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Effects of maternal hydration status on the osmolality of maternal milk

María José Soto-Méndez[1,2], Alejandra Maldonado[1], Lauren Burgunder[1], Laura Scieszka[1], Ángel Gil[2] and Noel W. Solomons[1]

[1]Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM). Guatemala City, Guatemala. [2]Department of Biochemistry and Molecular Biology II. Institute of Nutrition and Food Technology. Center of Biomedical Research. University of Granada. Granada, Spain

Abstract

Background: It has been demonstrated that human milk osmolality (Mosm) is regulated within an established range, typically 290 to 300 mOsm/kg, and appears to be resistant to effects of maternal dehydration, as reflected by high urinary osmolality (Uosm).

Objective: To determine the degree of association between Mosm and Uosm at a common point in time, as well as the reproducibility of both measures over a one-week interval of sampling.

Methods: Mosm and Uosm were measured with a Vogel Löser 450 osmometer on samples of the respective biological fluids collected concurrently in 31 lactating women, with infants aged between 30 and 340 days. In the first 15 women recruited, collections were repeated 7 days after the initial ones.

Results: The median Mosm for the 46 samples collected was 308 mOsm/kg with a range from 288 to 448 mOsm/kg. The corresponding values for Uosm were 598 mOsm/kg with a range from 93 to 1,678 mOsm/kg. The Spearman rank-order correlation coefficient for within-individual association of Mosm and Uosm was $r = 0.214$ ($p = 0.153$). The median Mosm for the 15 repeat-subjects was 309 mOsm/kg on both occasions, with a within-individual Spearman coefficient of $r = 0.326$ ($p = 0.118$). By contrast, for the Uosm, the within-subject association was much stronger, with $r = 0.699$ ($p = 0.002$).

Conclusions: The osmometry technique proved to be a highly stable and reproducible measurement technique. Mosm and Uosm are not significantly associated at a point in time. Intra-subject Mosm varies more across time than intra-subject Uosm.

Key words: Breastfeeding. Milk osmolality. Urine osmolality. Hydration status. Guatemala.

BACKGROUND

Adequate hydration is an unrecognized aspect of human nutrition and health (1-3). In theory, this would be a special concern among lactating women, who not only have their normal daily water losses from renal, intestinal, lung and skin, but also from a volume of milk oscillating normally around 780 ml (4). In a certain way, additional water must be obtained from four sources: beverages, water in recipes, intrinsic moisture of foods, and metabolic water generated from macronutrients (5). Obviously, regarding water sources, it should be clarified that safe water (spring water, tap filtered and chlorinated water, etc.) as well as the liquid in beverages and that added to recipes would be the target sources for intervention to increase water intake.

Prentice et al. (6) imposed a 14.5-h total restriction of liquids on 10 lactating Gambian women, following plasma osmolality and total water balance. Women lost on average 7.6% of their body weight during abstinence and raised their plasma osmolality, but the turnover was twice as much as non-lactating controls. Water economy could not be explained by milk synthesis, which was not affected in the acute trial. Given the importance of this observation, subsequent literature on hydration status in lactating women was scarce in our literature search. A range of techniques can be used to estimate hydration status, from the color of the urine and its specific gravity, to daily liquid intake or urine output, to more refined laboratory techniques involving freezing-point depression osmometry of plasma or urine (7,8). The urine specimen can be derived from a causal spot sample or from a 24-h quantitative collection.

These different hydration indicators are neither mutually equivalent nor reflective of the same aspects of hydration status. Manz and Wentz (9) feel that urinary osmolality (Uosm) is the most integrative manner to assess human hydration, and they define normative "euhydration" as a Uosm in the range of 90-900 mOsm/kg. Plasma osmolality, by contrast, is tightly regulated in a narrow range around 300 mOsm/kg (9) and is generally only responsive to acute and profound hydration stress (7,10). Osmolality in human milk is also tightly regulated, and close to the range for plasma.

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Correspondence:

María José Soto-Méndez. Center for the Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM). 17 Avda. 16-89 Interior, Zona 11. 01011 Guatemala City, Guatemala
e-mail: clinicanutricion@gmail.com

How systemic hydration of lactating mothers might influence the osmolality of human milk remains unresolved so far. Experimental studies in cows, involving intravenous perfusion with saline, showed a temporal diluting effect on milk (11). There is a benefit to the infant from consuming beverages of lower solute loads, as the osmolality of fortified milk fed to premature infants can rise to unacceptably high levels (12,13). In Guatemala, the circumstances of availability of microbiologically-safe water (14) may be a disincentive to adequate consumption of beverages in lactating women in that nation.

OBJECTIVE

The availability of an osmometer in our laboratories in the Western Highlands allowed us to perform osmolality analyses on both urine and human milk. This enabled us to gather these background issues into a hypothesis-driven study to examine, in a cross-sectional manner, whether any significant association existed between maternal systemic hydration status, as represented by Uosm, and the osmolality of her breast milk. We present here the experience of that investigation.

METHODS

RECRUITMENT OF SUBJECTS

A total of 31 women, meeting the criteria for mother age (18 to 49 years) and of lactation age (days of life of the infant) varying from 30 to 365 days, were recruited into the study from the Quetzaltenango Health Center. Additional inclusion criteria included general good health of the dyad and absence of acute illness in either party on the day of study. It did not matter if the child was exclusively or predominantly breastfed or had already begun mixed feeding. The first 15 enrollees underwent collections twice at a one-week interval, whereas the remaining 16 subjects participated on a single occasion.

The study had been approved by the Human Studies Committee of the Center for Studies of Sensory Impairment Aging and Metabolism (CeSSIAM), and subjects gave informed written consent after the nature, purpose, inconveniences, risks and benefits had been described in Spanish. Subjects received the cost of their transportation, a snack and a meal, and set of dishes in compensation for their participation.

COLLECTION OF MILK SAMPLES

Milk samples were collected by the full-breast extraction method. One breast was reserved during at least 90 min, while the infant was allowed to nurse *ad libitum* from the contra-lateral breast. Using a breast-pump, the extractable volume was collected from the reserved side while the infant suckled from the opposite side. The specimen was thoroughly homogenized and two samples of up to 15 ml were aliquoted into a 15 ml conical tube and frozen at -20 °C until analysis.

COLLECTION OF SPOT URINE SAMPLES

Either just before or just following the milk extraction, the subject evacuated the urine in her bladder into a receptacle. After thorough mixing, an aliquot of 250 ml volume was transferred into a 15 ml conical tube and froze at -20 °C until analysis.

MEASUREMENT OF OSMOLALITY IN BIOLOGICAL FLUIDS

Osmolality was measured on a calibrated Vogel Löser 450 osmometer (Giessen, Germany). The respective sample was thawed to room temperature and remixed for 3 min. A 100 µL volume of the biological fluid was transferred by pipette into the receiving vessel of the instrument, and the osmolality was measured by the freezing-point depression principle of Peltier. Results were expressed in milliosmoles per kilogram of urine or milk (mOsm/kg).

DATA HANDLING AND STATISTICAL ANALYSIS

The database and subsequent calculations were made using SPSS Version 20 (IBM, Chicago, IL, USA). Descriptive statistics of median, 95% confidence limits, and maximum and minimum values were displayed for all samples collected, as well as for appropriate sub-groupings. Repeated measures Friedman test was used to make comparisons.

The Spearman rank-order and Pearson product-moment correlation coefficients were generated to assess the association between paired values from the same subject. A probability level of 5% was accepted as the criterion for statistical significance.

RESULTS

CHARACTERISTICS OF THE MATERNAL-INFANT DYADS

A total of 31 lactating mothers were recruited for the donation of urine and milk samples. The characteristics of maternal age and offspring age are shown in the upper rows of table I; 26 were legally married or living in a partnership union and 5 were single and unaccompanied. Three were unschooled, 6 completed elementary school and 9 middle-school, 7 had completed high-school and 6 were university graduates.

DESCRIPTIVE STATISTICS OF MILK OSMOLALITY

Milk osmolality ranged from 291 to 465 mOsm/kg, with a median of 308 mOsm/kg (n = 46) (Table I). Although the distribution is somewhat right-shifted, 39 values (85%) fell between 292 and 324 mOsm/kg, which represents a ± 5% boundary around the median. There were no differences in osmolality between milk fed to girl or boy babies (p = 0.769).

Table I. Descriptive statistics of the dyads and aggregated and disaggregated central tendency values for milk (Mosm) and urinary (Uosm) osmolality

Grouping	Variables	n	Median (min-max)
All subjects	Maternal age (y)	31	24 (18-41)
	Lactational age (d)	31	121 (30-340)
	Milk volume, all samples (ml)	46	13.5 (2-100)
			mOsm/kg
	Mosm all samples	46	308 (288-448)
	Mosm 1st samples	31	307 (288-448)
All subjects by sex	Mosm 1st sample, girls	14	306 (292-448)
	Mosm 1st sample, boys	17	309 (288-415)
	p value (Mann-Whitney U test)		0.769
Duplicate specimens subsample	Mosm 1st sample	15	309 (292-448)
	Mosm 2nd sample	15	309 (300-329)
	p value (Mann-Whitney U test)		0.902
All subjects	Uosm all samples	46	598 (93-1,678)
	Uosm 1st sample	31	606 (93-1,072)
Duplicate specimens subsample	Uosm 1st sample	15	530 (93-1,072)
	Uosm 2nd sample	15	586 (297-1,678)
	p value (Student t-test)		0.319

Mosm: Milk osmolality; Uosm: Urinary osmolality.

DESCRIPTIVE STATISTICS OF URINARY OSMOLALITY

Urinary osmolality ranged from 93 to 1,678 mOsm/kg, with a median of 598 mOsm/kg (Table I). If we accept 90 mOsm/kg as a convenient cut-off for overhydration (9), none of the lactating women fell into this category. With respect to the cut-off for hypohydration of 900 mOsm/kg (9,15), on 4 occasions (9% of all samplings) a participant had a Uosm in excess of that criterion on the day of sampling.

CORRELATES OF MILK OSMOLALITY

The Spearman rank-order correlation coefficient for the association of milk osmolality with urinary osmolality at the same time as the milk sample donation was r = 0.214 (p = 0.153) when all collected samples are considered (Table II). Equivalent and non-significant associations are seen when the correlations of women with duplicate sampling are considered for the first and second collections, separately (p = 0.093; p = 0.939). Moreover, Mosm was not related to the volume of milk accumulated in the breast (p = 0.920). Spearman's correlation coefficients for Mosm with maternal and offspring age, respectively (p = 0.137; p = 0.194).

CORRELATES OF URINARY OSMOLALITY

As shown in table II, Uosm similarly had no significant association with either maternal age (p = 0.662) or with offspring age (p = 0.180).

ASPECTS OF REPRODUCIBILITY OF MILK AND URINE OSMOLALITY

We have examined both biological and methodological aspects of the reproducibility of osmolality states. The median Mosm values for the 15 women providing two samples at a week's interval were identical at 309 mOsm (p = 0.902) (Table I), but the respective Pearson and Spearman correlation coefficients were not significant (p = 0.233; p = 0.118) (Table III).

We also examined the stability of Mosm measurements if the milk was stored for different intervals and defrosted in different manners. In a subgroup of 23 samples, collected in August 2015, an initial Mosm measurement was made within 1 to 6 days of their being collected and stored at -20 °C. For analysis, samples were simply thawed to room temperature prior to thorough mixing and injection into the osmometer. On a second occasion, in October 2014, with -20 °C freezing intervals varying from 6 to 81 days, an identical defrosting and analysis protocol was conducted on a second aliquot of the same 23 samples. On yet a third aliquot, the preparatory phase included thawing to room temperature and then raising the temperature to 37 °C in a warm-water bath, then cooling back to room temperature prior to thorough mixing for analysis. The Pearson correlation coefficients for the various paired comparisons were, however, not statistically significant (Table IV). When the October measurements for both the direct thawing and the water bath approaches were compared for the common 23 samples with the early measurements made in August as well, the median Mosms were 309, 305 and 288 mOsm/kg, respectively (p = < 0.001 by Friedman's test). Figure 1 shows the scattergram for the Mosm values with the two preparatory protocols for all 46 milk samplings in the study; the Pearson correlation coefficient was r = 0.966 (p = < 0.001).

Table II. Correlates of milk and urinary osmolality for all specimens and by subgroups, using the Spearman rank-order correlation coefficient

	Number of specimens and sampling frame	r-value	p-value
Associations between milk and urinary osmolality			
Mosm *vs* Uosm	all samples (n = 46)	0.214	0.153
Mosm *vs* Uosm	1st subsample (n = 15)	0.449	0.093
Mosm *vs* Uosm	2nd subsample (n = 15)	0.021	0.939
Other associations with osmolality values			
Mosm *vs* volume collected	all samples (n = 46)	0.015	0.920
Mosm *vs* maternal age	1st samples (n = 31)	0.273	0.137
Uosm *vs* maternal age	1st samples (n = 31)	-0.092	0.622
Mosm *vs* lactational age	1st samples (n = 31)	-0.240	0.194
Uosm *vs* lactational age	1st samples (n = 31)	-0.247	0.180

Table III. Within individual reproducibility of milk osmolality in duplicate subgroup

Statistic	n = 15 in each sub-sample	
	r-value	p-value
Milk osmolality		
Pearson correlation subsample 1st *vs* 2nd	0.204	0.233
Spearman correlation subsample 1st *vs* 2nd	0.326	0.118
Urinary osmolality		
Pearson correlation subsample 1st *vs* 2nd	0.642	0.005
Spearman correlation subsample 1st *vs* 2nd	0.699	0.002

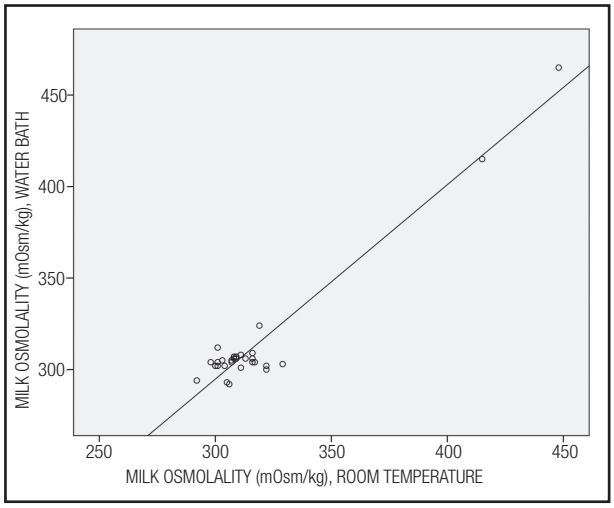

Figure 1.

Scattergram of 46 paired values of milk osmolality for frozen milk aliquot thawed to room temperature prior to analysis (x axis) vs for a second aliquot of frozen milk aliquot thawed to room temperature then raised to 37 °C and cooled to room temperature prior to analysis (y axis). The Pearson correlation coefficient was r = 0.966 (p < 0.001).

Table IV. Reproducibility expressions (of medians and of correlation) of milk osmolality measurements in relation early after collection and after prolonged freezing with two procedures for thawing

Statistic	n = 23 in each sub-analysis			
	Pearson's r value			p value
Original *vs* post-freezing with normal thaw	0.863			< 0.001
Original *vs* post-freezing with thaw, heat and cool	0.853			< 0.001
Post-freezing with normal thaw *vs* post-freezing with thaw, heat and cool	0.927			< 0.001
Median concentrations	Osmolality (mOsm/kg)			
	288[a]	309[b]	305[c]	< 0.001[d]

[a]Measured shortly after collection. [b]Post-freezing, with normal thaw. [c]Post-freezing with thawing, heating and cooling cycle. [d]Repeated measures Friedman test.

With respect to the reproducibility of Uosm on repeat collections in the subsample, table I shows the values for first and second samples; the difference in mean values was not significant ($p = 0.319$), and the respective Pearson and Spearman correlations showed significant reproducibility association ($p = 0.005$, $p = 0.002$). Our previously studies (16) demonstrated the stability of Uosm in urine samples during long-term frozen storage. This was confirmed with the August and October repeat measurements (data not shown).

DISCUSSION

Breastfeeding is the means to satisfy thirst and hunger of the infant and to provide the child with adequate hydration and nourishment. In the theory of lactation biology, it is the nursing demand of the child that determines the output of milk (17,18). Limitation of nutrients to the mother, however, may compromise the nutrient quality of the milk (19). It is logically sound to postulate that limited intake or retention of water will compromise the quantity, and similarly limit the flow of nutrients to an infant. Confirming a relationship of maternal hydration to the quality or quantity of her milk would have actionable consequences for public health programming.

COMPARATIVE PERSPECTIVE ON HYDRATION FINDINGS

The general tenor of hydration for these highland-dwelling lactating women may be that of adequate status. This conclusion comes from the evaluation of casual urine samples, however. It might be considered as preferable to collect formal 24-h urine samples to best assess hydration by Uosm measurement, but the logistics for such a tedious assignment were not reasonable, given our mode of subject recruitment. Many studies have employed spot urine samples for osmolality when 24-h collections were inconvenient or logistically impossible (15,20-24), but how they compare with concurrent full-day collections for rank-ordering of subjects' hydration status is not completely understood. Notwithstanding the caveats regarding a casual urine sample, lactating women, overall, have a Uosm that is ~600 mOsm/kg, which is some 75 mOsm/kg higher than the 530 mOsm/kg median for preschoolers of the same region (16). In addition, we can make comparison with a series of 15 casual samples collected from the female staff members and associates in Quetzaltenango at the same time of day, 09:00-11:00 am, as those of the lactating subjects (data not shown). The median was 492 mOsm/kg (110-1,020); this value is 106 mOsm/kg and 18% lower than the median for lactating women shown in table I.

Our findings from this small sample of women show, moreover, that, on any given day, lactating women can show hydration states at the extremes of the euhydration range (9). High values of Uosm, signifying hypohydration, are seen sporadically in our lactating women. At least in the few subjects in the repeated-collections series, however, the altered hydration state had abated on the other measurement opportunity. A value of 93 mOsm/kg, just short of the hyperhydration criterion of Manz and Wentz (9), was seen on one occasion, but the same subject had a Usom value of 321 mOsm/kg on the second collection. Without a larger series of lactating women, or a greater number of repeated values, no stable estimate for the frequency of Uosm values at the extremes of the hydration-status range in this population can be made.

COMPARATIVE PERSPECTIVE ON MILK OSMOLALITY FINDINGS

Typical osmolality for human milk is reported as 300 mOsm/kg, in a rounded-off fashion. This is coincidentally -or not coincidentally- identical to the 300 mOsm/kg, which is the value cited for human plasma osmolality (9). The median of 308 mOsm/kg for breast milk osmolality is close to that value. Although 85% of values were in a ± 5% osmolality band of the median, strictly tight regulation cannot be inferred, as a residual 15% had a higher ($n = 6$) or lower ($n = 1$) value. Fortified milk or infant formula with an osmolality of > 400 conveys a risk of osmotic diarrhea in the baby (12). At least at the moment of collection, three samples of milk had a Mosm in this excessive range, but it was not constant in the mother on her other sampling.

THE ASSOCIATION OF SYSTEMIC HYDRATION STATUS AND MILK OSMOLALITY

The medullar finding in response to the main hypothesis of the present inquiry is the failure to detect any significant association between osmolality in the urine and the human milk at a common moment in time. In a mathematical sense, however, the narrow, core of distribution of Mosm, with an effective width of 32 osmolality unite, militates against any rank-order scaling that could reveal associations with another variable. Nevertheless, we can tentatively conclude that the maternal hydration status was adequate to maintain appropriate hydration in the nursing offspring. Brown et al. (25) found that all 40 exclusively breast-feeding Peruvian mothers maintained infants with adequate milk volumes as gauged by adequate infant urinary outputs with a 1.003 to 1.017 urine specific gravity.

INDIVIDUAL AND GROUP STABILITY OF OSMOLALITY

Uosm is well documented to be mutable and responsive to water balance (26,27). Likewise, as would be expected, Mosm is a state, not a trait, as the day-to-day fluctuation, even within the narrow range, was marked. On a group basis, for the constant group of 15 women with two collections separated by a week, however, the collective distribution remained very firm and solid. This suggests that a powerful intervention, such as a beverage-of-

fering intervention of a substantial magnitude, could shift the distribution of Mosm to the left in a detectable manner. Similarly, in a cohort of lactating mothers, drought conditions with severe thirst might shift this variable to the right.

Maternal age and lactation duration are factors known to affect other aspects of lactation and milk quality (28-30), so it was reasonable to probe our data for evidence of an association with osmolality. Likely, due to the intrinsically narrow distribution of Mosm it is not a readily scalable variable. Hence, the lack of association is not surprising.

EFFECT OF DURATION OF FROZEN STORAGE ON MILK OSMOLALITY

Freezing and storing of human milk has been shown to affect a host of analytes (31). Table IV shows a significant difference of a 7.2% increase in the median of analyzed samples. Neither median value for Mosm, nor that from timely or that from delayed analysis, is outside reported normative range, however. On the one hand, Neubauer et al. (32) found a range of 290-299 mOsm/kg in 33 control mothers in Connecticut; this is in line with the central tendency for the freshly analyzed samples, i.e. with a short duration of frozen preservation. On the other hand, breast milk from 84 healthy Viennese mothers had a median *osmolarity* of 297 mOsm/l. If converted to osmolality by applying the specific gravity of breast milk of 1.031 (33), this converts to a median of 306 mOsm/kg, i.e., expressed as *osmolality* in terms of *kilograms* of sample weight. In many ways, however, most comparable in setting and population make-up with our Guatemalan women is a study from an urban settlement of Brasilia, published in 1988 by Dorea et al. (34); in breast-feeding women across a range of lactation age, the mean ± SEM of human milk was 319 ± 4.5 mOsm/kg, with median values not reported.

These values are all in general accordance with the analysis of samples after up to 2.5 months of frozen storage. The additional water-bath treatment in thawing had no influence on assay results. It is worth mentioning that, despite a narrow intrinsic distribution making associations with extrinsic variables such as maternal or infant age difficult to discern (Table II), the stability of the osmometry methodology finds correlation coefficients of > 0.85 for repeated measurement of the same milk collection (Table IV). Specifically, as shown in figure 1, the correlation coefficient reached 0.97 for all 46 samples thawed in the two procedures. With the 23 samples measured shortly after collection and later after freezing, however, we see a significant increase in osmolality with the prolonged freezing.

STRENGTHS AND LIMITATIONS OF THE STUDY

The major strength was the nature of the primary hypothesis addressed. We found no evidence for or against an influence of systemic hydration on milk osmolality using Uosm as the hydration status index. A limitation might be found in the number of participants and specimens. A sample-size of 31 women is not uncommon for a hydration study, as this was exactly the number of young women subjects of a comprehensive assessment of hydration markers by Anderson et al. (35). There are limitations in the statistical power to encounter associations. With 29 degrees of freedom for a two-tailed test of linear association, the lowest r value that would be significant at a 5% probability level would be 0.335. With a focus on analytical or short-term biological reproducibility, however, associations on the order of > 0.80-90 are generally anticipated, independent of the number of pairs. We can attribute the narrow range of distribution in Mosm for the limitation in detecting strong associations with extrinsic variables, whereas in the methodological domain, this distribution has little effect on high associations between split-sample repeated analyses.

CONCLUSIONS

Human milk has evolved to present an osmolality near to 300 mOsm/kg, which approximates to average plasma osmolality. Although Mosm values were narrowly clustered around the median, an occasional sample shows a sporadic high value exceeding 400 mOsm/kg. The hydration status of lactating mothers as a group is in the upper range of the euhydration range, and is inferior to local non-lactating professionals. Albeit as adequate in its nature, hydration status may not be optimal for lactation; a woman is occasionally seen to express under-hydration states on a given day. We find no significant association between hydration and osmolality in human milk, but recognize the poor capacity for stable rank-ordering within the condensed distribution of Mosm. Variation in the duration of frozen storage is small, but may add additional attenuation in efforts to associate Mosm with other variables.

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