breast-fed neonates and infants is a significant issue. The concentration of antioxidants in milk depends on mother's diet, vitamins supplementation during pregnancy and lactation and geographical area of domicile (Szalagatys-Sidorkiewicz et al., 2004).

As Asghar et al. reported (2009), we observed an increase in the total antioxidant status at the beginning of the night period opposite to the diurnal period, probably as consequence of the immunological activity of the mother and the amount of vitamins and proteins (Yamawaki et al., 2005) in the mothers' diet during the day.

In the future, this study should be improved by 24h-questionnaries from the breastfeeding women, at the point to observe which the origin of the antioxidants substances is in human milk. Probably, a specific antioxidant test should be used.

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