

Review Article

Dual Benefits, Compositions, Recommended Storage, and Intake Duration of Mother's Milk

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Abstract

Background: Breastfeeding benefits both infants and mothers. Mother's milk provides infants with immunity and protections from multiple diseases.

Objective: To comprehensively review the benefits and composition of mother's milk.

Methodology: 31,496 mother's milk-related abstracts (published until March 6th, 2019) from PubMed were analyzed using text mining and cited articles were studied through careful perusing.

Results: Our results suggest that mother's milk compositions (e.g. fatty acids) vary according to maternal diet and responses to infection in mothers and/or infants. Milk of malnourished mothers lacks certain important nutrients (e.g. DHA, vitamin B12). Since more mothers are expressing milk, we summarized the recommended storage and the effects on mother's milk compositions. Additionally, we examined the duration of mother's milk intake, concerns associated with mother's milk and the need and the possibility of relactation.

Conclusions: Healthy and unwell infants need mother's milk. Individualized mother's milk fortification and proper maternal supplementations may be needed to address the variability observed in mother's milk.

Keywords: breastfeeding; breastfeeding duration; exclusive breastfeeding; infant growth; lactation; maternal nutrition; milk composition; relactation

Introduction

Mother's milk help infants and preemies to develop immunity (Le Huërou-Luron et al., 2010) and long-term, dose-dependent protections from respiratory infections, gastrointestinal diseases, sudden infant death syndrome (SIDS), childhood cancers (leukemia and lymphoma) (Karimi et al., 2016), obesity, diabetes, multiple sclerosis (Conradi et al., 2013), behavior problems (Liu et al., 2013), and hand, foot and mouth disease (HFMD) (Supplementary).

In infants born to HIV-infected women, exclusive breastfeeding reduces diarrhea and respiratory infections (Mwiru et al., 2011). Breastfeeding

duration also influences the protections against eczema, allergic rhinitis, rhino conjunctivitis, and asthma (Lodge et al., 2015; van Odijk et al., 2003). Even partial breastfeeding can reduce SIDS (Thompson et al., 2017). Mother's milk may reduce the risk of obesity and diabetes (Pereira et al., 2014); breastfeeding (for ≥ 3 months) may reduce overweight in children of gestationally-diabetic women (Schaefer-Graf et al., 2006). Mother's milk, especially its longer intake (Angelsen et al., 2001), promotes brain growth and cognition (Lucas et al., 1992);

For mothers, breastfeeding reduces their risks for breast cancer (Islami et al., 2015), endometrial

cancer, ovarian cancer (Cramer, 2012), obesity-related diseases, postpartum depression and bone loss. Breastfeeding duration is inversely associated with risks for breast cancer (Lipworth et al., 2000). Breast cancer risks are lower for parous women (regardless of birth numbers) who have cumulatively breastfed for 12/> months (Anothaisintawee et al., 2013), as breastfeeding alters hormones, molecular histology and immunity (Islami et al., 2015) through undiscovered mechanisms. Breastfeeding lowers endometrial cancer risk through reduced estrogen (Okamura et al., 2006). Long-term breastfeeding protects against ovarian cancer (Jordan et al., 2010). Intense breastfeeding helps with managing pregnancy-gained weight and reducing risks for metabolic syndrome (Ram et al., 2008), hypertension (Schwarz et al., 2009), type 2 diabetes (Stuebe et al., 2005) and heart disease (Perrine et al., 2016). Lactating mothers secrete prolactin and oxytocin that help them to adapt to motherhood (Torner, 2016).

Despite these numerous benefits of breastfeeding, globally, less than half of the infants are exclusively breastfed for the initial six months. Studies suggest that knowledge on breastfeeding can improve this rate (Gupta et al., 2013; Valdés et al., 1993). This may be because mothers who understand the benefits of breastfeeding are more

likely to exclusively breastfeed (Arage and Gedamu, 2016). Although there are many published articles reviewing the benefits of mother's milk, given the importance of educating mothers in promoting breastfeeding, it is important to update the information. This motivated us to review the literature on mother's milk by including results from text mining analysis of PubMed abstracts.

The results from text mining particularly suggest that ensuring maternal nutrition has a very important role in ensuring infant health during breastfeeding. We also reviewed literature on mother's milk storage, intake duration, associated concerns (Supplementary) and relactation (Supplementary).

Methodology

We retrieved PubMed abstracts related to mother's milk using several keywords and their combinations on March 6th, 2019 (Table 1). Using the results from the keyword search that returned the largest number of articles, i.e. 31,496 articles, we created a dataset and a word cloud (Figure 1), that suggested relationship between mother's milk compositions and the health of infants including preemies (see Results). A review of the literature was conducted by including the results from text mining.

Table 1: Number of Articles Retrieved using Different Keywords of Mother's Milk.

Keywords Used	Number of articles retrieved
"mother's milk"	1327
"human milk"	21.351
"breast milk"	12.275
"breastmilk"	1655
"breastfeed*"	4398
"breast milk" OR "breastmilk"	13.773
"mother's milk" OR "human milk" OR "breast milk" OR "breastmilk" OR "breastfeed*"	31.496

level of zinc (i) is observed in mother's milk of women with loss-of-function mutation in zinc transporters and (ii) causes transient neonatal zinc deficiency (TNZD) in exclusively breastfed infants (Golan et al., 2017). The risk of TNZD is at least 1 in 2334 infants (Golan et al., 2017); high mother's milk cytokine (TGF β 2) amounts were associated with high risks for eczema (Munblit et al., 2017).

Mother's milk also lacks sufficient vitamin D (González and Visentin, 2016) and this increases infant's risks for rickets, respiratory diseases, type 1 diabetes (Dawodu and Wagner, 2012), hypocalcemic seizures and dilated cardiomyopathy (Omotobara-Yabe et al., 2018). These studies suggest that early detection of nutrient deficiencies as well as proper supplementation of nutrients are necessary for breastfeeding women.

Timing of nutrient deficiency is important, also in case of the brain development of children (Nyaradi et al., 2013; Prado and Dewey, 2014). Some neurodevelopmental processes start during pregnancy and complete within the first few years of life and nutrient deficiencies in this time period can affect the process (Prado and Dewey, 2014). Mother's milk from women who do not consume food from animal sources may lack some important micronutrients in sufficient quantities such as vitamin B12 and DHA (Kuratko et al., 2013; Nyuar et al., 2010; Venkatramanan et al., 2016; Williams et al., 2016). The severity of nutritional deficiencies determines their negative effects on infants (Prado and Dewey, 2014). Sometimes, the effects may be irreversible when treated later (Lozoff et al., 2000, 2006; Prado and Dewey, 2014; Wise, 2016). Moreover, nutrients act synergistically causing deficiency of one nutrient to affect another (González and Visentin, 2016; Nyaradi et al., 2013). Recovery from the negative effects may only be possible, if the nutrients are available during the time the affected growth process is still occurring (Prado and Dewey, 2014). Therefore, improving maternal nutrition in malnourished pregnant and lactating women should be given increasing attention.

The variabilities observed in mother's milk are particularly important in case of preemies since they have higher nutritional requirements and risks for diseases and growth retardation (Boquien, 2018). However, mother's milk is undeniably the

best food for the preemies as it offers protection and ensures their proper development (Boquien, 2018). Therefore, individualized mother's milk fortification (de Halleux and Rigo, 2013) can be used to address this variability.

Next, we discuss (i) the mother's milk compositions and the variability in greater details, (ii) effects of storage on expressed mother's milk, (iii) recommended durations for breastfeeding and factors affecting the duration. See Supplementary for discussion on (i) concerns associated with mother's milk and (ii) relactation.

Discussion

Mother's milk compositions and their variability

To meet infant developmental needs, mother's milk changes from colostrum, transitional milk, to mature milk (Supplementary) with distinct compositions. Mother's milk nutrients (proteins, fats, carbohydrates, vitamins) can promote infant health and survival (Ballard and Morrow, 2013) (Supplementary). Mature milk contains 60-75 kcal/100 ml and its fat, protein and carbohydrate contents are ~3-5%, 0.8-0.9% and 6.9-7.2%, respectively (Jenness, 1979). During early lactation, mother's milk contains more whey (protein that remains as liquid, e.g. lactoferrin, alpha-lactalbumin, immunoglobulins, lysozyme) than casein (protein that becomes curds in stomach and harder to digest) (Lönnerdal et al., 2017; Martin et al., 2016). Subsequently, their proportions equalize to facilitate nutrient absorption and immunity in infants (Lönnerdal et al., 2017). Mother's milk provides essential fatty acids (linoleic and alpha-linolenic acids) for infant growth and brain development (Lauritzen et al., 2016). Mother's milk fats (>98% triglycerides of palmitic and oleic acids) increase throughout a single nursing (Institute of Medicine (US) Committee on Nutritional Status During Pregnancy and Lactation, 1991). More than 98% of the fat is in the form of triglycerides (Pons et al., 2000). Main mother's milk carbohydrates include lactose, that induces innate immunity by triggering antimicrobial peptides production (Cederlund et al., 2013), and oligosaccharides, that shape microbiota and protect against Group B *Streptococcus* infection (Andreas et al., 2016).

Mother's milk completely provides carotenoids (Lipkie et al., 2015) for immunity and vision.

Although mother's milk has all essential fatty acids, its DHA level is variable and depends on maternal diet, particularly on fish products that are rich in preformed DHA (Nyuar et al., 2010). The rate of synthesis of DHA from alpha-linolenic acid, its precursor, is also inefficient for infants and the DHA level depends on maternal supply through mother's milk (Nyuar et al., 2010). DHA is essential for neurovisual development; its deficiency can negatively affect mental development and learning abilities (Nyuar et al., 2010). Maternal diets also influence vitamin composition in mother's milk, especially the composition of vitamins A, B1, B2, B3, B6, B12, C and D (Allen, 2012; Ares Segura et al., 2016; Dror and Allen, 2018; Kominiarek and Rajan, 2016; Neerven and Savelkoul, 2017; Nommsen et al., 1991; Valentine and Wagner, 2013). Deficiency of vitamin B6, vitamin B12, and thiamine causes growth stunting (Allen, 2012). Mother's milk minerals are important for infant growth, development and immunity (Klein et al., 2017). While mother's milk calcium, magnesium, iron, zinc, copper amounts are independent of maternal mineral status (Domellöf et al., 2004; Dorea, 2000; Li et al., 2016), selenium amount is dependent (Kumpulainen et al., 1985).

Since maternal nutrients affect mother's milk compositions, maternal supplementations are available. While folic acid intake is recommended in the first trimester to avoid fetal neural tube defects, it can alter gene expression epigenetically (McStay et al., 2017) and cause respiratory allergies in mice (Hollingsworth et al., 2008). Studies of the association between maternal folic acid intake beyond the first trimester and childhood allergic diseases remain inconclusive (McStay et al., 2017). While some studies reported their positive association (Dunstan et al., 2012; Håberg et al., 2011), maternal intake of folic acid might promote cow's milk allergy and childhood immunoglobulin E antibody-mediated allergic diseases (McStay et al., 2017; Tuokkola et al., 2016). In contrast, pregnancy supplementations of omega-3 polyunsaturated fatty acids (i.e. EPA and DHA) protect against childhood allergies (Munblit et al., 2015).

Mother's milk anti-inflammatory cytokines offer passive protection to infants from intestinal inflammation (Chatterton et al., 2013) whereas pro-inflammatory cytokines stimulate active immunity (Brenmoehl et al., 2018). TGF β , the dominant mother's milk cytokine (Ballard and Morrow, 2013), promotes mucosal immunity by promoting immunoglobulin A production and may decrease allergic risk (Kalliomäki et al., 1999). Colostrum has the most leukocytes (Hassiotou et al., 2012), that significantly increase upon infection.

Expressed milk storage

More mothers are expressing milk while being away from their baby due to illness, premature delivery, or returning to work. Expressing milk also relieves engorgement. To prevent microbes' growth, expressed mother's milk (if not for immediate consumption) needs cooling/freezing. Storage temperature, freezing, thawing, light exposure, pasteurization and storage container affect mother's milk composition/quality (Supplementary) (Hamosh et al., 1996; Lawrence, 2007).

Creatamocrit and human milk analyzer (HMA) are for measuring mother's milk fat and caloric contents. The methods employ different parameters. To account for the effects of freezing on fat contents in mother's milk, Wang *et al.* used the following regression equations to calculate caloric contents in fresh and frozen mother's milk: for fresh mother's milk, energy (kcal/dl) = 5.99 x creatamocrit(%) + 32.5; for frozen mother's milk, energy (kcal/dl) = 6.20 x creatamocrit(%) + 35.1 (Wang et al., 1999). On the other hand, in HMA, protein, lactose and fat in mother's milk can be measured (O'Neill et al., 2013). In a study that used HMA (García-Lara et al., 2011), caloric contents in mother's milk was calculated using this equation: energy (kcal/dl) = 9.25 x fat + 4.40 x total nitrogen + 3.95 x lactose (García-Lara et al., 2011). While several studies suggest that creatamocrit correlated strongly with fat and energy content in mother's milk (Lemons et al., 1980; Lucas et al., 1978; Wang et al., 1999), Neill et al. reported that creatamocrit overestimates fat and energy content in mother's milk and HMA has increased accuracy (O'Neill et al., 2013). There are

also inconsistencies in results from studies that used the different methods.

A study on frozen (-20°C) mother's milk samples for 28 days, that used the creamatocrit method, reported that caloric contents did not change much (Silprasert et al., 1987). However, in a study that used the HMA method, Garcia-Lara et al. observed that frozen (at less than -20°C) mother's milk contains less fat and calories than fresh mother's milk (García-Lara et al., 2011). They observed that fat and caloric contents decrease as duration (7, 15, 30, 60, and 90 days) of freezing increases. In their study, fresh mother's milk had 4.52-5.64 g/100 ml of fat and 0.72-0.82 kcal/ml of calories, whereas frozen mother's milk had only 3.84-5.14 g/100 ml of fat and 0.65-0.77 kcal/ml of calories after 90 days of freezing. Freezing for 7, 15, 30, 60, and 90 days, did not significantly change the nitrogen content in mother's milk, and the decrease of lactose in frozen mother's milk was only significant after 90 days of freezing.

Mother's milk fat and protein content are reduced by pasteurization (Vieira et al., 2011). Fat content is significantly reduced while delivery using continuous infusion feeding, due to the adherence of fat to the delivery system (Vieira et al., 2011). Protein denaturation occurs during freezing and thawing (Chang et al., 2012). Freezing mother's milk can change and destabilize casein micelles and change the quaternary structure of whey proteins, resulting in the formation of precipitates (García-Lara et al., 2011). However, storage temperatures have not shown to influence the protein concentration in mother's milk (Chang et al., 2012). Reduction in fat and protein concentration is a serious concern as it can affect the growth rate of preterm infants (Jarjour et al., 2015).

Milk fat globule membranes (MFGMs) in mother's milk are broken by freeze, thawing procedures (García-Lara et al., 2011; Ogundele, 2002). The triglycerides released with rupturing of MFGMs can come in contact with the lipase enzymes in mother's milk (Ogundele, 2002), which are observed to be active at freezing temperature (-20°C), and room temperatures (5°C , 25°C and 38°C) (García-Lara et al., 2011; Hamosh et al., 1996; Ogundele, 2002). The lipase catalyzes the hydrolysis of triglycerides (also known as

lipolysis) into diglycerides, monoglycerides, and free fatty acids (Bitman et al., 1983). Triglycerides are esters derived from glycerol and three fatty acids. In a diglyceride, its glycerol is ester-linked to two fatty acids, whereas in a monoglyceride, its glycerol is ester-linked to only a fatty acid. The fatty acids produced by this hydrolysis have antimicrobial activity (Hernell et al., 1986; Isaacs and Thormar, 1991; Thormar et al., 1994). On the other hand, rupturing of MFGM may reduce the bactericidal properties of mother's milk as MFGMs have bacterial sequestration abilities (Ogundele, 2002). However, at -20°C , a significant reduction in bactericidal activity of mother's milk is not observed after one month (Ogundele, 2002). Freezing at -70°C should be optimal as fat hydrolysis does not occur at this temperature (Berkow et al., 1984).

Another matter of concern is lipid peroxidation. Lipid peroxidation is the process in which free radicals (most commonly reactive oxygen species) remove electrons from lipids producing reactive intermediates (Lipid Peroxidation | IntechOpen, n.d.). Turoli et al. observed lipid peroxidation in fresh and frozen mother's milk (-20°C), and formula milk (Turoli et al., 2004). They observed highest lipid peroxidation in frozen mother's milk. This finding may be related to the higher contents of free fatty acids in frozen mother's milk due to hydrolysis activity of lipase. Lipid peroxidation is also observed in mother's milk at 4°C (Miranda et al., 2004) and -80°C (Silvestre et al., 2010). However, lipid peroxidation is minimal in frozen mother's milk (-20°C and -80°C) compared to that stored in refrigerator (4°C) (Miranda et al., 2004; Silvestre et al., 2010). To minimize lipid peroxidation, freezing temperature of -80°C is preferable over -20°C (Silvestre et al., 2010). Presence of lipid peroxidation products in infant feeds may play roles in development of diseases including necrotizing enterocolitis and bronchopulmonary dysplasia (van Zoeren-Grobbe et al., 1993). Studies to determine the effect of consuming lipid peroxides in infants including preemies are needed.

The antioxidant activity of mother's milk, which could protect against the effects of lipid peroxidation, also decreases with storage at refrigeration (4°C) and freezing temperatures (-8°C , -20°C , -80°C) (Hanna et al., 2004; Silvestre

et al., 2010; Xavier et al., 2011). The complete list of antioxidant components in mother's milk and their contribution to this variation are unknown. However, the carotenoid (major antioxidants in mother's milk) contents in frozen mother's milk were reported to be stable at -18°C for 28 days (Tacken et al., 2009). Storage at -80°C for a period of less than 30 days is recommended to maximally preserve antioxidant activity (Silvestre et al., 2010).

Freezing ($-20^{\circ}\text{C}/-70^{\circ}\text{C}$) and heat treatment have little effects on concentration of vitamins A, D and E in mother's milk (Hanna et al., 2004; Moffatt et al., 1987; Van Zoeren-Grobben et al., 1987). On the other hand, storage for more than one month in a freezer (-16°C) or 24 hours in a refrigerator ($4^{\circ}\text{C}-6^{\circ}\text{C}$) may substantially reduce vitamin C concentration (Buss et al., 2001). Holder pasteurization may affect the vitamin B6 concentration in mother's milk (Van Zoeren-Grobben et al., 1987).

Many protective immunologic components of frozen mother's milk kept at -20°C remain stable for a duration of up to 365 days while lactoferrin and live cells amounts are affected by storage container, freezing and/or thawing (Lawrence, 2001). Mother's milk lactoferrin significantly decreases after 3 months of freezing (-20°C) (Raouf et al., 2016). Cycles of freezing and thawing can rupture cells in mother's milk (Chantry et al., 2006) and longer storage time reduces the cell function (Lawrence, 2001). Frozen mother's milk kept at -20°C has lower number of cells and functions of surviving cells than fresh mother's milk does (Lawrence, 2001). Cells in mother's milk can also adhere to the surface of the storage container particularly Pyrex glass container (Lawrence, 2007).

Duration of mother's milk intake

In an Australian study, only 45.9% of babies received any mother's milk at age 6 months; 19.2% at age 12 months (Scott et al., 2006). Maternal attitudes are among factors lengthening the duration of mother's milk intake (Casal et al., 2017; Mehta et al., 2017; Scott et al., 2006). Opposing factors include early breastfeeding difficulties (Scott et al., 2006), inadequate mother's milk (Kimani-Murage et al., 2011), lack of family support (Lauwers and Swisher, 2005), early

introduction of pacifier (Scott et al., 2006), smoking (Scott et al., 2006), social stigma (Kendall-Tackett and Sugarman, 1995) and short maternity leave (Scott et al., 2006). Since duration and exclusivity of mother's milk intake are influencing health benefits (Supplementary), lactation counseling can promote breastfeeding exclusivity and its total duration (Morrow et al., 1999). A USA study comparing exclusive mother's milk intake for 6 months/more versus 4-<6 months, found that the former has lower respiratory tract infections (Chantry et al., 2006). Breastfeeding beyond 6 months can better protect against respiratory and gastrointestinal tract infections (Tromp et al., 2017).

Besides studies supporting World Health Organization (WHO)'s recommendations of *exclusive* breastfeeding for the first six months (Dewey, 2001; Lessen and Kavanagh, 2015) and continued breastfeeding up to 2 years/beyond along with complementary foods, some studies suggested that exclusive breastfeeding may be insufficient for 6-month-old infants (Fewtrell et al., 2007; Reilly et al., 2005). However, early introduction of complementary foods may negatively affect infants (Cohen et al., 1994; Gupta et al., 2017). Prolonged breastfeeding after 6 months along with complementary foods is beneficial for mother-child bonding (Weaver et al., 2018), healthy dietary patterns at age 6 (Perrine et al., 2014), and decreasing asthma risk (Lodge et al., 2015).

After infants turn 6 months old, complementary foods are essential, because zinc, calcium, vitamin B6 and vitamin C levels in mother's milk subsequently decrease (Garza et al., 1983; Karra et al., 1986), whereas mother's milk continues providing vitamin A (first 3 years) (Onyango et al., 2002; Persson et al., 1998) and fat (first 2 years) (Mandel et al., 2005; Onyango et al., 2002). In the second lactating year, mother's milk provides significantly more protein, lactoferrin, immunoglobulin A and lysozyme (Perrin et al., 2017).

Conclusion

Mother's milk protects against various diseases in infancy, childhood and later life, by promoting proper and long-term immunity. More future studies are needed to understand the molecular

mechanisms of how mother's milk influences maternal hormonal levels and renders immunity to infants. A strategy that builds on our current work, will be exhaustively mining the literature on mother's milk and hormones including estrogen, prolactin, and oxytocin. Infants, including infected ones, require mother's milk. Knowledge of mother's milk compositions, storage and intake duration can help mothers to make better efforts in breastfeeding and lactation.

Supplementary information

Supplementary materials are available from <https://tinyurl.com/mothersmilksupplementary>.

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