Human Breastmilk Storage and the Glutathione Content

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Summary

Human breastmilk storage for use later in infant feeding is on the increase as a result of the economic activities of nursing mothers. This study investigated glutathione (GSH) status of stored human breastmilk due to its major antioxidant role and as a cofactor for enzymes in detoxification of carcinogens. In newborns, human breastmilk becomes an important source of dietary GSH since their GSH synthetic capacity may not be well developed. The results showed that the total GSH content of human breastmilk obtained from apparently healthy lactating mothers was 192.2 ± 148.3 μmol/l (mean ±SD). Early breastmilk (fed to infants up to 4 weeks old; GSH content of 252.5 ± 173.9 μmol/l) was significantly higher ($p < 0.05$) when compared with their mature counterpart (milk from mothers with infants older than 1 month of age; GSH content 163.9 ± 128.0 μmol/l). Substantial loss of GSH occured when breastmilk was kept at either −20°C. 4°C or at room temperature for 2 h. When compared with fresh unstored breastmilk, the extent of the loss was 80.6, 79.1 and 73.0 per cent respectively. It is suggested that feeding infants on stored human milk could weaken the antioxidant and toxin refractory capacity of those in early childhood.

Introduction

Glutathione (GSH) is a tripeptide comprising glutamine, cysteine and glycine and is of much importance to health. GSH performs several important physiological functions. These include inactivation of oxygen-derived highly reactive substances, as a cofactor for enzymes in the detoxication of various types of toxins and carcinogens of endogenous and exogenous types,[1–4] and in the elimination of malonic dialdehyde (MDA), a known toxicant and carcinogen, formed from oxidation of polyunsaturated fatty acids.[5–8] GSH also maintains other important antioxidants, such as ascorbic acid, in their functional state and improves the cellular immune response by its role in lymphocyte activation.[9,10] GSH is formed in all cells, but the capacity of cells to synthesize GSH *in vivo* is not well developed in the very young.[11,12] Dietary GSH, therefore, would be expected to provide the GSH required early in life to maintain health. Feeding on breastmilk thus becomes the principal source of GSH for the human infant in early childhood. This practice would be expected to render such infants less susceptible to the destructive effects of oxidants and other toxins[2–4,8] in addition to the provision of colostrum for anti-infective action.

For convenience, the habit of storing human breastmilk by working nursing mothers for later use in infant feeding is on the increase because of its known anti-infective property. It had been reported that GSH is preserved in frozen foods,[13] but whether this is the same for stored human breastmilk is not certain. The results of the present investigation showed that storage of human breastmilk results in a substantial decrease of GSH and hence a major loss of the antioxidative and anti-toxic properties of the milk.

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Subjects and Methods

Subjects

The subjects were randomly recruited from apparently healthy breastfeeding mothers attending a postnatal clinic at the University of Ghana Hospital, Legon. A total of 47 breastfeeding mothers (age, 19–40 years; mean 27.6 years) volunteered to participate in the study. Fifteen of them (mean age, 26.2 years) had infants less than 4 weeks old and breastmilk from this group was classified as early human milk. Breastmilk from the remaining 32 mothers (mean age, 28.4 years) with infants who were more than 4 weeks old was classified as mature human milk. Health was defined as an absence of a current major medical illness or medication and a subjective perception of good health.

Human breastmilk collection

A 1.0 ml sample of human breastmilk (henceforth referred to as human milk) was collected from each

subject between the hours of 7 : 00 a.m. to 8 : 30 a.m. into sterile polypropylene tubes and capped. Aliquots of the human milk were treated immediately (0-h sample) with 5 per cent ice-cold meta phosphoric acid or after it had been kept either at the existing room temperature (24–32°C, at 4°C or at −20°C for up to 2 h. A few ml of early morning spot urine was collected from each subject.

GSH analysis

The acid treated human milk was kept in ice for a minimum of 15 min and centrifuged. Aliquots of the resulting supernatant were collected and assayed for GSH as follows: the sample, alongside calibrators prepared from GSH obtained from Sigma Chemical Co. (St. Louis, MO, USA), was assayed by the method of Cohn and Lyle.[14] Briefly, GSH was reacted with 0.1 per cent of 0-phthalaldehyde (WAKO Chemicals, Osaka, Japan) in 100 mM phosphate buffer, pH 8.0, and read fluorometically (excitation, 350 nm; emission, 420 nm) using the SFM-25 spectrofluorometer (Kontron, Zurich, Switzerland).

MDA analysis

The MDA content of the milk and urine samples was analysed by the method of Slater and Sawyer[6] but with a slight modification as described by Halliwell and Gutteridge.[15] To 0.4 ml of either the human milk supernatant fraction or urine specimen was added an equivolume of 0.67 per cent (w/v) of 2-thiobarbituric acid (Sigma Chemical Co., St Louis, MO) dissolved in 50 mM sodium hydroxide. After heating the mixture at 100°C for 15 min, the suspension was read at 532 nm ($\epsilon = 1.49 \times 10^5$ l/mol cm) alongside blank samples. Total thiol(-SH) content of the urine specimens was also determined by the method of Ellman ($\epsilon =$ 13.600 M cm).[16]

Statistics

Statistical analysis was based on Student's *t*-test, two-tailed; $p < 0.05$ was considered significant.

Results and Discussion

GSH was detected in all 47 milk samples. The mean GSH concentration was 192.2 μmol/l ranging from 20.3 to 498.4 μmol/l. The results showed that the mean human milk GSH level declined significantly ($p < 0.05$) as the age of the child increased: 252.5 and 163.9 μmol/l, respectively in early and mature human milk. This pattern was not observed with measurement of total thiol (-SH) levels in the urine of mothers (mean ±SD: 28.4 ± 10.8 μmol/l for mothers with early human milk vs. 32.8 ± 10.3 μmol/l for mothers with mature human milk). MDA was detected in each human milk specimen but no significant difference was seen between the early and mature milk samples (Table 1). A similar observation was made for urine MDA content of the mothers (mean ±SD: 2.5 ± 1.5 μmol/l for mothers with early human milk and 3.5 ± 3.5 μmol/l for mothers with mature human milk). The observed inverse relationship between higher human milk GSH and an early age (4 weeks or less) of the child is strategic since the milk provides much more GSH at a period when the newborn may lack the capacity to synthesize GSH or do so efficiently.[11,12] Thus, soon after birth, maternal milk is an essential source of GSH for the infant which, among others, functions as a major antioxidant and detoxification agent of various toxicants.[1,2,4] From the present results human milk GSH enters the child's intestinal lumen along with MDA. The latter is a product of cellular lipid peroxidation that alkylates proteins and DNA and is a carcinogen. GSH is known to facilitate the elimination of MDA and hence its toxic actions.[2,3,13] This protective role of GSH is also known to perate for drugs[17,18] and other toxic and carcinogenic chemicals, such as aflatoxins, that may contaminate human milk fed to infants.[4,19]

Figure 1 shows that when human milk was stored at existing room temperatures, 4°C or −20°C for 2 h there was a substantial (between 73.0 and 80.6 per cent) loss of its GSH content. The obtained GSH levels (mean ±SD) were 51.8 ± 56.6, 40.2 ± 51.0 and 37.3 ± 56.8 μmol/l, respectively vs. 192.2 ± 148.3 μmol/l for fresh unstored human milk. The decrease in the GSH level was highly significant ($p < 0.001$) irrespective of the storage temperature of the milk and is in contrast to the reported preservation of GSH in frozen foods.[13] Unlike the anti-infective properties of human milk which may not be affected by the storage conditions as used in the present study, human milk antioxidative action is likely to be largerly destroyed by the loss of GSH.[1,2] It is, therefore, in the total health interest of infants to be fed on fresh unstored human milk in order to supply them with as much GSH as available. It is worth noting that the GSH

TABLE 1
Glutathione (GSH) and malonic dialdehyde (MDA) content of early and mature human breastmilk

Analyte (μmol/l)	Early milk ($n = 15$)	Mature milk ($n = 32$)	Total ($n = 47$)
Milk GSH	252.5 ± 173.9*	163.9 ± 128.0*	192.2 ± 148.3
Milk MDA	5.8 ± 5.3	4.6 ± 3.0	4.9 ± 3.8

*Significantly different, $p < 0.05$.
Values given as mean ±SD.

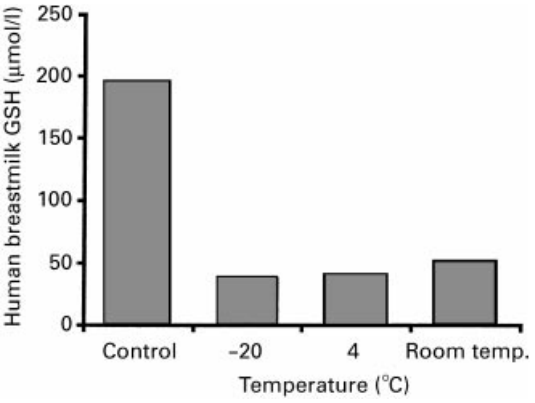

FIG. 1. Loss of glutathione (GSH) in stored human breastmilk. The milk was kept at the indicated temperatures for 2 h before assay of the GSH as described in the methods.

level was relatively higher in the early milk, obtained during a period in the life of the baby when the recommended diet is human milk and when biosynthesis of GSH may not have started or reached an optimum level.[11,12] The first weeks after birth may, therefore, be critical with respect to GSH insufficiency if an infant is fed mainly on stored human milk. This may lead to the possibility of developing chronic disease(s) at a later age due to susceptibility to adverse biological actions of toxic chemicals.

In conclusion, the results emphasize the need to avoid feeding infants with stored human milk since this could result in deficits in GSH and adversely affect the need to optimize the antioxidant capacity and detoxification mechanisms of chemical toxins and carcinogens (endo- and exo-genous in origin) of infants, especially in early childhood.

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