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Carla Manuela Soares de Matos

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Breastfeeding: antioxidative properties of breast milk.

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Abstract

Birth is an event that exposes the newborn baby to a high concentration of free radicals (reactive oxygen species (ROS) / reactive nitrogen species (RNS)), which can contribute to several diseases. The balanced equilibrium between ROS / RNS and the antioxidant system of the newborn is a key factor in preventing a plethora of diseases. The antioxidant system involves endogenous and exogenous molecules, from vitamins (A, E, C), to enzymes (glutathione peroxidase, superoxide dismutase), metals (copper (Cu), zinc (Zn), and selenium (Se)) and other molecules (coenzyme Q_{10}, melatonin) that can act in a synergistic manner to deactivate free radicals. A competent antioxidant system of the baby is strongly dependent on the intake of free radical deactivating molecules from feeding, either maternal or formula milk, with several studies pointing that breast milk has more powerful antioxidant effects on lowering the infant´s oxidative status. An improved understanding of the antioxidant molecules, their mechanism of action, and the relationships between them, are key factors to comprehend all the potential benefits of human breastfeeding in this matter. The purpose of this review is to describe different research efforts and methodologies of evaluation of total antioxidant system (TAS) in human milk, and to evaluate and summarize the contribution of different antioxidant molecules in TAS, with special emphasis in the last 10 years.

Keywords:

Total antioxidant status, breast milk, free radicals, antioxidative molecules, oxidative stress

Abbreviations: AAPH or ABAP (2,2´-azobis (2-methyl-propionamidine) hydrochloride); ABTS (2,2´-azinobis(3-ethylbenzothiazole-6-sulphonate); CUPRAC (cupric ion-reducing antioxidant capacity; DPPH (2,2-diphenyl-1- picrylhydrazyl radical-scavenging capacity); FRAP (ferric reducing antioxidant power); ORAC (oxygen radical absorbance capacity); RNS (reactive nitrogen species); ROS (reactive oxygen species); TAC (total antioxidant capacity); TAS (total antioxidant status); TE (Trolox equivalents); TEAC (Trolox equivalent antioxidant capacity); TRAP (total radical trapping antioxidant parameter).

Introduction

Breast milk is the most valuable nourishment available to the newborn. It is an easily digestible, readily accessible, low cost, natural food, which contains all the nutritional elements, fluids and energy required for growth and development of the newborn, besides having many other benefits in developmental, psychological, social and economic aspects for both baby and mother.

Breast-feeding is the best nutrition for the majority of infants during their first months of life, being recommended in exclusivity for 6 months, and possibly prolonged for up to two years of age or beyond along with appropriate complementary foods (WHO-World Health Organization).

Besides, as is widely acknowledged, having a high nutritional value and supporting the immune function, breast milk also has important antioxidative properties. Oxidative stress results from a balance between the oxidation induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the antioxidative defense system, composed by endogenous and exogenous (obtained from the diet) molecules. Breastfed children acquire a more competent antioxidant defense, and experience less severe oxidative stress than formula-fed children (Aycicek et al., 2006).

The child's exposure to the environment outside the womb is an oxidative challenge, and requires an adaptation from a low-oxygen to a high-oxygen environment. This high oxygen concentration increases aerobic metabolic pathways and causes toxic effects on tissues, pathologic cellular changes and increase in free radical products (Gitto et al., 2012; Sandal et al, 2013; Mutinati et al., 2014).

Material and methods

Web of Science service was used in December 2014 to search and to download research papers, using suitable keywords ((antioxidant or antioxidative) and ("human milk" or "breast milk" or breastfeeding)). From a total of 222 titles a selection was performed excluding abstracts, patents, non-English papers, animal studies and papers that were not available. Other research papers not included in this first search were incorporated due to their interest and add value to the theme.

Theory

Free radicals and Antioxidants

Free radicals are highly reactive molecules, originated chiefly from the mitochondrial electron transport chain, containing one or more unpaired electrons, which makes these species highly reactant, in order to gain or donate electrons from other molecules, in an effort to pair their electrons and produce a more stable species (Gitto et al, 2012; Alfadda and Sallam, 2012; Mutinati et al., 2014). They are mainly oxygen (ROS) or nitrogen (RNS) centered species, such as the peroxyl radical ($ROO^{\bullet}$), the hydroxyl radical ($HO^{\bullet}$), nitric oxide (NO), the superoxide radical ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), peroxyl radical ($ROO^{\bullet}$), hypochlorous acid (HClO) and singlet oxygen ($^1O_2$), each of these molecules being a separate entity with its own reaction, reaction kinetics, sites of production or degradation, and diffusion characteristics in biological systems. Consequently, the biological role of a free radical depends on the molecule involved and on the particular physiological environment in which it is being generated (Murphy et al., 2011; Power et al., 2013).

Deactivation and scavenging of ROS is critical, since these products are implicated in the processes of chronic inflammation and carcinogenesis (Zagierski et al., 2012), and in disease states such as insulin resistance and diabetes mellitus, atherosclerosis and cardiovascular diseases, cancer, and aging (Alfadda and Sallam, 2012; Halliwell, 2012). Generally, ROS species can cause damage in proteins or polyunsaturated fatty acids, mutations in DNA, oxidation of membrane phospholipids and modification in low density lipoproteins (LDL) (López-Alarcón and Denicola, 2013). In newborns and infants, oxidative stress has been related to medical conditions such as bronchopulmonary dysplasia, retinopathy of prematurity, and necrotizing enterocolitis (Mentro, 2004; Vogelsang et al., 2009; Knuppel et al., 2012; Gitto et al, 2013).

On the other hand, ROS are also linked to various physiological processes and essential protective mechanisms: immune defense, antibacterial action, vascular tone, and signal transduction (Alfadda and Sallam, 2012; Graves, 2012; Halliwell, 2012). Consequently, equilibrium must be achieved between ROS production and antioxidant activity. Oxidative stress appears if the excessive free radicals production is not properly balanced by an adequate antioxidative system.

An antioxidant molecule can be defined as a molecule that experimentally prevents the oxidation of a biological or chemical system, or that has a chemical structure that allows a free radical scavenging reaction and/or the chelation of redox-active metals (Fraga et al., 2014). The classical mechanism of action of antioxidants can be described by the following reaction (López-Alarcón and Denicola, 2013):

$AH + FR\bullet \rightarrow A\bullet + FRH$

where AH and FR• represent an antioxidant and a free radical, respectively.

The majority of nutrient-based antioxidants acts by stopping or slowing the oxidative

process once it has begun (chain breaking antioxidants). They can do this by reacting with a radical before vital biomolecules are damaged (sacrificial antioxidants) or as donor antioxidants, in which they are oxidized to products with insufficient reactivity to propagate the radical chain reaction (such as vitamin C, E). The physicochemical properties of an antioxidant molecule can dictate its action and efficacy *in vivo.* Hydrophilic antioxidants, such as ascorbic acid, scavenge free radicals in the aqueous phase. Lipophilic antioxidants, such as vitamins E, quench radical species present in cell membranes or lipid bilayers and provide protection against lipid peroxidation of polyunsaturated fatty acids (Power et al., 2013).

Determination of total antioxidant capacity in human milk

Determination of the total antioxidant capacity (TAC) (or total antioxidant system (TAS)) is one of the most frequently used strategies to evaluate the free radical-antioxidant balance in biological systems, and a multitude of research papers have described the determination of TAC in human milk. Several methods have been developed to assess TAC, most of them based on the reaction of an oxidizable substrate with an oxidant to obtain a maximum level of oxidation in the presence or absence of the "antioxidant sample" in evaluation (Fraga et al., 2014).

Based on the chemical reactions involved, the assays for measuring TAC can be classified in two major groups:

1-**Competitive methods**, in which a substrate and an antioxidant compete for peroxyl radicals generated by thermal decomposition of 2,2´-azobis (2-methyl-propionamidine) hydrochloride (AAPH or ABAP). Included in such methods one can find (López-Alarcón and Denicola, 2013; Fraga et al., 2014):

- ORAC (oxygen radical absorbance capacity): the peroxyl radicals formed react with a fluorescent probe to form a nonfluorescent product. In the presence of antioxidant, this reaction is inhibited. TAC is determined by a decrease in the rate and amount of fluorescent product formed over time.
- TRAP (total radical trapping antioxidant parameter): based on measuring oxygen consumption during a controlled lipid peroxidation reaction. The presence of an antioxidant in the test sample interferes with the reaction.

2-**Electron transfer methods**, that measure the ability of an antioxidant to reduce an oxidant, which also is the probe for monitoring the reaction by a change in spectrophotometric absorbance, used to quantify the reducing capacity of the antioxidant. Examples are (López-Alarcón and Denicola, 2013):

- TEAC (Trolox equivalent antioxidant capacity): the mechanism is based on scavenging of the 2,2´-azinobis(3-ethylbenzothiazole-6-sulphonate (ABTS) radical cation .
- DPPH (2,2-diphenyl-1- picrylhydrazyl radical-scavenging capacity): DPPH is a stable free radical, with an unpaired electron at one atom of the nitrogen bridge. The DPPH radical has a violet color and is widely used to monitor the radical scavenging ability of various antioxidants.
- FRAP (ferric reducing antioxidant power): measures the ability of an antioxidant to reduce a ferric ion complex to a ferrous complex, detected spectrophotometrically.
- CUPRAC (cupric ion-reducing antioxidant capacity): determines the ability of a sample to reduce the neocuproine-cupric complex

The TEAC (ABTS) and DPPH assays are amongst the most used methods to quantify

TAC in human milk. Researchers compared the 2 methods and reported a lack of linear correlation between the results obtained by both techniques, and that TAC levels determined by the ABTS method were significantly higher than those reported in the DPPH assay. The reasons pointed for that were the limitation of DPPH assay: lower sensitivity, a slower reaction with most antioxidants, DPPH dissolves only in polar matrices and human milk contains components whose spectra overlap with DPPH•. These limitations question the DPPH assay as suitable for the determination of TAC values in human milk (Martysiak-Żurowska and Wenta, 2012).

Other in vitro methods were described that measure specific ROS and RNS species. These tests include superoxide, hydrogen peroxide, hydroxyl, single oxygen and peroxynitrite scavenging assays. However, TAS determination may give more precise information than the measurement of each component separately, since the decrease in one component may be compensated by the increase in another, so that all the antioxidant machinery behaves as a self-regulating system (Sandal et al., 2013). Nevertheless, *in vitro* measurement of TAS cannot be straight forwardly related to their antioxidant capacity in the *in vivo* situation, since their bioavailability, distribution, or metabolism are not evaluated in these assays.

Approaches closer to the *in vivo* situation can be attempted with in vitro studies such as the use of cell culture models, as the model of the human intestinal mucosa, Caco-2 cells. These cells were used to study the antioxidant activity of breast milk after its in vitro digestion, mimicking the physiological microenvironment of intestinal mucosa and digestion conditions, confirming that human milk reduces the oxidative stress in the studied system (Yao et al. 2010).

Colostrum is the first fluid produced by the mammary gland after delivery. It is

produced in low quantities in the first few days postpartum, is rich in immunologic components, such as secretory immunoglobulin (Ig)A, lactoferrin, leukocytes, and developmental factors, such as the epidermal growth factor (EGF) (Ballard and Morrow, 2013; Golinelli et al., 2014). Transitional milk is produced from 5 days to 2 weeks postpartum, and meets the mounting nutritional requirements of the rapidly growing infant. Maturation increases in the next weeks, and by 4 to 6 weeks postpartum, human milk is considered fully mature (Ballard and Morrow, 2013).

Results

Several studies to determine the antioxidant potential in the different types of breast milk were conducted. These studies are usually designed so that the milk is harvested in different periods of breastfeeding, and then TAC is determined in the different portions collected.

A resume of some of the research efforts aiming to determine TAC/TAS in human milk published in the last years are depicted in Table 1.

Table 1. Studies evaluating TAS/TAC (Total Antioxidant System/Total Antioxidant Capacity) in human milk and the main conclusions withdrawn.

Sample characteristics	Method employed	Results for TAS/TAC	Conclusion
a. 30 healthy women with term (39 weeks) and preterm (32 weeks) babies (15+15); milk collected at 3 days (colostrum), 8 days (transition milk) and 30 days (mature milk)	TEAC (ABTS)	Fulterm: colostrum: 12; transitional milk: 9; mature milk: 7 mM; Preterm: colostrum: 10; transitional milk: 7; mature milk: 7 mM (results taken from graph)	TAC decreased during lactation time; the fullterm group had a higher TAS than the preterm group, for all the collecting periods
b. 145 women with full term newborns; milk collected at: colostrum (2 days), transitional milk (7 days) and mature milk (at 30, 90 and 180 days post partum); maternal plasma at 90 days	FRAP assay	Colostrum: 589.8; transitional 508.5; mature milk: 453.5, 479.3 and 402.6 μmol/l	Colostrum showed a higher antioxidant capacity than the transitional and mature milks. Positive correlation between the antioxidant content of breast milk and maternal plasma.
c. 56 samples of breast milk collected from mothers of premature infants (33 weeks), between 4 and 145 days after birth	assay similar to FRAP	Formula milk: 2671; Breast milk: 3807 μmol/L	TAC in breast milk was negatively correlated with the postnatal age and significantly higher than in formula milk. A reason appointed was that human milk has endogenous antioxidants, not presented in formula
d. 31 samples of mature milk collected by electric breast pump, subjected to 2 different pasteurization techniques: Holder pasteurization (63ºC for 30 min) high pasteurization (75ºC for 15 sec)	Commercial kit PAO-DELTACLON	in fresh milk: aprox. 0.25 mM equiv uric acid	There was a decrease in total antioxidant capacity in milk samples subjected to thermal processing versus fresh milk samples. High pasteurization offers superior preservation of TAC than Holder pasteurization
e. Pool of 100 samples from a milk bank (mature milk)	ORAC (with fluorescein as the probe)	3.41 to 2.46 μmolTE/mL	ORAC with fluorescein can be used to determine TAS in human milk
f. 60 women, 1 mo. after birth; milk collected after 5 minutes of feeding start (hindmilk)	ORAC (with fluorescein as the probe)	3.41 μmolTE/mL	TAC was correlated with α-tocopherol concentration

g. 115 samples of colostrum (2 days), 97 samples of transition milk (7 days) and 293 samples of mature milk at 3 times (30, 90 and 180 days); samples taken by manual expression of each breast at morning	FRAP assay	Colostrum: 1061.6 μmolTE/L; Transitional milk: 915 μmolTE/L; Mature milk: 816; 862; 724 μmolTE/L	The level of TAC decreased from colostrum to mature milk.
h. 102 lactating women (31 for the entire study time); 1,4,8,12,and 16 weeks; milk collected by breast pump/manual expression before and after each fed on the same day	Commercial kit (Randox ® assay) (TEAC)	7 days: 0.497 4 weeks: 0.399 8 weeks: 0.423 12 weeks: 0.401 16 weeks: 0.375 mmolTE/L	TAS decreased throughout the lactation time; Significant correlations were found between TAS and Cu, Zn and Se
i. 7 subjects; milk collected between 1 and 5 days postpartum (colostrum), at several different times during the day	TEAC (ABTS)	Values for TE varied throughout the day, from aprox. 0.004 TE at 0h to 0.014 TE at 21.00h (values taken from a graph)	A variation was reported for TAC day vs. night; lowest TAC measured at 0h and highest at 21.00 h
j. 20 women (10 term and 10 preterm deliveries) in the 3rd, 7th and 30th day postpartum and 20 women (10 term (40 weeks) and 10 preterm (36 weeks) deliveries) 5 months postpartum; TAC evaluated at 0h, 48h and 1 week (kept at 4 and -8 ºC)	Phosphomolybdenium method	In fresh milk: Fulterm: colostrum: 112.9; Transitional milk: 103.93; Mature milk: 101.82 Preterm: colostrum: 104.6; Transitional milk: 98.47; Mature milk: 97.45 μg/dL	Higher antioxidant levels measured in colostrum; no difference between term and preterm deliveries; TAC reduced with time, with refrigeration and freezing
k. 30 postpartum women who declared smoking more than 5 cig./day during pregnancy and lactation; milk collected the 3rd day (colostrum) and 30th-32nd day (mature milk) by electric pump, 2 h after the 1st morning feeding	Commercial kit (Randox ® assay) (TEAC)	Non smokers: Colostrum: 4.15; Mature milk: 5.25; Smokers: Colostrum: 2.99; Mature milk: 4.66 (mmol/L)	This study revealed inferior antioxidant properties of breast milk from smokers; determination of maternal 8-isoprostane (a biomarker for oxidative stress in urine and milk): correlated negatively with milk TAS for non-smokers but not for smokers.
l. 49 postpartum women, milk collected	Commercial	Colostrum: 3.59;	The phase of lactation does not affect the

the 3rd day (colostrum) and 30th-32nd day (mature milk) by electric pump, 2 h after the 1st morning feeding	kit (Randox ® assay) (TEAC)	Mature milk: 4.92 (mmol/L)	degree of lipid oxidative damage in human milk.
m. 80 lactating women in exclusively breastfeeding for 90 days, were given a symbiotic for 30 days; milk collected by manual expression before the first feeding in the morning	Commercial kit (Randox ® assay) (TEAC)	Supplemented group: 0.312 to 0.255; Placebo group: 0.317 to 0.255 mmol TE/L	Symbiotic (probiotic and prebiotic) supplementation increased TAC levels
n. 184 infants, divided in 2 groups: I (term-38.6 weeks) and II (preterm-33.7 weeks); colostrum samples collected using a vacuum pump before infant feeding	TEAC (ABTS)	Group I: 2.62 Group II: 2.41 mmol TE/L	In newborns (preterm and term) small for gestational age, an increase in plasmatic oxidative stress was observed; the differences found in TAS levels in breast milk were not significantly different
o. 20 assymptomatic HIV-infected lactating women and and 30 age‑matched HIV‑free lactating mothers, 15 days to 2 mo. post partum	FRAP assay	Infected: 850 μmol/L; Non-infected: 884 μmol/L;	It could be concluded from this study that hypoalbuminemia is a feature of HIV‑infected lactating mothers and that their breast milk of has low antioxidant capacity.
p. 60 samples from 15 women; milk collected at aprox. 7, 14, 21 and 28 days afer birth at the end of the morning feeding	TEAC (ABTS)	TAC decreased from aprox. 1.2 at 7 days to 0.9 mmol TE/L at 28 days	TAC decreases significantly in the 1st month of lactation and TAC in breast milk is mainly due to the presence of bioactive proteins
q. 98 women with a single preterm (31 weeks) infant; milk collected at day 4 (colostrums) with a vacuum pump from one breast before the feedind and used fresh or stored at -80ºC for 3 mo	TEAC (ABTS)	Fresh milk: 3.2 mmol TE/L Stored milk: 3.8 mmol TE/L	Storage at -80ºC does not change the antioxidant content of human milk

a) Quiles et al., 2006; b) Zarban et al., 2007; c) Ezaki et al., 2008; d) Silvestre et al., 2008; e) Sáenz et a., 2009; f) Tijerina-Sáenz et al., 2009; g) Zarban et al., 2009; h) Matos et al., 2009; i) Cubero et al., 2009; j) Xavier et al., 2011; k) Zagierski et al., 2012; l) Szlagatys-Sidorkiewicz et al., 2012; m) Nikniaz et al., 2013; n) Sandal et al., 2013; o) Rahamon et al., 2013; p) Mehta and Petrova, 2014; q) Akdag et al., 2014

Several research efforts have been conducted in order to determine whether the breast milk has a stronger antioxidant power when compared to formula milk. Aycicek and co-workers (2006) studied the antioxidant effects of breast milk in 3 and 6 months old children, by measuring plasma TAC, total peroxide levels and oxidative stress index (percentage of total peroxide to TAC), and showed that plasma TAC was significantly higher (while total peroxide levels and oxidative stress index were significantly lower) in the breast-fed group, when compared to the formula-fed group (TAC: 2.3 vs 2.0 mmol TE/L). Positive significant correlations were found between uric acid, albumin and total bilirrubin and plasma TAC in both groups, and a negative correlation between TAC and plasma iron. These results demonstrate that human milk provides better antioxidant power than formula. Similar results were obtained by Ledo et al., in 2009, by comparing 29 breastfed and 34 formula fed babies (using several urinary markers for oxidative stress) and concluding that breast milk has more powerful antioxidant effects on lowering the infant´s oxidative status. In 2010, Oveisi and colleagues measured total antioxidative activity by FRAP assay in 140 human breast milk samples and 80 infant formulas and showed that breast milk has a higher TAC level and provides better antioxidant power than formula. Later, Alpinar et al. (2012) studied 16 healthy breastfed infants and 18 formula fed, 3 to 6 months old, and reported a higher level of antioxidants in the plasma of the breastfed group. In a posterior study, Lugonja et al. (2013) performed an *ex vivo* study on the effect of human milk and formula on the contraction of the uterine rat muscle, and concluded that breast milk induced the relaxation of calcium-initiated muscle contractions, while the infant formulas did not have such an effect. Besides, a higher level of superoxide dismutase activity and a higher content of reducing agents were found in human milk.

On the other hand, Korchazhkina et al. (2006) collected samples of milk from 20

mothers who had had premature babies and urine of the milk fed babies (10 breast-fed and 10 formula-fed, 4-6 days old) and reported the malondialdehyde levels (a marker of lipid peroxidation) in those samples. The conclusion withdrawn was that both breast milk and formula were sufficient to prevent lipid peroxidation in healthy preterm newborns. In a more recent study, Friel and co-workers (2011) studied 65 premature babies and did not find a correlation between ORAC results or lipid peroxidation and breastfed or formula fed children, and concluded that the differences in the antioxidant role of these two milk sources are subtle, and may take long to manifest.

Discussion

These studies have demonstrated that Total antioxidant status (TAS) is higher in colostrum, in harmony with a higher oxidative stress in the newborn caused by the delivery, and decrease throughout lactation period, as the baby ´s own defense system matures (Ezaki et al., 2008; Zarban et al., 2009; Matos et al, 2009).

From the described studies, one can observe that the obtained results comparing formula and breast milk are dubious and diffident, and depend on the type of analysis conducted for determining TAS. Indeed, most adapted milks produced today are fortified with vitamins and other compounds with antioxidative activity, which leads to high antioxidant levels when chemical methods are used for its determination. However, the fact that a mixture is characterized chemically as having a strong redox activity, does not necessarily imply that it has the same behavior in a biological environment; on the other hand, human milk is produced to meet more completely the child's requirements.

The oxidant challenge seems to be more evident in premature babies, more susceptible to the effects of oxidative stress, because its antioxidant defense mechanism is still

undeveloped, and have a higher incidence of oxidative increasing circumstances, such as hyperoxia, reperfusion or inflammation (Perrone et al., 2007; Mehta and Petrova, 2014). As described above, breastfeeding preterm babies can have a positive impact in their development, and plays an important role in protecting the premature infant. The results that compare milk from mothers with premature and fullterm babies are also baffling; Quiles et al. (2006) described a higher TAS level in milk from mothers with fullterm babies, but posterior research works did not corroborate those findings (Xavier et al., 2011; Sandal et al., 2013).

Other studies have focused on the effect of lifestyle of the mother on the antioxidant status of her milk. The scarce results published suggest that supplementation with non-alcoholic beer (Codoner-Franch et al., 2013) or symbiotics (Nikniaz et al., 2013) may be beneficial, while smoking appears as clearly detrimental (Zagierski et al., 2012).

Types of antioxidants in human milk

Vitamins

Vitamins A, E and C have strong antioxidative activity. Vitamins A and E were found to be more concentrated in colostrum as compared to mature milk.

Vitamin A

Vitamin A is a liposoluble compound with antioxidative action, acting against reactive oxygen species (ROS). Carotenoids are a group of molecules structurally similar to vitamine A, some of them being its dietary source (provitamin A), as β- and α-carotenes. The antioxidative activity of vitamin A and carotenoids is conferred by the conjugated carbon-carbon double bonds in the hydrophobic chain, which can quench singlet oxygen, neutralize thiol radicals, and combine with and stabilize peroxyl

radicals, and in this way protect the cells against peroxidative damage (Palace et al, 1999; Rutkowski and Grzegorczyk, 2012). In general, the longer the polyene chain, the greater the peroxyl radical stabilizing ability (Landete, 2013). Moreover, vitamin A apparently suppresses the activity of enzymes participating in propagation of peroxidation of lipids, as well as prevents oxidative disorders of protein glycosylation in cell membranes (Rutkowski and Grzegorczyk, 2012; Sommer and Vyas, 2012).

Vitamin A has a limited transplacental transfer, so mammal newborns have low stores of this compound, relying on milk consumption to maintain a suitable amount (Debier and Larondelle, 2005). Colostrum has an important role in providing initial protection to the newborn against deficiency, and it usually contains higher vitamin A concentrations than mature milk. Macias and Schweigert (2001) reported a vitamin A concentrations of 1.02 ± 0.56 lg⁄mL in colostrum, which declined to 0.33 ± 0.14 lg⁄mL in the first weeks of lactation. Nevertheless, according to Tijerina-Sáenz (2009), vitamin A concentration did not significantly contribute to milk antioxidant capacity at 1 month of breast-feeding and only a modest role of this compound was found in improving clinical outcomes and incidence of bronchopulmonary dysplasia (Mentro, 2004).

The β-carotene and lutein are the most abundant carotenoids in human milk samples, and a sharp decrease in carotenoid content is also noted as a function of lactation stage (Song et al., 2013). β-carotene also quenches $^1O_2$, and neutralizes various free radicals, deactivates the earlier formed peroxy radicals of the body's lipids, preventing their propagation and participation in peroxidative processes (Rutkowski and Grzegorczyk, 2012).As a result of the above mentioned reactions, the decrease in plasma β-carotene concentration in newborns may be implicated in oxidative stress related diseases (Vogelsang et al., 2009).

Vitamin E

The terms α-tocopherol and vitamin E are often used alternatively; in fact, though vitamin E has eight forms (four tocopherols (α, β, γ, δ) and four tocotrienols (α, β, γ, δ), α-tocopherol constitutes 90% of all the forms and is described as having the strongest biological activity (Rutkowski and Grzegorczyk, 2012; Landete, 2013).

Vitamin E deactivates ROS, including $^{1}O_2$ (singlet oxygen), $O_2^{\bullet-}$ (superoxide radical anion) and $HO^{\bullet}$ (hydroxyl radical) (Rutkowski and Grzegorczyk, 2012). This powerful antioxidative activity of α-tocopherol makes it the main fat-soluble vitamin responsible for protecting cell membranes against peroxidation. As a lipophilic compound, it accumulates in circulating lipoproteins, cell membranes, and fatty deposits, reacting with free radicals and molecular oxygen, protecting polyunsaturated fatty acids (PUFAs) and lipoproteins from peroxidation. Vitamin E inhibits lipid peroxidation primarily by trapping peroxyl radicals ($LOO^{\bullet}$) through the following reaction (Debier and Larondelle, 2005):

$LOO^{\bullet}$ + α-tocopherol → LOOH + α-tocopherol$^{\bullet}$ (α -tocopheroxyl radical)

The antioxidant function of vitamin E is located in the chromanol nucleus, where the phenolic hydroxyl group donates an H atom to quench lipid radicals, becoming an α-tocopheroxyl radical, which, contrary to fatty acids, is fairly stable, because the unpaired electron of the O atom is delocalized throughout the aromatic ring. α-tocopherol can be regenerated from the tocopheroxyl radicals by cellular reductants, such as ascorbic acid (Debier and Larondelle, 2005).

Vitamin E also regenerates the antioxidatively used up β-carotene and protects vitamin A against oxidation. Generally it plays such an important role in oxidative stress counteraction that it is considered to be the main lipophilic antioxidant of the body (Traber and Atkinson, 2007).

Vitamin E is extremely important from conception to postnatal development, but. during pregnancy placental transfer of vitamin E to the fetus is limited, making breast milk the only source of this nutrient for infants. Stimming et al. (2014) have determined α-tocopherol acetate, tocopherols, and tocotrienols from breast milk and infant formulas and showed that breast milk tends to contain higher ratios of vitamin E to unsaturated fatty acids than long chain polyunsaturated fatty acids (LC-PUFA) enriched formulas. Furthermore, higher concentrations of α-tocopherol were found in colostrum milk when compared to transitional and mature milk (Szlagatys-Sidorkiewicz et al., 2012), coincident with a significant reduction in the amount of some components of the fat globule, since most of the vitamin E is secreted as a constituent of the membrane of these globules (Lima et al., 2014). Still, vitamin E (and α-tocopherol in particular) seems to be highly accountable for the antioxidative capacity of breast milk at 1 month post-partum (Tijerina-Sáenz et al., 2009). Parity, anthropometric nutritional status, socioeconomic status, and habitual dietary intake of vitamin E by the mother do not appear to affect the levels of this nutrient in breast milk. Nevertheless, variables such as maternal age, α -tocopherol biochemical nutritional status, gestational age, and maternal vitamin E supplementation appear to have some degree of influence (Lima et al., 2014).

Vitamin C

Vitamin C (L- ascorbic acid) can act as an antioxidant since it has low reduction potential and can react with radicals and oxidants. The oxidation of ascorbate by one electron originates ascorbyl radical, that has a low reactivity due to resonance stabilization of the unpaired electron, and readily dismutates to ascorbate and dehydroascorbic acid (DHA) (Carr and Frei, 1999). The latter can regenerate back in ascorbate by enzyme-dependent pathways and nonenzymatically by glutathione and

lipoic acid. This reversible oxidation of ascorbate to DHA creates a redox system with the potential of 0.08 V, which allows it to act as an antioxidant. So, vitamin C has an important role in eliminating oxidative stress, being, due to its hydrosolubility, the main antioxidant of extracellular fluids. It plays an important role in breast milk antioxidative properties, and was associated with a reduced risk of atopy in high-risk infants (Hoppu et al., 2005).

Proteins and enzymes

The principal proteins in human milk are casein and whey protein. The β-casein is the predominant casein in human milk, which produces a lighter gastric curd with digestible floccules and reduces gastric emptying time. The major whey proteins are α-lactalbumin, lactoferrin, immunoglobulin A (IgA), and serum albumin (Golinelli et al., 2014).

The protein content composition of human milk changes considerably during the course of lactation. Colostrum and transitional milk contain high concentrations of protein, but there is little casein, which increases with duration of milk production. During the first month of lactation, the protein content in human milk decreases to concentrations of 9–11 g/L, and then decreases more slowly until the sixth month of breastfeeding. The concentration of whey proteins remains high during the whole lactation period (Stam et al., 2013). Several enzymes are also present in the human milk

There are two sources of proteins in the milk: whey proteins are synthesized in the mammary gland, and other proteins such as serum albumin, various enzymes and protein hormones are transferred to milk from plasma; a dimer of the secretory IgA is synthesized by epithelial cells of the mammary gland from the connection of two IgA molecules produced locally by resident lymphocytes (Golinelli et al., 2014).

The mechanism of antioxidative activity of peptides and proteins is the result of the combination of three main actions: scavenging of free radicals, inhibition of lipid peroxidation and chelating of transition metal ions (Sarmadia and Ismail, 2010). Moreover, other antioxidant mechanisms were attributed to these compounds, such as reducing hydroperoxides, enzymatically eliminating specific oxidants, and altering the physical properties of food systems in a way that separates reactive species (Elias et al., 2008). The induction of genes is another indirect mechanism by which peptides can keep cells safe from damage by ROS (Kruzel et al., 2013).

Several studies suggest that the antioxidative activity of proteins and peptides is related to their amino acid composition: tyrosine (Tyr), tryptophan (Trp), methionine (Met), lysine (Lys), cysteine (Cys), phenylalanine (Phe) and histidine (His) are examples of amino acids that have antioxidative activity. Amino acids with aromatic residues structure (Tyr, Trp and Phe) can donate protons to electron deficient radicals, which improves their radical-scavenging properties. The antioxidative activity of His-containing peptides is related to hydrogen-donating, lipid peroxyl radical trapping and/or the metal ion-chelating ability of the imidazole group, while the sulfhydryl (SH) group in cysteine has an independently crucial antioxidative action due to its direct interaction with radicals (by H donating). Hydrophobic residues (valine (Val), leucine (Leu) and Tyr improve the accessibility of proteins to hydrophobic radical species or polyunsaturated fatty acids (Elias et al., 2008; Sarmadia and Ismail, 2010; Power et al., 2013).

Lactoferrin

Lactoferrin is a basic (pI 8.6) iron-binding protein that has antimicrobial and immunotrophic functions (Yen et al., 2011; Kruzel et al., 2013; Golinelli et al., 2014). It

consists of a single polypeptide chain that is folded in two highly homologous lobes, namely the N and C-lobe, with a Fe^{3+}-binding site contained in each lobe (Sabatucci et al., 2007). This ability to bind iron is also the main mechanism of action of its antimicrobial activity, since ferric iron is essential for bacterial growth (Yen et al., 2011); also, it appears that lactoferrin´s anti-inflammatory action depends on its ability to take up iron in the inflammation focus, where low pH inhibits iron retention by transferrin (Sokolov et al., 2009). Moreover, the high proportion of iron present in breast milk is bound to lactoferrin, which facilitates the mineral uptake by the intestinal cells, as this protein is relatively resistant to proteolytic degradation in the gastrointestinal tract when compared to other milk proteins, such as casein, hence facilitating the absorption of iron from milk by the neonate (Golinelli et al., 2014).

This protein plays an important role in cellular redox modulation (Kruzel et al., 2013) and is found in high concentrations in the serum of human milk (Golinelli et al., 2014). Lactoferrin binds free iron, which initiates and catalyzes free-radical processes (as lipid peroxidation in biomembranes), since this metal enhances the Fenton reaction causing the production of the highly toxic hydroxyl radical (Belizi et al., 1999). Furthermore, lactoferrin forms a complex with ceruloplasmin, increasing its ferroxidase activity, being this complex found in breast milk, which is more evidence of the protective effect of these two proteins (Sabatucci et al., 2007; Sokolov et al., 2014).

Glutathione peroxidase

Selenium glutathione peroxidase is an enzyme that contains selenium as an essential component. It is a tetrameric protein composed of four apparently identical subunits, each containing one atom of selenium. Cellular glutathione peroxidase was the first enzyme recognized as a selenoprotein, the selenium component being present in the

form of selenocysteine. The plasma is identical but it is a glycosylated protein, and is kinetically, structurally and antigenically distinct from the cellular enzyme (Torres et al., 2003). Milk is another extracellular fluid known to contain glutathione peroxidase activity that confers protective effects against oxidative damage (L'Abbe and Friel, 2000). This enzyme increases during lactation time (L'Abbe and Friel, 2000), although other studies have reported that its activity decreases (Torres et al., 2003). A reported positive association between glutathione peroxidase and long-chain polyunsaturated fatty acids in human milk can indicate that it may be involved in protecting milk lipids from oxidative stress by reducing fatty acid hydroperoxides (L'Abbe and Friel, 2000).

Glutathione peroxidase catalyses the decomposition of hydrogen peroxide (H_2O_2) and organic hydroperoxides (L-OOH) using reduced glutathione (GSH), according to the following reaction (Torres et al., 2003; Elias et al., 2008):

$$H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$$

or

$$LOOH + 2GSH \rightarrow LOH + H_2O + GSSG$$

where GSSG is oxidized glutathione and LOH is a fatty acid alcohol.

Superoxide dismutase (SOD)

Mn- superoxide dismutase and Cu/Zn- superoxide dismutase have been identified in human milk, and both catalyze the conversion of superoxide anion to hydrogen peroxide by the following reaction (Elias et al. 2008; Tsukahara, 2013):

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$$

The hydrogen peroxide thus formed, then suffers the action of the enzyme catalase.

The amount of Cu/Zn- superoxide dismutase decreases throughout the lactation (França et al., 2010), although a peak around the 3rd week was described for both superoxide dismutases by some authors (L'Abbe and Friel, 2000; Kasapović et al., 2005).

Catalase

Catalase is a heme-containing enzyme found in many biological systems that catalyzes the conversion of hydrogen peroxide to water by the reaction (Elias et al. 2008):

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

Catalase was identified as the most important factor in bovine milk that maintains its oxidative stability (Silanikove et al., 2014). In human milk, its concentration decreases with lactation time (Friel et al., 2002).

Ceruloplasmin

Ceruloplasmin is a multi-functional copper containing protein that belongs to the family of multicopper oxidases and accounts for 95% of human plasma copper (Sabatucci et al., 2007; Samygina et al., 2013). Ceruloplasmin is described as having antioxidative properties due to a series of enzymatic properties, such as being a ferroxidase (inhibiting the formation of hydroxyl radicals in the Fenton reaction and ferrous stimulated lipid peroxidation) (Sokolov et al, 2014). These properties make ceruloplasmin an effective antioxidant, able to prevent oxidative damage to proteins, DNA and lipids (Atanasiu et al., 1998). Alternative mechanisms of antioxidative properties have been described, such as the decomposition of lipid peroxides or the scavenging of superoxide, hydroxyl and H_2O_2 radicals in a stoichiometric manner. It was described that ceruloplasmin was far more effective as a peroxyl radical scavenger than superoxide dismutase, but slightly less effective than catalase, and is an effective chain-breaking antioxidant for a variety

of radicals, independently of its catalytic ferroxidase activity (Atanasiu et al., 1998). As stated above, lactoferrin forms a complex with ceruloplasmin, which increases the ferroxidase activity of the latter (Sabatucci et al., 2007; Sokolov et al., 2014).

Other proteins, peptides and aminoacids have also antioxidative properties and were described as human milk components. Thioredoxin is an ubiquitary multifunctional protein that has a redox active region within the sequence Cys-Gly-Pro-Cys, protecting against oxidative stress by scavenging ROS, as well as having an anti-inflammatory activity. Birth stimulates the release of thioredoxin by the neonates, and early human milk is a rich source of this protein that contributes to the redox balance (Todoroki et al., 2005). Published data suggest that 60% of the variability of breast milk TAC is accounted for by leptin, adiponectin, lactoferrin and lysozyme (Mehta and Petrova, 2014). Mandal et al. (2014) have fractionated and purified several peptides from human milk and have identified two peptides has having antioxidative properties; lactoferricin (a fragment of lactoferrin), which appears to have also an excellent iron withholding capacity that accounts for inhibiting the oxidation of DPPH and ABTS radicals; the other antioxidant peptide identified was kappa casein, containing a short N-terminal containing repeated tyrosine, which may be the cause of preventing oxidation.

Other highly potent antioxidant peptides can be generated by enzymatic hydrolysis (pepsin and pancreatin) of human milk proteins (Hernández-Ledesma et al., 2007; Tsopmo et al., 2009; Tsopmo et al., 2011).

Other molecules

Coenzyme Q_{10}

Coenzyme Q_{10} or ubiquinone is a lipossoluble vitamin-like 1, 4-benzoquinone that has a

very potent action as an antioxidant and a free radical scavenger. It is an essential component of oxidative phosphorylation at the mitochondrial level, where it helps to stabilize cell membranes. Coenzyme Q_{10} is acquired both by endogenous biosynthesis and from dietary intake (Quiles et al., 2006; Knuppel et al., 2012). Several studies have been conducted to determine coenzyme Q_{10} throughout the lactation (Niklowitz et al., 2005; Quiles et al., 2006; Tang et al., 2006). Tang et al. (2006) have determined the concentration of ubiquinone in breast milk by HPLC and have reported a mean concentration 0.315 ± 0.205 μmol L−1 (ranging from 0.073 to 1.93 μmol L−1). Quiles et al. (2006) reported a concentration of 0.8 μmol/L for coenzyme Q_{10} in colostrum (HPLC), and a decrease with breastfeeing duration. Moreover, the concentration of this antioxidant molecule is lower in milk of preterm babies (for colostrum and transition milk, whilst equivalent for mature milk) and its concentration is directly correlated with the antioxidant capacity of the milk (Quiles et al., 2006). Higher increases in coenzyme Q_{10} blood levels have been observed in breast-fed infants when compared with formula-fed infants during the first days of life, which is likely related to the exogenous supply from breast milk (Compagnoni et al, 2004).

Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is a serotonin derivative, sensitized endogenously in the pineal gland that acts highly effectively as an antioxidant and free radical scavenger. It interact with various free radicals *in vitro* and *in vivo,* stimulates the activity of several antioxidative enzymes (catalase, copper/zinc superoxide dismutase, glutathione peroxidase), down-regulates pro-oxidative enzymes (myloperoxidase, nitric oxide synthase) (Reiter et al., 2009), and has demonstrated a positive effect in a multitude of neonatal diseases (Gitto et al., 2013). Melatonin has

been quantified in human milk by several researchers (Kimata, 2007; Honorio-França et al., 2013; Karunanithi et al., 2014) and has proven as to have a positive effect in the neonate´s wellbeing (Engler et al., 2012).

Trace elements and metals

The antioxidant system may involve also trace elements, such as iron (Fe), copper (Cu), zinc (Zn), and selenium (Se), elements that exist in small concentrations but exhibit redox potentials that exert their antioxidant effects. Besides acting as redox catalysts, these trace elements, can form part of the active site or are cofactors of antioxidant enzymes, and play an important part on the newborn´s antioxidant defense in the early post-birth period. Fe is important for the catalase activity, Cu and Zn are cofactors in the expression of Cu/Zn superoxide-dismutase, and Se is an essential component of glutathione peroxidase (Oshiro et al., 2001).

Matos and coworkers have studied 102 lactating women in a longitudinal study for 4 months (only 31 women completed the study throughout the entire time) and reported the concentrations of Cu, Zn, Mn (manganese) and Se (determined by inductively coupled plasma-mass spectrometry) from 7 days to 4 months. The concentration of all these elements decreased with the progress of lactation, and a positive correlation with TAS were observed for all elements, except for Mn. In a recent study, the role of the trace elements (cobalt, vanadium, rubidium and tellurium) in the antioxidant system was also put in evidence (Matos et al., 2014).

Conclusion

The antioxidant status of the offspring is highly dependent on the antioxidant status of the mother during pregnancy and, after birth, maintained by the synthesis of endogenous molecules by the newborn and infant and at the cost of exogenous molecules conveyed by the milk. The antioxidant capacity of human milk comprises endogenous and exogenous molecules, from vitamins (A, E, C), to enzymes (glutathione peroxidase, superoxide dismutase), metals (copper (Cu), zinc (Zn), and selenium (Se)) and other molecules (coenzyme Q_{10}, melatonin) that can act in a synergistic manner to deactivate free radicals. Several studies have demonstrated that colostrum has the highest antioxidant potential, and that there is a reduction in total TAC during the course of lactation. The concentration of specific antioxidants also varies during the lactation: the activity of molecules such as vitamins and coenzyme Q10, or elements such as copper (Cu), zinc (Zn), and selenium (Se) decrease, while for other enzymes the concentration varies (for superoxide dismutase a peak in the 3rd week was described and glutathione peroxidase´s concentration increases). Formula milk also has a strong antioxidative activity, with modern formulas being highly supplemented with reducing molecules, but several studies point that human milk has a stronger capacity to decrease oxidative biomarkers in the plasma of infants studied.

A healthy diet and lifestyle during pregnancy seem to be crucial for an adequate Total Antioxiant Status (TAS), increasing with the mother´s supplementation with non-alcoholic beer and symbiotic intake, and decreasing with smoking and infection.

All these findings convey the certainty that human milk is important to decrease the oxidative stress that can lead to multiple dysfunctions, and that its composition in antioxidant molecules varies during lactation to proportionate the best care to the newborn.

References

Akdag, A., Sari, F.N., Dizdar, E.A., Uras, N., Isikoglu, S., Erel, O., Dilmen, U., 2014. Storage at −80◦C Preserves the Antioxidant Capacity of Preterm Human Milk. J. Clin. Lab. Anal. 28, 415–418.

Alfadda, A.A., Sallam, R.M., 2012. Reactive Oxygen Species in Health and Disease. J. Biomed. Biotechnol. Article Number: 936486, 1-14. DOI: 10.1155/2012/936486.

Alpinar, A., Torun, E., Özcaya, E., Uzuner, S., Erenberk, U., 2012. A comparison of total antioxidant levels in breast-fed and formula-fed infants. Turk. Arch. Ped. 47, 97-100.

Atanasiu, R.L., Stea, D., Mateescu, M.A., Vergely, C., Dalloz, F., Briot, F., Maupoil, V., Nadeau, R., Rochette, L., 1998. Direct evidence of caeruloplasmin antioxidant properties. Mol. Cell Biochem. 189, 127–135.

Aycicek, A., Erel, O., Kocyigit, A., Selek, S., Demirkol, M.R., 2006. Breast milk provides better antioxidant power than does formula. Nutrition 22, 616–619.

Ballard, O., Morrow, A.L., 2013. Human Milk Composition. Nutrients and Bioactive Factors. Pediatr. Clin. N. Am. 60, 49–74.

Belizi, S., Nazarova, I.A., Klimova, I.A., Prokof'ev, V.N., Pushkina, N.V., 1999. Antioxidant properties of lactoferrin from human milk. Bull. Exp. Biol. Med. 127 (5), 471-473.

Carr, A., Frei, B., 1999. Does vitamin C act as a pro-oxidant under physiological conditions? FASEB J. 13 (9), 1007-1024.

Codoner-Franch, P., Hernandez-Aguilar, M.T., Navarro-Ruiz, A., Lopez-Jaen, A.B., Borja-Herrero, C., Valls-Belles, V., 2013. Diet Supplementation During Early Lactation with Non-alcoholic Beer Increases the Antioxidant Properties of Breastmilk and Decreases the Oxidative Damage in Breastfeeding Mothers. Breastfeed. Med. 8 (2), 164-169.

Compagnoni, G., Giuffre, B., Lista, G., Mosca, F., Marini, A., 2004. CoQ(10) plasmatic levels in breast-fed infants compared to formula-fed infants. Biol. Neonate 86 (3), 165-169.

Cubero, J., Sánchez, C.L., Bravo, R., Sánchez, J., Rodriguez, A.B., Rivero, M., Barriga, C., 2009. Analysis of the Antioxidant Activity in Human Milk, day vs. night. Cell Membranes and free radical research 1 (3), 100-101.

Debier, C., Larondelle, Y., 2005. Vitamins A and E: metabolism, roles and transfer to offspring. Br. J. Nutr. 93, 153–174.

Elias, R.J., Kellerby, S.S., Decker, E.A., 2008. Antioxidant Activity of Proteins and Peptides. Crit. Rev. Food Sci. Nutr. 48, 430–441.

Engler, A.C., Hadash, A., Shehadeh, N., Pillar, G., 2012. Breastfeeding may improve nocturnal sleep and reduce infantile colic: potential role of breast milk melatonin. Eur. J. Pediatr. 171 (4), 729-32.

Ezaki, S., Ito, T., Suzuki, K., Tamura, M., 2008. Association between Total Antioxidant Capacity in Breast Milk and Postnatal Age in Days in Premature Infants. J. Clin. Biochem. Nutr. 42, 133–137.

Fraga, C.G., Oteiza, P.I., Galleano, M., 2014. In vitro measurements and interpretation of total antioxidant capacity. Biochim. Biophys. Acta 1840, 931–934.

Franca, E.L., Nicomedes, T.D., Calderon I.D.P., Franca, A.C.H., 2010. Time-dependent alterations of soluble and cellular components in human milk. Biol. Rhythm Res. 41(5), 333–347.

Friel, J.K., Martin, S.M., Langdon, M., Herzberg, G., Buettner, G., 2002. Milk from Mothers of Both Premature and Full-Term Infants Provides Better Antioxidant Protection than Does Infant Formula. Pediatr. Res. 51 (5), 612-618.

Friel, J.K., Diehl-Jones, B., Cockell, K.A., Chiu, A., Rabanni, R., Davies, S.S., Roberts II, L.J., 2011. Evidence of Oxidative Stress in Relation to Feeding Type During Early Life in Premature Infants. Pediatr. Res. 69 (2), 160-164.

Gitto, E., D'Angelo, G., Cusumano, E., Reiter, R.J., (2012). Oxidative Stress of Newborn, Complementary Pediatrics, Dr. Öner Özdemir (Ed.), ISBN: 978-953-51-0155-0, InTech, Available from: http://www.intechopen.com/books/complementary-pediatrics/oxidative-stress-of-newborn.

Gitto, E., Marseglia, L., Manti, S., D'Angelo, G., Barberi, I., Salpietro, C., Reiter, R.J., 2013. Protective Role of Melatonin in Neonatal Diseases. Oxidative Medicine and Cellular Longevity Article ID 980374, 6 pages, http://dx.doi.org/10.1155/2013/980374

Golinelli, L.P., Del Aguila, E.M., Paschoalin, V.M.F., Silva, J.T., Conte-Junior, C.A., 2014. Functional Aspect of Colostrum and Whey Proteins in Human Milk. J Hum Nutr Food Sci 2(3): 1035, 1-9.

Graves, D.B., 2012. The emerging role of reactive oxygen and nitrogen species in redox

biology and some implications for plasma applications to medicine and biology. J. Phys. D: Appl. Phys. 45, 263001, 1-42.

Halliwell, B., 2012. The antioxidant paradox: less paradoxical now? Br. J. Clin. Pharmacol, 75 (3), 637–644.

Hernández-Ledesma, B., Quirós, A., Amigo, L., Recio, I., 2007. Identification of bioactive peptides after digestion of human milk and infant formula with pepsin and pancreatin. Int. Dairy J. 17, 42–49.

Honorio-Franca, A.C., Hara, C.C.P., Ormonde, J.V.S., Nunes, G.T., Franca, E.L., 2013. Human colostrum melatonin exhibits a day-night variation and modulates the activity of colostral phagocytes. J. Appl. Biomed. 11(3), 153-162.

Hoppu, U., Rinne, M., Lampi, A.M., Isolauri, E., 2005. Breast milk fatty acid composition is associated with development of atopic dermatitis in the infant. J. Pediatr. Gastr. Nutr. 41 (3), 335-338.

Karunanithi, D., Radhakrishna, A., Sivaraman, K.P., Biju, V.M.N., 2014. Quantitative determination of melatonin in milk by LC-MS/MS. J. Food Sci. Technol. 51(4), 805–812.

Kasapović, J., Pejić, S., Mladenović, M., Radlović, N., Pajović, S., 2005. Superoxide dismutase activity in colostrum, transitional and mature human milk. Turk. J. Pediatr. 47, 343-347.

Kimata, H., 2007. Laughter elevates the levels of breast-milk melatonin. J. Psychosom. Res. 62, 699– 702.

Knuppel, R.A., Hassan, M.I., McDermott, J.J., Tucker, J.M., Morrison, J.C., 2012. Oxidative Stress and Antioxidants: Preterm Birth and Preterm Infants, Preterm Birth - Mother and Child, Dr. John Morrison (Ed.), ISBN: 978-953-307-828-1, InTech, Available from: http://www.intechopen.com/books/preterm-birth-mother-and-child/oxidative-stress-and-antioxidants-pretermbirth-and-preterm-infants.

Korchazhkina, O., Jones, E., Czauderna, M., Spencer, S.A., 2006. Effects of exclusive formula or breast milk feeding on oxidative stress in healthy preterm infants. Arch Dis Child 91, 327–329.

Kruzel, M.L., Actor, J.K., Zimecki, M., Wise, J., Płoszaj, P., Mirza, S., Kruzel, M., Hwang, S., Ba, X., Boldogh, I., 2013. Novel recombinant human lactoferrin: Differential activation ofoxidative stress related gene expression. J. Biotech. 168, 666– 675.

L'Abbe, M.R., Friel, J.K., 2000. Superoxide Dismutase and Glutathione Peroxidase

Content of Human Milk From Mothers of Premature and Full-Term Infants During the First 3 Months of Lactation. J. Pediatr. Gastroenterol. Nutr. 31 (3), 270–274.

Landete, J.M., 2013. Dietary Intake of Natural Antioxidants: Vitamins and Polyphenols. Crit. Ver. Food Sci. Nutr. 53, 706–721.

Ledo, A., Arduini, A., Asensi, M.A., Sastre, J., Escrig, R., Brugada, M., Aguar, M., Saenz, P., Vento, M., 2009. Human milk enhances antioxidant defenses against hydroxyl radical aggression in preterm infants. Am. J. Clin. Nutr. 89 (1), 210-215.

Lima, M.S.R., Dimenstein, R., Ribeiro, K.D.S., 2014. Vitamin E concentration in human milk and associated factors: a literature review., J Pediatr (Rio J) 90(5), 440-448.

López-Alarcón, C., Denicola, A., 2013. Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. Anal. Chim. Acta 763, 1– 10.

Lugonja, N., Spasic, S.D., Laugier, O., Nikolic-Kokic, A., Spasojevic, I., Orescanin-Dusic, Z., Vrvic, M.M., 2013. Differences in direct pharmacologic effects and antioxidative properties of mature breast milk and infant formulas. Nutrition 29, 431–435.

Macias, C., Schweigert, F.J., 2001. Changes in the Concentration of Carotenoids, Vitamin A, Alpha-Tocopherol and Total Lipids in Human Milk throughout Early Lactation. Ann. Nutr. Metab. 45, 82–85.

Mandal, S.M., Bharti, R., Porto, W.F., Gauri, S.S., Mandal, M., Franco, O.L., Ghosh, A.K., 2014. Identification of multifunctional peptides from human milk. Peptides 56, 84-93.

Martysiak-Żurowska, D., Wenta, W., 2012. A comparison of ABTS and DPPH methods for assessing the total antioxidant capacity of human milk. Acta Sci. Pol., Technol. Aliment. 11(1), 83-89.

Matos, C., Moutinho, C., Balcão, V., Almeida, C., Ribeiro, M., Marques, A.F., Guerra, A., 2009. Total antioxidant activity and trace elements in human milk: The first 4 months of breast-feeding. Eur. Food Res. Technol. 230 (2), 201-208.

Matos, C., Moutinho, C., Almeida, C., Guerra, A., Balcao, V., 2014. Trace element compositional changes in human milk during the first four months of lactation. Int. J. Food Sci. Nutr. 65 (5), 547-551.

Mehta, R., Petrova, A., 2014. Is variation in total antioxidant capacity of human milk

associated with levels of bio-active proteins? J. Perinatol. 34, 220–222.

Mentro, A.M., 2004. Vitamin A and bronchopulmonary dysplasia: research, issues, and clinical practice. Neonatal netw. 23 (4), 19-23.

Murphy, M.P., Holmgren, A., Larsson, N.G., Halliwell, B., Chang, C.J., Kalyanaraman, B., Rhee, S.G., Thornalley, P.J., Partridge, L., Gems, D., Nystrom, T., Belousov, V., Schumacker, P.T., Winterbourn, C.C., 2011. Unraveling the Biological Roles of Reactive Oxygen Species. Cell Metab. 13, 361-366.

Mutinati, M., Pantaleo, M., Roncetti, M., Piccinno, M., Rizzo A., Sciorsci R.L., 2014. Oxidative Stress in Neonatology. A Review. Reprod. Dom. Anim. 49, 7–16.

Niklowitz, P., Menke, T., Giffei, J., Andler, W., 2005. Coenzyme Q10 in maternal plasma and milk throughout early lactation. BioFactors 25, 67–72.

Nikniaz, L., Mahdavi, R., Ostadrahimi, A., Hejazi, M.A., Vatankhah, A.M., 2013. Effects of Synbiotic Supplementation on Total Antioxidant Capacity of Human Breastmilk. Breastfeed. Med. 8 (2), 217-222.

Oshiro, M., Mimura, S., Hayakawa, M., Watanabe, K., 2001. Plasma and erythrocyte levels of trace elements and related antioxidant enzyme activities in low-birthweight infants during the early postnatal period. Acta Pediatr, 90, 1283-1287.

Oveisi, M.R., Sadeghi, N., Jannat, B., Hajimahmoodi, M., Behfar, A., Jannat, F., Nasaba, F.M., 2010. Human Breast Milk Provides Better Antioxidant Capacity than Infant Formula. Iran. J. Pharm. Res. 9 (4), 445-449.

Palace, V.P., Khaper, N., Qin, Q.I., Singal, P.K., Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease. Free Rad. Biol. Med. 26 (5/6), 746–761.

Perrone, S., Salvi, G., Bellieni, C.V., Buonocore, G., 2007. Oxidative Stress and Nutrition in the Preterm Newborn. J. Pediatr. Gastroenterol. Nutr. 45, S3, S178-S182.

Power, O., Jakeman, P., FitzGerald, R.J., 2013. Antioxidative peptides: enzymatic production, in vitro and in vivo antioxidant activity and potential applications of milk-derived antioxidative peptides. Amino Acids 44, 797–820.

Quiles, J.L., Ochoa, J.J., Ramirez-Tortosa, M.C., Linde, J., Bompadre, S., Battino, M., Narbona, E., Maldonado, J., Mataix, J., 2006. Coenzyme Q concentration and total antioxidant capacity of human milk at different stages of lactation in mothers of preterm and full-term infants. Free Radical Res. 40 (2), 199-206.

Rahamon, S.K., Arinola, G.O., Akiibinu, M.O., 2013. Total antioxidant potential and essential trace metals in the breast milk and plasma of Nigerian human immunodeficiency virus infected lactating mothers. J. Res. Med. Sci. 18 (1), 27-30.

Reiter, R.J., Paredes, S.D., Manchester, L.C., Tan, D., 2009. Reducing oxidative/nitrosative stress: a newly-discovered genre for melatonin. Crit. Rev. Biochem. Mol. Biol. 44(4), 175–200.

Rutkowski, M., Grzegorczy, K., 2012. Adverse effects of anioxidative vitamins. Int. J. Occup. Med. Environ. Health 25(2), 105-121.

Sabatucci, A., Vachette, P., Vasilyev, V.B., Beltramini, M., Sokolov, A., Pulina, M., Salvato, B., Angelucci, C.B., Maccarrone, M., Cozzani, I., Dainese, E., 2007. Structural Characterization of the Ceruloplasmin: Lactoferrin Complex in Solution. J. Mol. Biol. 371, 1038–1046.

Sáenz, A.T., Elisia, I., Innis, S.M., Friel, J.K., Kitts, D.D., 2009. Use of ORAC to assess antioxidant capacity of human milk. J. Food Comp. Anal. 22, 694–698.

Samygina, V.R., Sokolov, A.V., Bourenkov, G., Petoukhov, M.V., Pulina, M.O., Zakharova, E.T., Vasilyev, V.B., Bartunik, H., Svergun, D.I., 2013. Ceruloplasmin: Macromolecular Assemblies with Iron-Containing Acute Phase Proteins. PLOS ONE 8 (7), e67145.

Sandal, G., Uras, N., Gokmen, T., Oguz, S.S., Erdeve, O., Dilmen, U., 2013. Assessment of oxidant/antioxidant system in newborns and their breast milks. J. Matern. Fetal Neonatal Med. 26 (5), 540-543.

Sarmadia, B.H., Ismail, A., 2010. Antioxidative peptides from food proteins: A review. Peptides 31, 1949–1956.

Silanikove, N., Merin, U., Leitner, G., 2014. Nitrite and catalase levels rule oxidative stability and safety properties of milk: a review. RSC Adv. 4 (50), 26476-26486.

Silvestre, D., Miranda, M., Muriach, M., Almansa, I., Jareno, E., Romero, F.J., 2008. Antioxidant capacity of human milk: effect of thermal conditions for the pasteurization. Acta Paediatr. 97, 1070–1074.

Sokolov, A.V., Ageeva, K.V., Pulina, M.O., Zakharova, E.T., Vasilyev, V.B., 2009. Effect of lactoferrin on oxidative features of ceruloplasmin. Biometals 22, 521–529.

Sokolov, A.V., Zakahrova, E.T., Kostevich, V.A., Samygina, V.R., Vasilyev, V.B., 2014. Lactoferrin, myeloperoxidase, and ceruloplasmin: complementary

gearwheels cranking physiological and pathological processes. Biometals 27, 815–828.

Sommer, A., Vyas, K.S., 2012. A global clinical view on vitamin A and carotenoids. Am. J. Clin. Nutr. 96(suppl):1204S-6S.

Song, B.J., Jouni, Z.E., Ferruzzi, M.G., 2013. Assessment of phytochemical content in human milk during different stages of lactation. Nutrition 29 (1), 195-202.

Stam, J., Sauer, P.J.J., Boehm, G., 2013. Can we define an infant's need from the composition of human milk? Am. J. Clin. Nutr. 98(suppl):521S–8S.

Stimming, M., Mesch, C.M., Kersting, M., Kalhoff, H., Demmelmair, H., Koletzko, B., Schmidt, A., Böhm, V., Libuda, L., 2014. Vitamin E Content and Estimated Need in German Infant and Follow-On Formulas With and Without Long-Chain Polyunsaturated Fatty Acids (LC-PUFA) Enrichment. J. Agric. Food Chem. 62, 10153−10161.

Szlagatys-Sidorkiewicz, A., Zagierski, M., Jankowska, A., Łuczak, G., Macur, K., Bączek, T., Korzon, M., Krzykowski, G., Martysiak-Żurowska, D., Kamińska, B., 2012. Longitudinal study of vitamins A, E and lipid oxidative damage in human milk throughout lactation. Early Hum. Dev. 88, 421–424.

Tang, P.H., Miles, M.V., Steele, P., Davidson, B.S., Geraghty, S.R., Morrow, A.L., 2006. Determination of coenzyme Q10 in human breast milk by high-performance liquid chromatography. Biomed. Chromatogr. 20, 1336–1343.

Tijerina-Sáenz, A., Innis, S.M., Kitts, D.D., 2009. Antioxidant capacity of human milk and its association with vitamins A and E and fatty acid composition. Acta Paediatr. 98, 1793–1798.

Todoroki, Y., Tsukahara, H., Ohshima, Y., Shukunami, K., Nishijima, K., Kotsuji, F., Hata, A., Kasuga, K., Sekine, K., Nakamura, H., Yodoi, J., Mayumi, M., 2005. Concentrations of thioredoxin, a redox-regulating protein, in umbilical cord blood and breast milk. Free Radical Res. 39 (3), 291-297.

Torres, A., Farré, R., Lagarda, M.J., Monleón, J., 2003. Determination of glutathione peroxidase activity in human milk. Nahrung/Food 47 (6), 430– 433.

Traber, M.G., Atkinson, J., 2007. Vitamin E, antioxidant and nothing more. Free Radic. Biol. Med. 43, 4–15.

Tsopmo, A., Diehl-Jones, B.W., Aluko, R.E., Kitts, D.D., Elisia, I., Friel, J.K., 2009. Tryptophan Released From Mother's Milk Has Antioxidant Properties. Pediatr. Res. 66 (6), 614-618.

Tsopmo, A., Romanowski, A., Banda, L., Lavoie, J.C., Jenssen, H., Friel, J.K., 2011. Novel anti-oxidative peptides from enzymatic digestion of human milk. Food Chem- 126, 1138–1143.

Tsukahara, H., 2013. Redox modulatory factors of human breast milk. Handbook of Dietary and Nutritional Aspects of Human Breast Milk Ed 5, Human Health Handbooks, ISSN 2212-375X Ed. Sherma Zibadi, Ronald Ross Watson, Victor R. Preedy

Vogelsang, A., van Lingen, R.A., Slootstra, J., Dikkeschei, B.D., Konen, B.J., Schaafsma, A., van Zoeren-Grobben, D., 2009. Antioxidant Role of Plasma Carotenoids in Bronchopulmonary Dysplasia in Preterm Infants. Int. J. Vitam. Nutr. Res. 79 (5-6), 288-296.

WHO (World Health Organization), Health topics, Breastfeeding, Available from: http://www.who.int/topics/breastfeeding/en/ [Accessed: 27th February 2015]

Xavier, A.M., Rai, K., Hegde, A.M., 2011. Total Antioxidant Concentrations of Breastmilk—An Eye-opener to the Negligent. J. Health Popul. Nutr. 29(6), 605-611.

Yao, L., Friel, J.K., Suh, M., Diehl-Jones, W.L., 2010. Antioxidant Properties of Breast Milk in a Novel In Vitro Digestion/Enterocyte Model. J. Pediatr. Gastroenterol. Nutr. 50 (6), 670-676.

Yen, C., Shen, C., Hsu, W., Chang, Y., Lin, H., Chen, H., Chen, C., 2011. Lactoferrin: an iron-binding antimicrobial protein against *Escherichia coli* infection. Biometals 24, 585–594.

Zagierski, M., Szlagatys-Sidorkiewicz, A., Jankowska, A., Krzykowski, G., Korzon, M., Kaminska, B., 2012. Maternal smoking decreases antioxidative status of human breast milk. J. Perinatol. 32 (8), 593-597.

Zarban, A., Taheri, F., Chahkandi, T., Sharifzadeh, G., 2007. Pattern of total antioxidant capacity in human milk during the course of lactation. Iran. J. Pediatr. 17 (S1), 34-40.

Zarban, A., Taheri, F., Chahkandi, T., Sharifzadeh, G., Khorashadizadeh, M., 2009. Antioxidant and Radical Savenging Activity of Human Colostrum, Transitional and Mature Milk. J. Clin. Biochem. Nutr. 45, 150–154.

Anexo

Normas para submissão de manuscritos da revista Journal of Applied Biomedicine

Comprovativo de submissão

JOURNAL OF APPLIED BIOMEDICINE

AUTHOR INFORMATION PACK

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