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The effect of water abstention on milk synthesis in lactating women

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Summary

1. The effects of dehydration on mechanisms of water balance and milk synthesis were investigated in ten lactating Gambian women who were fasting during Ramadan. Ten non-pregnant, non-lactating women acted as controls. Fasting consisted of total water abstention from 05.00 hours to 19.30 hours and was accompanied by high insensible water losses.

2. Lactating women lost 7.6% of their total body water between 07.00 hours and 19.00 hours. Control subjects lost significantly less.

3. Plasma indices of dehydration (osmolality, sodium, uric acid) showed a greater rise in the lactating women than in the control subjects over the period of fasting. However, the 19.00 hours values remained in the normal range obtained on non-Ramadan days.

4. During Ramadan the lactating women restricted their urinary output to a lesser degree than the controls, and for much of the day their urine was also less concentrated. The lactating women appeared to have adapted by super-hydrating themselves overnight. This resulted in very low urine concentrations (osmolality, sodium, urea, creatinine) in morning samples. Urine concentrations approached, but did not exceed, non-Ramadan levels by late afternoon.

5. The daily water turnover of 6.4 litres in the lactating women was 2 litres greater than in the controls. This difference was much greater than that required for milk synthesis (500 ml) and may represent a further protective mechanism.

6. Fasting caused changes in milk osmolality, lactose, sodium and potassium concentrations indicative of a marked disturbance of milk synthesis. The results suggested that the normally tight junctions between the mammary secretory cells had become permeable to small ions and formed a paracellular pathway.

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Introduction

It is a widely held clinical belief that successful lactation in women is dependent on a high maternal fluid intake. However, only two studies have attempted to investigate the effect of fluid restriction on milk output. Olsen [1] found that variations in liquid intake, other than that in food, from 600 to 2775 ml/day over 3–4 day experimental periods had no effect on milk supply. Lelong *et al.* [2] reported two extended studies on one subject in which breast-milk output was not significantly altered when the subject's total water intake was reduced to 1765 ml/day over a 10 day period. In reviewing these studies Morrison [3] has concluded that "there is a strong impetus to milk secretion and a surprising tolerance of water restriction".

When lactating women fast during Ramadan in a tropical environment they undergo a 14 h period of total water abstention accompanied by high insensible fluid losses. In this study we have utilized this natural experimental model to study the effects of dehydration on water balance and mechanisms of water economy in lactating women, and to determine whether there is any disturbance of the normal process of breast-milk synthesis.

Methods

Subjects

Ten lactating women (L) and ten non-pregnant, non-lactating (NPNL) women in the village of Keneba, The Gambia, were each studied by an

identical protocol on 3 days separated by an interval of 1 month. The middle study day occurred between days 14 and 28 of Ramadan. The two groups compared as follows (means ± SEM): body weight, 50.9 ± 2.5 and 51.5 ± 3.0 kg; height, 157.6 ± 2.5 and 156.1 ± 1.8 cm; age, 27.2 ± 1.9 and 30.8 ± 2.9 years; parity 4.8 ± 0.7 and 1.1 ± 1.9 for the lactating and NPNL women respectively. With the exception of parity, which should not influence their response to dehydration, the groups were well matched. The lactating women were 3, 4, 6, 9, 10, 11, 11, 14, 15 and 21 months *post partum* on the pre-Ramadan study day. The period of high variability of milk composition in early lactation was therefore avoided. All subjects remained amenorrhoeic throughout the study.

All women appeared to adhere strictly to the fasting customs during Ramadan and consumed no food or water between a pre-dawn bowl of cereal gruel (04.00–05.00 hours) and breaking their fast at 19.30 hours. Ramadan occurred during the farming season and the women worked exceedingly hard in their fields during the hottest time of the day. The mean durations spent farming were: pre-Ramadan, 6.7 h; Ramadan, 7.6 h and post-Ramadan, 7.2 h. The mean shade temperatures and relative humidities at the hottest time of the day were: pre-Ramadan, 36.3°C and 55%; Ramadan, 30.5°C and 82%; post-Ramadan, 30.5°C and 74%. A combination of this heavy work load and high environmental temperature therefore added to the dehydrating stress of water abstention. Further details of Keneba and the dietary intake of the women have been published elsewhere [4–6].

Study protocol

Only two subjects were studied each day since it was necessary to observe them continuously from 07.00 hours to 19.00 hours to ensure that a complete urine collection was obtained. Venous blood (2 ml) was collected at 07.00 hours and 19.00 hours (± 10 min) together with 2 ml samples of milk from each breast of the lactating women. Milk samples were collected by manual expression approximately mid-feed and after washing the nipple with deionized water. All subjects were weighed at both times and in the evening were questioned about defecation since 07.00 hours. Deuterium oxide (see below) was administered at 07.15 hours, except during Ramadan when it was necessary to give it at approximately 04.00 hours. Breast-milk output between 07.00 and 19.00 hours was measured by test-weighing as described elsewhere [5]. All urine was collected from 07.00 hours on the study day until 07.00 hours on the following day. During the day each collection was individually measured and a sub-sample was frozen for analysis. The subjects were entrusted to collect all their urine overnight and the volume of the pooled sample was recorded and a sub-sample taken. One NPNL subject admitted to an incomplete overnight collection on the post-Ramadan day and repeated the collection on the following day.

Methods for measurements

Serum from the blood samples was analysed for sodium and potassium by flame photometry (EEL Clinical Flame Photometer), osmolality by using a Wescor Osmometer (Wescor Inc., U.S.A.), energy metabolites as described elsewhere [7] and urea, creatinine and uric acid were determined with commercial test kits (Boehringer Mannheim GmbH). Prolactin was measured with a radio-immunoassay supplied by CIS (UK) Ltd. Milk samples were analysed for sodium, potassium and osmolality as above and for lactose by a manual modification of the *p*-hydroxybenzoic acid hydrazide method [8]. The average result from the two breasts has been used in the analysis. Urine samples were analysed for sodium, potassium, osmolality, urea and creatinine as above.

A deuterium oxide dilution technique was used to measure total body water and water turnover rates (= water consumption) as described previously [9]. The ^2H_2O dose used was 0.1 ml/kg body weight.

This study was approved by the MRC Gambian Ethical Committee.

Results

Degree of dehydration induced during Ramadan

The relative extent of the dehydration induced in the lactating and control groups was assessed by measuring changes in body weight and in serum composition from morning to evening. The changes in body weight summarized in Table 1 show a decrease of 2.5 kg in the lactating women and 2.0 kg in the NPNL controls during Ramadan. This difference was significant when expressed as a percentage of each individual's body weight ($P < 0.01$). The decrease represented a 7.6% decrease in total body water since the ^2H_2O measurements at 04.00 hours yielded an average value for total body water of 64.3% of body weight for the lactating women. This assumes that all weight loss was due to water loss once corrected for defecation. This assumption is not strictly correct since some weight loss will be due to fat and carbohydrate metabolism. However, when corrected for the amount of metabolic water

TABLE 1. *Change in body weight between 07.00 hours and 19.00 hours during Ramadan*

Values are means ± SEM and (*n*). $*P < 0.05$, $**P < 0.01$, by *t*-test.

	Weight decrement from 07.00 hours to 19.00 hours	
	(kg)	(% of total body wt.)
Non-defecators		
Lactating subjects	2.38 ± 0.15 (6)	4.77 ± 0.32 (6)
NPNL subjects	1.87 ± 0.33 (5)	3.46 ± 0.52 (5) *
All subjects†		
Lactating subjects	2.50 ± 0.16 (10)	4.90 ± 0.21 (10)
NPNL subjects	2.02 ± 0.22 (10)	3.81 ± 0.31 (10) **

† Body weight decrement of subjects who defecated between 07.00 hours and 19.00 hours was adjusted by an assumed fecal weight of 300 g.

produced, oxidation of fat and carbohydrate would account for less than 4% of the weight loss, a value which would be similar in both groups.

Results of the serum measurements are shown in Fig. 1(*a*) and 1(*b*). Serum osmolality was significantly increased during Ramadan in the lactating women only ($P < 0.05$). However, the 19.00 hours value of 280 mosmol/l was not higher than on the other study days and the difference between morning and evening was caused more by a low 07.00 hours value. The serum sodium concentration also increased significantly in the lactating, but not in the NPNL, group ($P < 0.02$). However, the evening value for the lactating women was no higher than that from the NPNL group, in whom the morning sodium concentration was also unexpectedly high. Serum potassium showed significant decreases on the pre- and post-Ramadan study days in the lactating women, but remained unchanged during Ramadan. Urea concentrations appeared to correlate with the time interval since the last meal and showed an increase from morning to evening in both L and NPNL groups on the non-Ramadan days and a reversal of this during Ramadan. Serum creatinine showed a non-significant increase during Ramadan in the lactating women and a slight decrease in the controls. Uric acid showed a large increase during Ramadan in the lactating women ($P < 0.02$), which contrasted with a decrease from morning to evening on the non-Ramadan days. The NPNL controls also had a significant increase in serum uric acid concentration during Ramadan ($P < 0.01$), but the evening value was 25% lower than that for the lactating women. In summary, the response to dehydration was more marked in the lactating women than in the controls, but with the exception of sodium none of the Ramadan 19.00 hours values fell outside the range observed at other times and the sodium concentration itself was high in both L and NPNL groups.

Urine output and concentration

Cumulative urine volumes and urine compositional data from the Ramadan study day analysed in four 3 h intervals during the daytime are shown in Table 2. (The full version of this Table including the pre- and post-Ramadan data is available as *Clinical Science* Table 83/4 from The Librarian, Royal Society of Medicine, 1 Wimpole Street, London W1M 8AE.) The NPNL women produced slightly more urine than the lactating women throughout the study, but this may have been due to individual differences in physiology rather than due to an inherent difference between the groups since the NPNL group contained two subjects with very high urine outputs. Three subjects produced low urine volumes (400–500 ml/day), but their urine was concentrated to such an extent that their 24 h outputs of sodium, urea and creatinine fell in the middle of the overall range. Incomplete collections were not therefore suspected.

The ability of the subjects to conserve water during Ramadan can be judged by their urine output between 13.00 hours and 19.00 hours. These values were: pre-Ramadan, 180 and 288 ml; Ramadan, 91 and 81 ml; post-Ramadan, 279 and 413 ml, for the L and NPNL groups respectively. Thus on a normal day the NPNL women produced 60% more urine during this period than the lactating women, but during Ramadan the output was similar in both groups. The NPNL women therefore showed a much greater restriction of their normal afternoon urine output in response to water abstention during Ramadan. The urine output of both groups during this period represents approximately 15 ml/h, which remained over

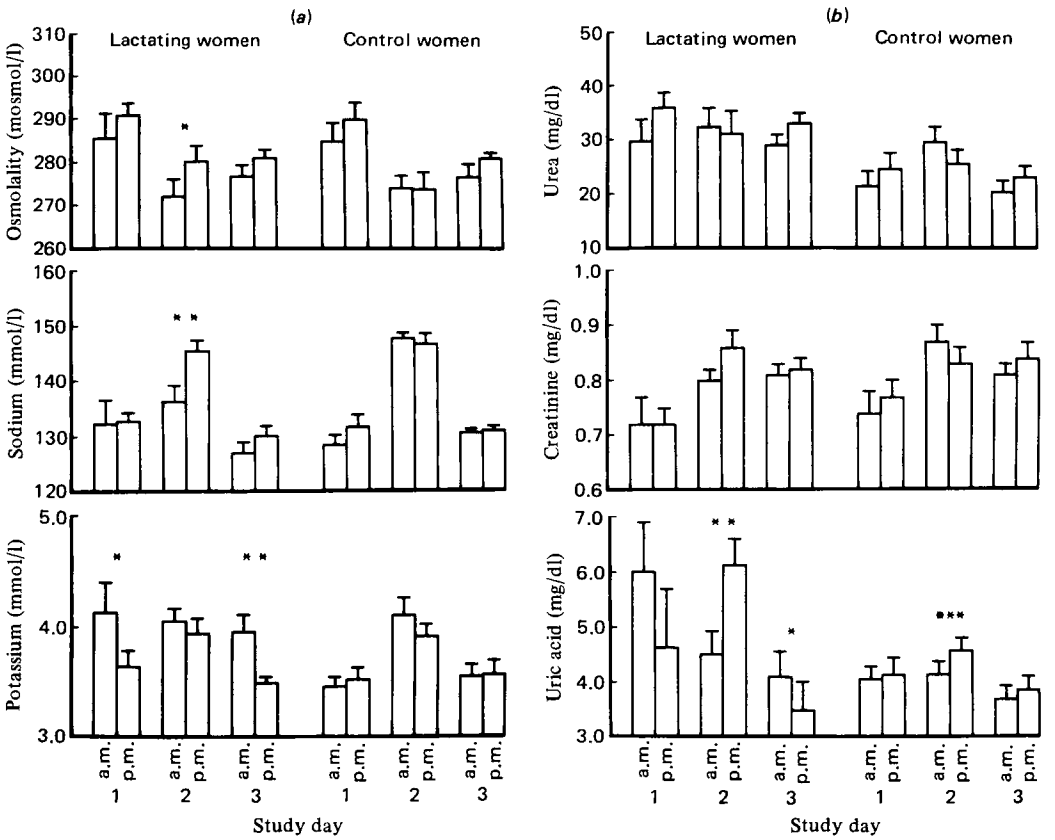

FIG. 1. Serum indices of dehydration. Values are means ± SEM ($n = 10$ throughout). Study day 1, pre-Ramadan; day 2, Ramadan; day 3, post-Ramadan. $*P < 0.05$, $**P < 0.02$, $***P < 0.01$, by paired t-test.

TABLE 2. *Urine volumes and composition during Ramadan*

Values are means with SEM in parentheses. Period 1, 07.00–10.00 hours; period 2, 10.00–13.00 hours; period 3, 13.00–16.00 hours; period 4, 16.00–19.00 hours. $*P < 0.05$, $**P < 0.02$, $***P < 0.01$, for L vs NPNL by t-test.

Time period	Cumulative urine volume (ml)		Osmolality (mosmol/l)		Sodium (mmol/l)		Potassium (mmol/l)		Urea (g/l)		Creatinine (mg/100 ml)		Number of samples†	
	L	NPNL	L	NPNL	L	NPNL	L	NPNL	L	NPNL	L	NPNL	L	NPNL
1	197	252	335	416	81	110	21	16	20	21	36	43	8	9
	(41)	(50)	(52)	(87)	(15)	(31)	(5)	(4)	(4)	(4)	(4)	(10)		
2	324	414	369**	646	88***	174	31	48	22	33	62*	88	5	9
	(68)	(44)	(18)	(70)	(12)	(20)	(6)	(11)	(5)	(3)	(6)	(8)		
3	393	448	592***	793	106*	212	79	58	33	26	94	106	3	5
	(62)	(49)	(8)	(41)	(3)	(34)	(6)	(9)	(8)	(5)	(25)	(14)		
4	415	494	635*	835	114	187	81	81	33	31	128	173	6	7
	(56)	(57)	(61)	(53)	(18)	(37)	(10)	(13)	(7)	(7)	(17)	(18)		
Overnight‡	991	1304	543	457	118	97	21	15	43	31	76	89	10	10
	(122)	(209)	(65)	(71)	(20)	(14)	(6)	(5)	(7)	(5)	(9)	(12)		

† Numbers refer to all columns except columns 1 and 2, where $n = 10$ throughout.
‡ Overnight cumulative total represents the 24 h total.

double the theoretical *volume obligatoire* calculated as 6 ml/h based on a maximal urine concentration of 1.4 osmol/l [10]. The subjects were therefore either physiologically unable to concentrate their urine sufficiently even to approach this minimum volume or, more likely, were insufficiently dehydrated to necessitate such a reduction.

The urine compositional data support the latter conclusion since the 16.00–19.00 hours values for both L and NPNL groups during Ramadan were not above the range of values observed on non-Ramadan days for any of the factors measured. This relative normality of urine composition after approximately 14 h of water abstention and high sweat losses was apparently achieved by means of super-hydration overnight. The Ramadan morning urine samples had a much lower solute content than in any of the non-Ramadan periods. The lactating women had the lower solute levels of the two groups in this period and the difference became accentuated in periods 2 and 3, and was significant for urine osmolality, sodium and creatinine concentrations in at least one of the 3 h time periods. The most important fact to note from the urinary data is that the sodium concentration and osmolality of the lactating women in Ramadan remained low even in period 4 and therefore suggested that the dehydration was not severe enough to stress the urinary concentrating capacity of these women.

Total body water and water turnover rates

Total body water measurements were made on each study day for the lactating women but only on the Ramadan day for the controls. The mean values (± SEM) were: 63.0 ± 1.7, 64.3 ± 1.5 and 63.2 ± 1.4% for the lactating women and 61.0 ± 2.0% for the NPNL controls during Ramadan. The slightly raised value in the lactating women during Ramadan compared both with the non-Ramadan days and with the NPNL controls may possibly again suggest that they are in a superhydrated state. However, the gradual reduction in body weight over the entire study period, presumably due to the loss of adipose tissue, makes further interpretation difficult.

The measurements of total water turnover rates, which include water of metabolism, showed a marked difference between the L and NPNL groups. Water turnover rates for the lactating women on the 3 study days were: 6.93 ± 0.35, 6.40 ± 0.34 and 5.56 ± 0.27 litres/24 h. Measurements in the NPNL group were made only during Ramadan when the turnover rate was 4.44 ± 0.32 litres/day ($P < 0.01$, vs Ramadan lactating women). The lactating women therefore increased their water turnover rate by an amount considerably greater than that needed to account for water loss in breast-milk. Together with the differences in total body water this further suggests that they may have developed a protective mechanism against potential dehydration.

Serum prolactin concentrations

Serum prolactin concentrations were measured since prolactin has a role in water and salt retention in some species [11, 12] and might possibly have such a role in man [13]. The prolactin levels shown in Table 3 are given as absolute values for the NPNL women, but as percentages of the population mean values for the lactating women to take into account the large variation with stage of lactation which exists [14]. The results demonstrate a diurnal variation in both groups with lower values in the evening. This pattern was not affected by Ramadan.

Breast-milk output

The breast-milk output of the lactating women is shown in Table 4. The relatively small milk volumes observed were due to the advanced stage

TABLE 3. *Serum prolactin concentrations*

Values are means ± SEM and (n). For lactating subjects concentrations are expressed as a percentage of the population mean value for the same stage of lactation [14]. $^{**}P < 0.05$ by paired t-test.

	Time	Lactating subjects (%)	NPNL subjects (μ-units/ml)
Pre-Ramadan	07.00 hours	95.1 ± 16.9 (9)	529 ± 136 (10)
	19.00 hours	73.3 ± 12.5 (9)	421 ± 65 (10)
Ramadan	07.00 hours	113.6 ± 31.1 (8)	409 ± 89 (10)
	19.00 hours	71.2 ± 10.3 (8)	326 ± 34 (10)
Post-Ramadan	07.00 hours	109.4 ± 22.0 (9)	534 ± 114 (10)
	19.00 hours	50.2 ± 5.5 (9)**	368 ± 64 (10)

TABLE 4. *Breast-milk output of the lactating subjects*

Values are means ± SEM and (n), where n = number of women in rows 1–3 and number of feeds in rows 4–6. Data from mothers who left their infants at home were excluded from columns 1–4. $^{**}P < 0.01$ for column 4 vs columns 1–3 by t-test.

	Output (g)				
Time interval (hours)..	07.00–10.00	10.00–13.00	13.00–16.00	16.00–19.00	07.00–19.00
Output per interval					
Pre-Ramadan	82.5 ± 19.6 (10)	74.0 ± 13.8 (10)	84.5 ± 14.9 (10)	71.5 ± 11.7 (10)	312.5 ± 38.1 (10)
Ramadan	41.7 ± 24.7 (6)	97.5 ± 29.4 (6)	95.8 ± 18.7 (6)	62.5 ± 18.2 (6)	254.5 ± 30.4 (10)
Post-Ramadan	81.7 ± 22.9 (6)	47.5 ± 6.6 (6)	33.3 ± 16.2 (6)	88.3 ± 27.0 (6)	221.1 ± 31.5 (10)
Output per feed					
Pre-Ramadan	63.5 ± 14.4 (13)	56.9 ± 7.3 (13)	60.4 ± 9.6 (14)	42.1 ± 7.1 (17)	54.8 ± 4.9 (57)
Ramadan	62.5 ± 14.4 (4)	73.1 ± 13.5 (8)	63.9 ± 4.3 (9)	37.5 ± 6.9** (10)	63.6 ± 5.6 (46)
Post-Ramadan	54.4 ± 7.4 (9)	47.5 ± 6.6 (6)	40.0 ± 17.5 (5)	66.3 ± 22.9 (8)	56.1 ± 7.6 (36)

of lactation and the fact that the infants were receiving supplementary feeds. In order to test whether milk output decreased towards the end of the period of fasting the results were again analysed in 3 h intervals. Women who left their children at home whilst farming were excluded from the analysis since they gave misleadingly large feeds when they returned to their children. Their results were, however, included in the analysis of 12 h intakes. The results indicate a slight reduction in milk output in the 16.00–19.00 hours period during Ramadan, which was significant when analysed in terms of volume per feed ($P < 0.01$). However, a reduction of similar magnitude occurred on the pre-Ramadan day also. The 12 h milk outputs decreased from 313 ml on the pre-Ramadan day to 221 ml on the post-Ramadan day, reflecting a combination of stage of lactation and seasonal effects seen in this community [5].

Breast-milk composition

Breast-milk osmolality, sodium and potassium concentrations were measured to determine whether maternal dehydration was reflected in milk compositional changes, and lactose was measured since it is the main contributor to osmolality. The results are shown in Fig. 2. Breast-milk osmolality increased by 3.2% ($P < 0.02$) from morning to evening during Ramadan. This increase was consistent with the increase in serum osmolality of 3.0% and reflects the direct association between the two variables. However, breast-milk sodium concentration increased by 51% ($P < 0.001$) during Ramadan, which contrasts with an increase in serum sodium of only 7.5%. The large increase in sodium content may have been caused by the 13% ($P < 0.01$) decrease in milk lactose concentration since these two components are usually inversely related. Mean breast-milk potassium concentration was unaffected during Ramadan.

Correlations between individual lactose and sodium concentrations were negative and significant throughout the study. Correlations between individual lactose and potassium concentrations were negative but non-significant at all collections except for the Ramadan evening collection, when the correlation was positive (+0.645, $n = 10$). Finally the normal breast-milk sodium/potassium ratio of 1:2.3 was reduced to 1:1.6 in the Ramadan evening samples owing to the large increase in sodium content.

Discussion

The question being tested by this study was whether a moderate degree of dehydration in lactating women interferes with the normal process of breast-milk synthesis and secretion. It is not possible to achieve a direct answer to this question by comparison of milk outputs in fasting and non-fasting mothers since milk output is influenced by so many factors. Examples of the confounding factors are, in the short term, changes in the mothers' day to day activity patterns, which influence their breast feeding pattern, and, in the long term, include the normal decrease in milk

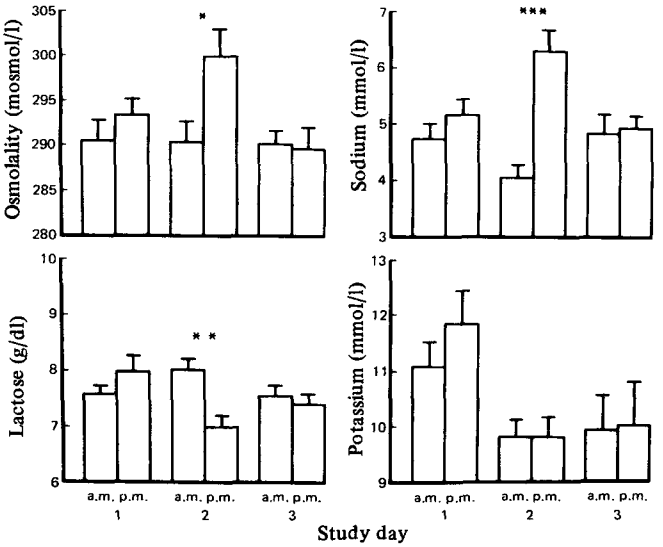

FIG. 2. Breast-milk composition. Values are means ± SEM ($n = 10$ throughout). Study day 1, pre-Ramadan; day 2, Ramadan; day 3, post-Ramadan. $*P < 0.02$, $**P < 0.01$, $***P < 0.001$, by paired t-test.

output with progressing lactation, variations in the amount of weaning food given to the infants and seasonal variations in milk output due to changes in maternal work load and dietary intake [4–6]. In this study we have therefore taken a more indirect approach and have tested, firstly, how lactating mothers respond to the dehydrating stress of Ramadan, and, secondly, whether changes in milk composition occur which would suggest that the process of milk synthesis is being altered or inhibited.

The lactating women lost more water during the period of fasting than the NPNL controls as judged by their changes in body weight. However, in spite of a 7.6% loss of total body water from morning to evening the dehydration was evidently not severe since urinary osmolality averaged only 630 mosmol/l in late afternoon urine samples. Similarly, although several of the serum indices of dehydration showed significant changes during the Ramadan fast, none of the 19.00 hours mean values was abnormally high. These results suggest that the subjects were highly adapted to the water restriction and that the main mechanism of adaptation was that of super-hydration overnight. The observation that the daily water turnover of the lactating women was 2 litres greater than that of the NPNL controls in spite of a daily milk output averaging only about 500 ml suggests the possibility of a second protective mechanism whereby lactating women built up a larger water buffer than non-lactating women. An increase in water intake which is disproportional to the quantity of milk produced also occurs in lactating dairy cows [15].

In spite of this apparently mild degree of dehydration there were marked changes in breast-milk composition after Ramadan fasting. The increase in breast-milk osmolality exactly matched the increase in plasma osmolality and can be considered to be a direct result of the latter. However, the increase in milk sodium concentration was far greater than that observed in plasma. The increase in sodium may have been caused by the decrease in lactose since both are major determinants of milk osmolality, and the reduced lactose concentration itself could be caused by reduced maternal glucose levels after fasting. However, this is most unlikely since, as reported elsewhere [7], the energy metabolite pattern in these lactating women after a Ramadan fast was not significantly different from that after an overnight fast and breast-milk compositional changes were not observed in any of the morning samples.

A more likely explanation of the changes comes from extensive work on lactating goats by Linzell & Peaker [16, 17], who have shown that under certain circumstances a paracellular pathway for milk secretion develops whereby small ions can pass through the normally tight intercellular junctions between secretory cells. When the paracellular pathway develops sodium and chloride ions pass into milk and lactose and potassium flow out of milk down simple concentration gradients.

Lactose and potassium, which are negatively correlated in normal milk, become positively correlated in the presence of the paracellular pathway. The decrease in lactose concentration together with the large increase in sodium concentration and the positive lactose–potassium correlation in the evening Ramadan samples strongly suggest that the paracellular pathway is active under these conditions. Although the potassium concentration in these samples was not actually lower than in the morning samples, as might be expected from the above hypothesis, the values were considerably lower than would be predicted on the basis of a constant sodium/potassium ratio as should exist in normal milk. The mean sodium/potassium ratio was 1:1.6 in the Ramadan evening samples and averaged 1:2.3 at all the other collections.

The paracellular pathway, which in goats occurs when milk is obtained from pregnant animals or by the use of oxytocin injections [16], has not been unequivocally demonstrated to occur in humans. However, there is increasing suggestive evidence that it can occur. In our studies in The Gambia the presence of abnormally high concentrations of sodium and of some serum specific proteins such as immunoglobulin G and transferrin in breast-milk from women with mastitis, involuting breasts or breast dysfunction suggests that 'leaky' junctions exist between the cells of the mammary epithelium (A. M. Prentice, unpublished results). In addition, Hartmann & Prosser [18] have recently demonstrated that in women with normal menstrual cycles two short periods of milk compositional change occur at 5–6 days before ovulation and 6–7 days after ovulation. The changes they observed were very similar to those observed in this study as a result of fasting and led the authors to conclude that the paracellular pathway also occurred at these two stages of the menstrual cycle. It is unclear whether there is any mechanistic link between these different observations.

Further studies are required to determine whether the transient appearance of the paracellular pathway is detrimental to breast-milk production in the long term, since with the exception of these compositional changes the lactating women in this study were capable of a remarkable degree of adaptation to the period of water abstention required by a Ramadan fast and did not show a marked disturbance of their normal physiology.

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