Vitamin C is reduced in human milk after storage

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In order to establish how cold storage of human milk affects levels of bioavailable vitamin C, 11 samples were stored for 24 h in the refrigerator or up to 2 mo in the freezer. Total vitamin C levels decreased on average by one-third in the refrigerator or after 1 mo of freezing, with wide variations between individuals (6 to 76% and 3 to 100%, respectively). After 2 mo of freezing, the average decrease was two-thirds (7–100%).

Conclusion: We recommend a change in human milk storage practices, to under 24 h in a refrigerator or under 1 mo in a freezer. Alternatively, vitamin C supplementation may be considered.

Key words: *Ascorbic acid, breast milk, human milk, vitamin C*

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Current recommendations for storing human milk in neonatal units and at home vary: from 24–48 h (1) to 3–5 d (2) to up to 8 d (3) for refrigerator storage and from 3 to 12 mo for freezer storage at −18°C (1–3). These time periods are aimed at avoiding bacterial growth rather than preserving nutritional properties. However, handling and storage can result in the loss of components sensitive to oxidation, such as the physiologically relevant forms of vitamin C, ascorbic acid (AA) and dehydroascorbic acid (DHAA). Losses of up to 40% of AA (4) and 20% of total vitamin C (5) from human milk after 24 h in the refrigerator have been reported, as well as minimal (in milk from mothers of term infants) or no loss (preterm infants) of total vitamin C after 3 mo of freezer storage (5). In the study by Bank et al. (5), however, a colorimetric method was used that measures total vitamin C including 2,3-diketoglutonic acid, a non-bioavailable oxidation product. Therefore, losses may have been underestimated. In contrast, measurement of AA without DHAA (as in (4)) may overestimate actual losses. The observation of decreases in AA levels during handling and storage of expressed human milk, assessed as part of a vitamin C supplementation trial of preterm infants, prompted us to investigate how storage affects levels of bioavailable vitamin C.

After informed consent had been given, we collected 11 samples of freshly expressed human milk from mothers whose babies were inpatients at Christchurch Women's Hospital. The babies were born with a gestational age of 23–29 wk and were 1 wk to 3 mo old at the time of sampling. Ameda AG pumps (Ameda Egnell, Switzerland) with Medela breast cups and tubing were used to collect the milk into sterile plastic bottles during the first expression in the morning. An aliquot of the final contents was received in the laboratory between 2 and 6.5 h after expressing, having been under refrigeration and in the dark in the meantime.

Six aliquots of each sample were transferred to 0.5ml Eppendorf tubes and analysed immediately or stored for a total of 24 h in the refrigerator (4–6°C) or for 1 wk, 1, 2 or 3 mo in the laboratory freezer (−16°C). Storage temperatures reflected realistic conditions in routine care and in the home. The laboratory refrigerator temperature was a little higher than the target of <4°C. The freezer unit had a separate door and was maintained at −16°C to −17°C.

AA was measured by high-performance liquid chromatography with electrochemical detection (6). Column, mobile phase and perchloric acid concentrations were modified from the original publication (Brownlee-Spheri-5-ODS 220 × 4.6 mm; 80 mM sodium-acetate-buffer, 0.54 mM disodium-EDTA, pH 4.8, flow rate 0.9 ml/min; 0.54 M perchloric acid to precipitate proteins and 77 mM for dilution of standards and samples). To analyse total vitamin C, DHAA was reduced to AA with dithiothreitol, final concentration 25 mM, for 15 min at room temperature before treating the sample with perchloric acid. AA could be detected down to 0.01 mg/100 ml.

The total mean vitamin C concentration in our samples was 5.31 mg/100 ml (SD 1.66) (Table 1). After 24 h in the refrigerator, the mean had decreased to 65% of the initial concentration, but the range of vitamin C losses varied widely, from 6 to 76%. One week of

Table 1. Total bioavailable vitamin C (ascorbic acid and dehydroascorbic acid) in human milk samples.

	Initial concentration (mg/100 ml)	% of initial concentration			
		Refrigerated, 24 h	Freezer, 1 wk	Freezer, 1 mo	Freezer, 2 mo
	6.40	94	91	70	17
	6.49	86	91	97	78
	6.10	80	92	58	49
	8.04	75	98	79	9
	4.73	70	84	67	36
	6.42	66	103	97	83
	5.36	57	83	82	n/a
	5.47	44	86	34	16
	2.33	27	83	45	39
	3.57	27	99	65	39
	3.51	24	97	0	0
Mean	5.31	65	92	63	38
SD	1.66	25	7	29	28
Median	5.47	66	91	67	37

freezer storage decreased total vitamin C to 92% of the initial concentration (range 83–100%), 1 mo to 63% (range 0–97%) and 2 mo to 38% (range 0–83%). The time-course was not extended beyond 2 mo because of the large losses already observed. After 2 mo of freezer storage, 4 out of 10 analysed samples had lost more than 80% of their bioavailable vitamin C, whereas 2 samples had lost only about 20%.

Initially, 91% of the total vitamin C was in the AA form (Table 2). After storage, the proportion of AA decreased, but this also varied widely for different milk samples.

To investigate the mechanism responsible for vitamin C loss, either potassium cyanide (KCN), a peroxidase inhibitor, or allopurinol, a xanthine oxidase inhibitor, was added to aliquots of human milk at respective final concentrations of 1 and 0.1 mM before 24-h storage in the refrigerator. The addition of allopurinol had no effect. KCN gave some protection against losses, one sample decreasing to 73% instead of 27% of the initial total vitamin C concentration (81% instead of 10% for AA), the other to 74% instead of 66% (80% instead of 25% for AA). Therefore, it appears that losses are partly due to lactoperoxidase activity.

Our findings are contrary to a previous publication (5), probably because that study used an analysis method that included an oxidized non-bioavailable product. The loss of, on average, 41% of AA after 24-h refrigeration confirms the results of Garza et al. (4).

The initial vitamin C content of our samples varied from 2.33 to 8.04 mg/100 ml before storage. We made no attempt to assess the mother's dietary intake or obtain milk after similar lactation periods, because this was not the focus of our study. Our results are nevertheless in line with previous published studies. Sneed et al. (7) reported mean AA concentrations of 5.31 mg/100 ml, SD 1.71, at 1 wk postpartum; Heinz-Erian et al. (8) reported mean total vitamin C concentrations of 5.81 mg/100 ml, SD 1.65, for mature milk and 5.32 mg/100 ml, SD 1.37, for transitory milk. Maternal supplementation increases human milk vitamin C only minimally, but not significantly (5, 7–9), unless the mother is vitamin C deficient, indicating the possible existence of a regulatory mechanism to avoid increase of vitamin C levels beyond a certain saturation point.

Recommended daily dietary allowances for infants have been estimated to be 30–35 mg/d, with 85% of dietary vitamin C actually being absorbed (2). Based on our average results and assuming a milk intake of 750 ml/d/infant, 41 mg vitamin C would be consumed with fresh milk, 27 mg after 24 h of refrigeration, 27 mg after 1 mo and 15 mg after 2 mo of freezer storage.

In neonatal units, infants may be fed stored milk for extended periods of time, with the oldest milk being given first. Many of these infants are supplemented with vitamin C, but with varying amounts. Term infants usually do not receive vitamin C supplementation. They may still be given stored milk, but usually not exclusively. Our main point of concern is the wide and unpredictable variation in vitamin C loss in stored samples, in some instances leading to a complete loss of vitamin C. The storage time should therefore be as short as possible. If long-term stored milk constitutes a major and regular proportion of consumption, vitamin C supplementation may be considered. Although ascorbic acid is particularly prone to oxidation, other nutrients may also follow a similar pattern, as has been reported for folacin, after freezer storage (5).

In conclusion, the extent of vitamin C loss during storage is considerable and can be extreme in individual

Table 2. Ascorbic acid in human milk samples.

	Initial concentrations		Refrigerated, 24 h (% of total[a])	Freezer, 1 wk (% of total[a])	Freezer, 1 mo (% of total[a])	Freezer, 2 mo (% of total[a])
	(mg/100 ml)	(% of total[a])				
Mean	4.86	91	59	64	43	41
SD	2.02	18	35	34	42	31
median	4.93	92	38	69	38	0[b]
Range	(1.19–7.84)	(51–100)	(1–99)	(2–97)	(0–100)	(0–78)

[a] Total = total vitamin C (AA + DHAA)

[b] Six out of 10 samples had no detectable AA.

samples. However, storage for 1 mo in a freezer and under 24 h in a refrigerator will usually preserve two-thirds of the initial vitamin C. Recommendations on the storage of human milk should be adapted accordingly.

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