Temporal Changes in Osmolality of Fortified Human Milk with Contemporary Human Milk Fortifiers

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Background

- Human breast milk has many benefits for both term and preterm infants including a decreased risk of mortality, lower rates of respiratory and ear infections, immune protection, and positive effects on neurodevelopment
- Fortification of breast milk is the standard of care for premature/low birth weight infants
- Fortified breast milk is considered safe to be consumed up to 24 hours after preparation
- Preparation times are not standardized across institutions—can be done at time of feeding or 12-24 hours in advance
- Many higher calorie fortification recipes are off-label and thus properties like osmolality have not been well-described
- Osmolality >450 mOsm/kg may cause concern due to a theoretical increased risk for necrotizing enterocolitis.

Objective

- To evaluate changes in osmolality over 24 hours in human milk prepared with contemporary HMFs at standard and higher calorie recipes

Methods

- 3 base types were studied: fresh (never frozen) unpasteurized human milk, previously frozen unpasteurized human milk, and pasteurized donor human milk. The unpasteurized milk was donated by multiple individuals through the CCHMC Human Milk Research Biorepository
- HMF agents utilized: Similac (SHMF; extensively hydrolyzed casein) and Enfamil (EHMF; partially hydrolyzed whey) in both high protein (HP) and standard protein (SP)
- EHMF and PHMF were prepared at caloric densities of 22, 24, 26, 28, and 30 kcal/oz following manufacturer's recipes. SHMF was prepared at 22, 24, and 26 kcal/oz. Of note, EHMF and SHMF recipes >24 kcal/oz are all off-label.
- Tested osmolality of fortified and unfortified milk in 3 samples per batch at 0, 12, and 24 hours after preparation. Osmolality was tested using a freezing-point depression osmometer (Advanced Instruments Osmo1). Milk samples were refrigerated at 4 degrees Celsius between time points.
- One-sided paired t-tests were used to compare whether percentage change in osmolality by 24 hours was greater than change at 12 hours.
- Mixed-effects linear regression models were used to study the effect of time, fortifier, and caloric density, with Tukey adjustment for post hoc multiple comparisons. Statistical analysis was performed using SAS version 9.4. P-values less than 0.05 were considered significant.

Results

		Fresh			Frozen			Donor		
		Osmolality (Initial)	% Change (12 hours)	% Change (24 hours)	Osmolality (Initial)	% Change (12 hours)	% Change (24 hours)	Osmolality (Initial)	% Change (12 hours)	% Change (24 hours)
Unfortified		297 ± 3	1 ± 1.2	0.9 ± 1.7	301 ± 10	-1.2 ± 1.5	0.5 ± 1.2	302 ± 18	0.4 ± 0.9	0 ± 2.7
Enfamil SP	22 kcal/oz	315 ± 8	0.5 ± 0.9	1.4 ± 0.7	324 ± 5	1.5 ± 2.7	3.4 ± 4.7	325 ± 11	1 ± 0.9	0.9 ± 1.3
	24 kcal/oz	340 ± 13	1.2 ± 0.6	1.2 ± 1.3	343 ± 9	-0.1 ± 1.2	0 ± 2.3	363 ± 17	-0.7 ± 1.3	0.7 ± 2
	26 kcal/oz	353 ± 1	2.5 ± 0.4	3.4 ± 1.9	350 ± 15	0 ± 1.1	0.9 ± 0.7	376 ± 22	-0.1 ± 0.4	0.6 ± 1.3
	28 kcal/oz	381 ± 6	1.4 ± 0.8	3 ± 1	371 ± 14	0 ± 5.8	3.6 ± 1.1	384 ± 16	-0.5 ± 3.3	0.5 ± 4.7
	30 kcal/oz	406 ± 4	1.8 ± 0.3	1.9 ± 1.7	404 ± 4	1.3 ± 3.1	1.6 ± 1.3	401 ± 8	0.2 ± 1.9	0.4 ± 2.3
Enfamil HP	22 kcal/oz	317 ± 5	-0.6 ± 1.9	0 ± 1.4	320 ± 3	3.9 ± 4.8	3.1 ± 2.7	309 ± 7	-0.1 ± 1.6	0.6 ± 2.3
	24 kcal/oz	334 ± 8	-0.8 ± 2.6	-1 ± 1.9	333 ± 3	0.4 ± 0.7	1.2 ± 1.6	330 ± 2	0.7 ± 0.9	0.3 ± 1.6
	26 kcal/oz	329 ± 6	1.5 ± 0.9	0.9 ± 0.6	335 ± 1	1.4 ± 1.1	2.3 ± 1.4	343 ± 5	0.4 ± 0.9	1.3 ± 1.2
	28 kcal/oz	352 ± 6	1 ± 2.2	0.5 ± 1.7	361 ± 4	0.4 ± 0.3	2.3 ± 3.9	355 ± 15	0 ± 0.7	-0.1 ± 0.6
	30 kcal/oz	385 ± 8	0.5 ± 0.9	-0.2 ± 1.7	376 ± 6	0.3 ± 1.8	1.3 ± 1.2	385 ± 12	-0.2 ± 0.7	-1 ± 0.7
Similac	22 kcal/oz	418 ± 10	1.2 ± 2.9	0.9 ± 2.8	428 ± 22	-3.6 ± 5	-4 ± 4.6	429 ± 2	0 ± 0.6	1.6 ± 0.8
	24 kcal/oz	516 ± 6	3.9 ± 0.9	6.1 ± 1.3	531 ± 6	1.8 ± 1.3	3.3 ± 2.1	529 ± 1	0.7 ± 0.9	1.5 ± 0.5
	26 kcal/oz	606 ± 23	1.9 ± 3	4.1 ± 3.8	620 ± 8	3.5 ± 3.2	-0.3 ± 1.5	619 ± 1	0.7 ± 0.2	2.4 ± 0.7
Prolacta	22 kcal/oz	345 ± 18	0.1 ± 1.3	0 ± 2.6	335 ± 16	0.4 ± 4.3	1.6 ± 3.9	349 ± 3	0.8 ± 1.9	-0.7 ± 2.3
	24 kcal/oz	404 ± 42	4.6 ± 5.9	-2.2 ± 3.4	375 ± 37	2 ± 0.9	0.7 ± 1.6	381 ± 5	0.6 ± 3.1	-1.1 ± 1.2
	26 kcal/oz	388 ± 10	2 ± 2.2	0 ± 2.5	380 ± 4	-0.1 ± 2	-1 ± 0.8	404 ± 11	-0.5 ± 1.1	-2.2 ± 1.1
	28 kcal/oz	400 ± 5	-0.3 ± 2.4	2.5 ± 1.8	416 ± 16	-0.4 ± 1.2	0.4 ± 2.3	415 ± 15	-0.3 ± 1.8	-1.3 ± 1.8
	30 kcal/oz	426 ± 19	-0.7 ± 4.8	0.1 ± 5.6	430 ± 12	-0.2 ± 3.5	2.4 ± 2.7	436 ± 12	2.8 ± 1.9	1.5 ± 4.5

X.

Table 1: Osmolality of fortified human milk and percentage change at 12 and 24 hours . N=3 for all recipes

Effect	p-value
Time	0.20
Fortifier	0.17
Milk Base	0.07
Time * Fortifier	0.02

Table 2: Mixed effects model of time, fortifier, and milk base on change in osmolality

Recipe (kcal/oz)	Comparison Fortifier	Reference Fortifier	Estimate Difference	Adjusted p-value
22	Enfamil HP	Enfamil SP	-5.9	1
	Enfamil HP	Prolacta	-27.8	0.003
	Enfamil HP	Similac	-109.6	<0.0001
	Enfamil SP	Prolacta	-21.9	0.07
	Enfamil SP	Similac	-103.7	<0.0001
	Prolacta	Similac	-81.8	<0.0001
24	Enfamil HP	Enfamil SP	-16.0	0.51
	Enfamil HP	Prolacta	-54.3	<0.0001
	Enfamil HP	Similac	-192.9	<0.0001
	Enfamil SP	Prolacta	-38.3	<0.0001
	Enfamil SP	Similac	-176.9	<0.0001
	Prolacta	Similac	-138.6	<0.0001
26	Enfamil HP	Enfamil SP	-24.0	0.02
	Enfamil HP	Prolacta	-55.3	<0.0001
	Enfamil HP	Similac	-279.4	<0.0001
	Enfamil SP	Prolacta	-31.3	0.0003
	Enfamil SP	Similac	-255.4	<0.0001
	Prolacta	Similac	-224.1	<0.0001
28	Enfamil HP	Enfamil SP	-22.3	0.05
	Enfamil HP	Prolacta	-54.2	<0.0001
	Enfamil SP	Prolacta	-31.9	0.0002
30	Enfamil HP	Enfamil SP	-21.3	0.08
	Enfamil HP	Prolacta	-48.7	<0.0001
	Enfamil SP	Prolacta	-27.3	0.0036

Table 3: Difference in initial osmolality by fortifier type

Results continued

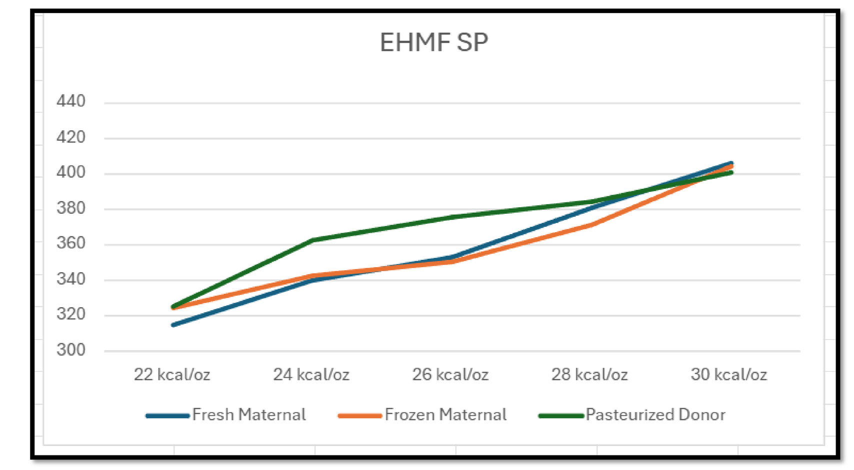

Figure 1: Degree of fortification (x axis) vs. initial osmolality (y axis) for EHMF-SP, n=3 for each recipe

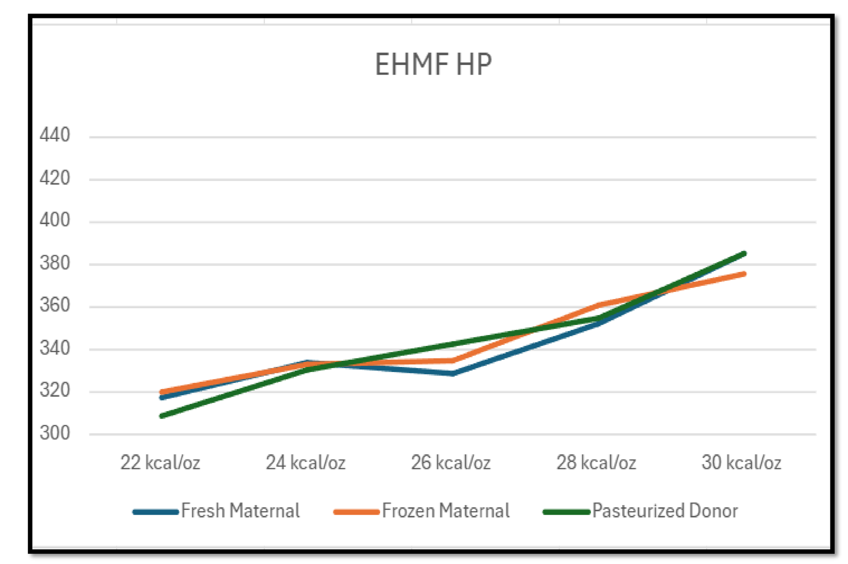

Figure 2: Degree of fortification (x axis) vs. initial osmolality (y axis) for EHMF-HP, n=3 for each recipe

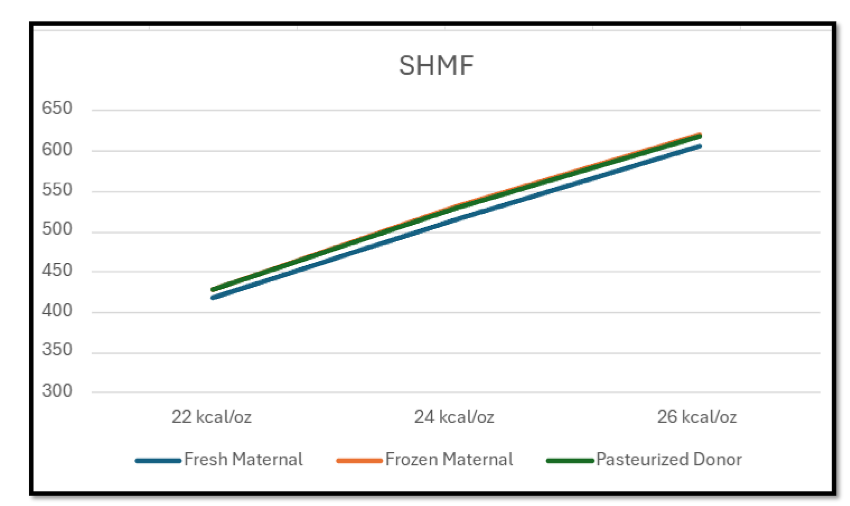

Figure 3: Degree of fortification (x axis) vs. initial osmolality (y axis) for SHMF, n=3 for each recipe

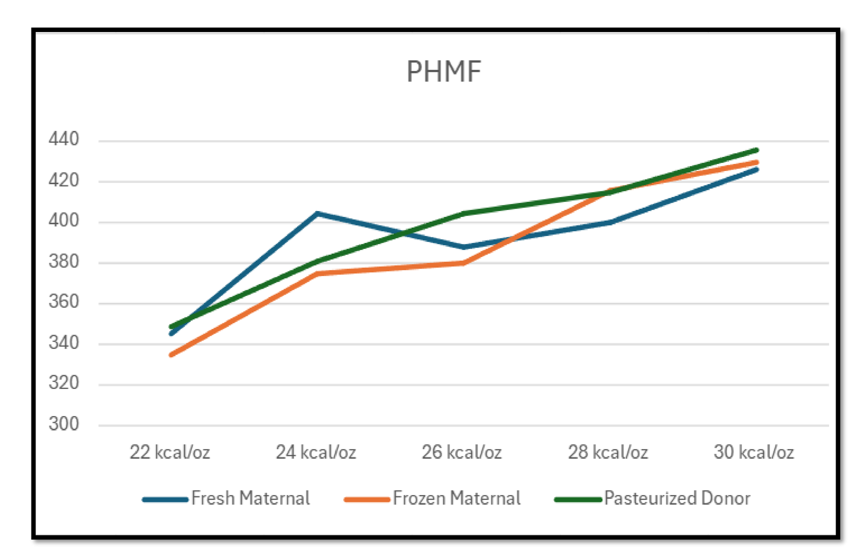

Figure 4: Degree of fortification (x axis) vs. initial osmolality (y axis) for PHMF, n=3 for each recipe

Discussion

- **Table 1** demonstrates the percent change in osmolality for all samples at 12 and 24 hours. When considering all samples, there was a statistically significant difference between change in osmolality at 24 vs. 12 hours (p = 0.02).

Discussion continued

- In final mixed effects model adjusting for fortifier and milk base and including the interaction between time and fortifier, time was not significantly related to change in osmolality **(Table 2)**. There was also not a statistically significant effect of fortifier or milk base on change in osmolality. There was an interaction between time and fortifier (p=0.02); however, in post hoc Tukey-Kramer adjustments, no pairwise comparisons remained significant.
- SHMF had the highest initial osmolality amongst all the tested fortifiers **(Table 3; Figures 1-4)**. We suspect this is due to its extensively hydrolyzed bovine milk casein, which likely contributes a greater proportion to osmolality compared to EHMF (partially hydrolyzed whey protein).

Conclusions

- In this limited set of fortified human milk samples, we found that commercially available HMFs do increase the osmolality of human milk to varying degrees and with varying temporal effects, although the clinical significance of this is not entirely clear.
- These data may support limiting fortified breast milk preparation times to 12 hours prior to feeding in order to minimize osmolality change.
- Future larger studies to compare the clinical outcomes of infants receiving these contemporary HMFs are needed to provide clinicians with increased knowledge and confidence with which to make nutritional decisions.

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