



# Relation between polyphenols, malondialdehyde, antioxidant capacity, lactate dehydrogenase and toxic elements in human colostrum milk



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## HIGHLIGHTS

- Antioxidant and toxicological parameters in human milk were studied.
- Total phenolic content was correlated with total antioxidant capacity.
- Malondialdehyde correlated with Pb, Al and lactate dehydrogenase.
- Antioxidant capacity and total phenols were correlated with vegetable consumption.
- Former smoking and place of living did not differentiate studied parameters.

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## ABSTRACT

The present study investigated the interrelationships between the concentration of total polyphenols (TP), malondialdehyde (MDA), total antioxidant capacity (TAC), lactate dehydrogenase activity (LDH) and content of toxic elements (Al, As, Cd, Ni, Pb) in human colostrum milk ( $n = 75$ ), and further assessed their potential association with maternal lifestyle characteristics. As and Cd were always below detection limits while Al, Ni and Pb were found at the level of 89.7, 6.2 and 1.3  $\mu\text{g L}^{-1}$ , respectively. Concentrations of TP and MDA, in the studied group were  $46.91 \pm 21.25 \text{ mg GAE L}^{-1}$  and  $0.66 \pm 0.27 \text{ nmol mL}^{-1}$ , respectively, and were inversely correlated ( $R_s = -0.32$ ;  $p < 0.01$ ). TP and TAC increased significantly with maternal consumption of vegetables ( $R_s = 0.25$  and  $R_s = 0.37$ , respectively;  $p < 0.05$ ). Concentration of Al was positively correlated with MDA ( $R_s = 0.21$ ;  $p < 0.01$ ) and negatively with TP ( $R_s = -0.28$ ;  $p < 0.01$ ). Positive correlation was also found between Pb and MDA ( $R_s = 0.32$ ;  $p < 0.01$ ). No association with place of living (urban/rural), women's age and former smoking were found for any studied milk parameter. The results add to the general understanding of factors influencing redox balance in milk and potentially affecting its quality.

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## 1. Introduction

Maternal milk is considered to be an excellent source of nutrients and non-nutritive bioactive factors such as anti-inflammatory

agents, growth factors, chronobiotic components, prebiotics and cells. The benefits of breastfeeding in the neonatal period and after weaning are widely documented, and exclusive human milk feeding has been recommended by the World Health Organization to be practiced for the first 6 months of life, with continued breastfeeding for 1–2 years of life or longer (World Health Organization, 2003).

Due to the essentiality of breastfeeding in proper child

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development, there is an ongoing interest in studies on the chemical composition of human milk and factors potentially modifying it. Over the years numerous studies have assessed the presence of various contaminants in human milk e.g. toxic metals, pesticides, polychlorinated biphenyls, endocrine-disrupting chemicals as well as molecules of specific biological role including macro- and micronutrients, hormones, microRNAs and antioxidants (Andreas et al., 2015; Poniedziałek et al., 2017). The latter consist of enzymes (e.g. superoxide dismutase, catalase), hydrophilic (e.g. glutathione, ascorbic and lipoic acid) and lipophilic metabolites (e.g.  $\alpha$ -tocopherol, carotenes, ubiquinol), and polyphenols (Yuksel et al., 2015; Vázquez et al., 2015). Their intake with breast milk is considered beneficial as they offer protection from oxidative stress, a phenomenon caused by concentrations of free radicals above the capacity of the biological system to readily detoxify them. Their potential detrimental outcomes include DNA damage, protein modifications, lipid peroxidation, and necrotic or apoptotic cell death (Halliwell and Gutteridge, 2007; Komosa et al., 2017). A high concentration of antioxidants in breast milk is particularly important for neonates as their antioxidant capacity is low while oxidative stress during the neonatal period has been associated e.g. with necrotizing enterocolitis if the site of the damage is in the digestive tract, periventricular leukomalacia and intraventricular hemorrhage and retinopathy (Papp et al., 1999; Inder and Volpe, 2000; Zhou et al., 2005). It has been reported that breast milk provides significantly higher antioxidant potency than infant formulas (Aycicek et al., 2006; Oveisi et al., 2010).

Recently some attention has been paid to the presence of polyphenols in human milk, a structural class of plant secondary metabolites. Based on number of phenol rings and structural elements that bind these rings they can be divided into flavonoids, phenolic acids, stilbenes and lignans (Manach et al., 2004). Their strong antioxidative activity has been well established and current evidence suggests that the intake of polyphenols, which may be as high as 1 g per day (Kuhnau, 1976), has a beneficial effect in the prevention of various disorders such as cardiovascular diseases, cancer or osteoporosis (Scalbert et al., 2005). Given the importance of polyphenols, it is imperative that their content in breast milk and factors influencing it be systematically investigated. However, current knowledge in this respect is still limited, although various methods for the determination of polyphenol content in milk from different species including humans have been developed (Li et al., 2009; Vázquez et al., 2015). Moreover, there is essentially no data on the association of these compounds with milk quality and function or with factors associated with maternal lifestyle. Considering the protective role of polyphenols in oxidative stress, it is important to assess whether and how their presence in milk may be associated with its pro- and antioxidative parameters.

The present study investigated the concentration and interrelationships of total polyphenols (TP), selected toxic elements, aluminum (Al), arsenic (As), cadmium (Cd), nickel (Ni) and lead (Pb), malondialdehyde (MDA), total antioxidant capacity (TAC) and activity of lactate dehydrogenase (LDH) in human colostrum milk collected from 75 healthy Polish mothers with no pregnancy complications. All of these metals are known to induce oxidative stress and cell death while MDA is, along with 4-hydroxy-2-nonenal, a major genotoxic product of lipid peroxidation, and LDH is one of the most convenient marker of cytotoxicity (Fotakis and Timbrell, 2006; Ayala et al., 2014). The potential associations between TP, MDA, TAC, LDH and metals in milk and maternal lifestyle factors such as place of living, smoking and diet (fish, vegetable and meat consumption) were also assessed.

## 2. Material and methods

### 2.1. Study recruitment

The study was conducted in 2016. Milk was collected from 75 white Caucasian women who had been admitted to the Gynecologic and Obstetrical University Hospital in Poznań (Poland) and were giving birth. The milk was taken three days after parturition, during the initiation of lactation. The inclusion criteria for the study included: birth by spontaneous delivery or cesarean section, birth weight  $\geq 2500$  g, no chronic maternal disease, no breast disease or inflammation requiring topical administration of drugs. Exclusion criteria included pregnancy complications (pregnancy-induced hypertension, anemia, intrauterine-growth restriction, gestational diabetes, premature rupture of membrane), low birth weight ( $< 2500$  g), history of musculoskeletal, or other disease requiring metal-based or artificial material implantation, no surgery with metal-based stapler use, chronic renal, gastrointestinal, endocrine (except previously treated and stable hypothyroidism in the euthyroid stage), genetic, musculoskeletal, metabolic, neural, psychiatric, pulmonary or cardiologic disease. Considering that maternal smoking decreases the antioxidative status of human breast milk and can cause other adverse changes to its composition (Zagierski et al., 2012; Napierala et al., 2016), all female smoking during pregnancy were also excluded from the study. All mothers who had not developed sufficient colostrum secretion to collect samples for the study were excluded.

Based on a questionnaire, place of living and history of cigarette smoking were specified for each enrolled participant. Fish, vegetable and meat consumption was recorded as a frequency of consumed servings (serv.) defined according to the [Dietary Guidelines for Americans \(2010\)](#) (medium-sized in case of meat) and specified using a six-point scale for each food group. The frequency of fish consumption was defined as: never,  $< 1$ serv./month, 1serv./month, 2serv./month, 1serv./week or  $\geq 2$ serv./week. The frequency of vegetable ranged from never, 1-2serv./week, 3-4serv./week, 1-2serv./day, 3-4serv./day to  $\geq 5$ serv./day. The frequency of red meat and poultry consumption was defined as: never, 1serv./week, 2-3serv./week, 4-5serv./week, 5-6serv./week,  $\geq 7$ serv./week. The studied group was divided into women living in urban and rural (population density below 150 inhabitants per km<sup>2</sup>) areas. The study was approved by the Local Bioethical Committee of the Poznan University of Medical Sciences (Poland) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All women undersigned a written consent.

### 2.2. Sample collection

Human colostrum milk (15–20 mL) was collected using a manual breast pump suction device from women who did not experience any difficulties in milk secretion. No high under-pressure, breast massage or topical/systemic drugs were used to stimulate milk secretion. Milk was collected directly into a 20 mL syringe (B. Braun, USA) by a professional lactation consultant (International Board Certified Lactation Consultant IBCLC No:309–75569) between 8:00 and 12:00 a.m. during postpartum hospitalization. After collection the milk samples were immediately frozen at  $-40$  °C prior to further procedures.

### 2.3. Determination of total phenolic concentration

The extraction and quantification of total phenolic concentration using the Folin–Ciocalteu method were performed according to Vázquez et al., (2015) with some modification. The milk samples

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