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EFFECT OF CALORIC SUPPLEMENTATION DURING LACTATION ON LEVELS AND AVIDITY OF IGA ANTIBODIES OF HUMAN MILK

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INTRODUCTION

Human breast milk contains several soluble and cellular components that may counteract microbial negative effects in the mucosae of the breastfed infants (1-3). The *in vitro* antimicrobial action of these milk factors, such as receptor analogues, lipids, lysozyme and cells of the defense system, has been well documented (4-7). Experimental studies using animals have proven that some of the components of human milk may be important *in vivo* (8-10). In the human, however, only specific IgA antibodies have been shown to be protective: Children infected with either *Vibrio cholerae* or heat-labile toxin producing *E. coli* have a decreased risk of developing diarrhea when they ingest milk with high titers of IgA antibodies, directed against the respective bacterial toxins and somatic antigens (11,12). High levels of IgA antibodies against cow's milk proteins also protect the breastfed child from developing allergy to the bovine proteins (13).

Several investigators have addressed the question of the protective potential of breast milk of women from different locations, by analyzing its content of total secretory IgA and of specific antibodies (14-17). Special attention has been given to the effect of maternal nutritional status on these parameters and, although one report presented data indicating decreased levels of specific antibodies in colostrum but not in mature milk of undernourished women (14), the information available shows that there is no impairment among underprivileged women to mount an IgA antibody response in milk (15-18). Nevertheless, none of these studies have

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investigated the quality of the specific antibodies. Recently, Roberton *et al* (19) reported that the avidity of milk IgA antibodies directed against *E. coli* somatic antigens and diphtheria toxin among Pakistani women, was lower than in Swedish mothers. The authors, however, clearly state that the mothers from Pakistan were not obviously undernourished.

Antibody avidity is an estimation of its binding strength and, therefore, of its functional capacity. Here we present information in regard to milk IgA antibody levels and avidity in samples obtained from Guatemalan undernourished women, and the effect on these parameters of caloric supplementation during 20 weeks of lactation. The original protocol, a randomized, double-blind, controlled study, was planned to evaluate the effect of supplementation of undernourished lactating women on their milk production (20).

MATERIALS AND METHODS

Women

The study population was drawn from several communities of Quetzaltenango, a department in the Northwest of Guatemala. Only undernourished women were considered as potential participants. Calf circumference was used to screen the women. Previous hospital-based comparisons showed that calf circumference was a more sensitive discriminator of undernourished women than arm circumference, or skin folds (20). Recruitment into the study was done during the last trimester of pregnancy, after informed consent was given. Women were randomized, stratifying by height, into one of two groups. Starting on the fifth week postpartum, mothers in group A were given high-calorie cookies (250 Kcal/unit) while mothers in group B received low-calorie ones (70 Kcal/unit). The supplements, two cookies per day, were delivered at the mothers' homes Monday through Saturday by community distributors. Compliance with supplement consumption was monitored daily. Women were advised to consume these cookies in addition to their usual diets. The cookies were based on wheat, corn and soy flours, and contained sugar, sesame seeds and chocolate or vanilla flavor. There were 111 mothers recruited into the main study (56 in Group A and 55 in Group B); of these, 67 were included in the immunological analyses but only 48 (28 in Group A and 20 in Group B) had determinations done when the study was initiated, and at the three follow-up points. The baseline characteristics of the women are presented in Table 1.

Field methodology

At five weeks postpartum, and before the supplementation was started, mothers were invited to the study clinic where they spent 28 consecutive hours. During this period, the 24hr milk volume was estimated by the test-weighing method. Two hours after the last feeding, the left breast was totally emptied by extraction with a mechanical pump. These procedures were repeated at 10, 20 and 25 weeks postpartum, that is, 5, 15 and 20 weeks after initiation of the nutritional treatments. Anthropometric measurements were also done at these times.

Table 1
Initial characteristics of the women

	Total group	With IgA Studies	
		All Subjects (37/30)	Repeated Measures 28/20
Weight (Kg)			
Diet A	42.2 ± 3 8*	41.8 ± 3 8	42.2 ± 4.0
Diet B	43.0 ± 3 3	43.0 ± 2.9	42 5 ± 2.8
Height (cm)			
Diet A	143.5 ± 4.8	143.1 ± 5.0	142.9 ± 4.6
Diet B	143 8 ± 5.2	143.6 ± 5.0	142.7 ± 4.7
Calf circumference (cm)			
Diet A	29.5 ± 1.4	29 5 ± 1.4	29.6 ± 1.3
Diet B	29.5 ± 1.3	29.5 ± 1.2	29.3 ± 1.3
Age (years)			
Diet A	24.6 ± 6.0	24.9 ± 6.4	25.3 ± 7.0
Diet B	26.4 ± 6.9	27.2 ± 6 9	25.7 ± 6.2

* Mean ± Standard Deviation
No difference between diets
No difference among groups

Laboratory methodology

The milk samples were placed in glass containers, frozen at -20°C and transported to INCAP's central laboratory. Frozen specimens were thawed, homogenized and sonicated. An aliquote was then taken, frozen again, and not thawed until immediately before its analysis. Levels of IgA antibodies against *E. coli 06* and against a pool of ten *E. coli* somatic antigens (01, 02, 04, 06, 07, 08, 018, 022, 025 and 075), hereafter called *E. coli 10*, were determined by the enzyme-linked immunosorbent assay (ELISA), as previously described (17). The avidity of the antibodies was also investigated by ELISA, using the thiocyanate elution technique (21).

Statistical methodology

Repeated measures multiple regression analyses were done (22, 23) to evaluate the effect of treatment on immunological parameters. Maternal nutritional status, antibody values, milk volume, and milk nutritional quality were taken into consideration when exploring the effect of treatment through time on antibody levels and avidity. To further discriminate the effect of initial nutritional status of the women on antibody levels and avidity, the mothers in each treatment group were divided into two additional categories: Those whose calf circumference was below the median (29.5 cm), and those with values above the median.

RESULTS

The levels of specific IgA antibodies against *Escherichia coli 06* were similar in Group A and Group B at baseline. These titers remained unchanged in the two groups of women at weeks 10 and 20 of the study. At week 25, however, there was a significant increase in the titers of the mothers in Group B, while that of women in Group A did not change, making the difference among groups highly significant ($p = 0.0168$, Table 2). Multiple regression analysis of repeated measures indicated that there was an effect of diet in time, with the women in Group B showing higher antibody levels than the supplemented ones (Table 6). The group with the highest anti-*E. coli 06* antibody levels at week 25 was that of the women with smaller calf circumference in Group B (43 ± 25; Table 4). The levels of anti-*E. coli 06* IgA milk antibodies depended on their initial values, related to the initial volume of milk produced by the mother, and to changes in milk output during the study period. Other variables that were found related to changes in anti-*E coli 06* antibodies in milk, were initial avidity of the specific IgA anti-*E. coli 06* antibodies, initial milk calorie content, and changes in caloric milk values.

At baseline, levels of IgA antibodies against *E. coli 10* were identical in the two groups of mothers (Table 2). Although there was a tendency for higher initial values among the mothers with larger calf circumference in the two diets, the trend did not attain statistical significance (Table 5). The levels of antibody changed significantly during the study period, but there was no difference in these titers between the two dietary groups (Table 6). Statistical analyses showed that the levels of anti-*E. coli 10* milk IgA antibodies were related to their initial value, to the initial milk output, and to changes in milk volume ($p = 0.0007$).

The avidity of milk IgA antibodies directed against *E. coli 06* among mothers in Group A and among those in Group B was similar (Table 3). The mean values showed time-related changes, but there were no differences between the two study groups of women (Table 6). The avidity of anti-*E coli 06* antibodies at week 10, 20 and 25 depended on the values observed at week 5 (Table 6).

There were differences among the two groups of women in the way the avidities of milk IgA antibodies against *E. coli 10* behaved in time: The mean avidity remained unchanged among subjects in Group A. In Group B, the tendency was towards lower avidity at weeks 20 and 25 ($p = 0.0009$, Table 3). This pattern was independent of calf circumference category within each group. Again, avidity values at weeks 10, 20 and 25 were dependent on the values observed at the beginning of the supplementation ($p = 0.0001$, Table 6).

Table 2
Milk IgA antibody levels (% of standard)

	Time Postpartum (Weeks)			
	5	10	20	25
Antibodies against *E. coli* 06				
Diet A	23 ± 14*	20 ± 15	23 ± 22	24 ± 13
Diet B	26 ± 23	19 ± 14	25 ± 16	37 ± 22
Antibodies against *E. coli* 10				
Diet A	51 ± 21	43 ± 16	42 ± 20	44 ± 21
Diet B	50 ± 28	39 ± 18	38 ± 21	42 ± 23

* Mean ± Standard Deviation

Table 3
Milk IgA antibody avidity (M KSCN)

	Time Postpartum (Weeks)			
	5	10	20	25
Antibodies against *E. coli* 06				
Diet A	1 6 ± 0 8*	1 6 ± 0 6	1.7 ± 0 8	1.7 ± 0.7
Diet B	1.3 ± 0 5	1 5 ± 0 5	1 5 ± 0.6	1.3 ± 0.6
Antibodies against *E. coli* 10				
Diet A	2.1 ± 0.6	1.9 ± 0 5	2.2 ± 0 6	2.1 ± 0 6
Diet B	1.8 ± 0.4	1.8 ± 0 6	1.4 ± 0 6	1 5 ± 0.7

* Mean ± Standard Deviation

Table 4
Antibody levels(%) in relation to nutritional status[1] and diet[2]

Nutritional Status	Diet	Antibody Levels[3] against *E. coli* 06			
		Time Postpartum (Wk)			
		5	10	20	25
Thin	A	23 ± 13	24 ± 20	25 ± 30	25 ± 17
Thin	B	21 ± 14	18 ± 11	23 ± 16	43 ± 25
Heavy	A	23 ± 15	18 ± 9	18 ± 10	23 ± 9
Heavy	B	30 ± 28	19 ± 16	26 ± 16	29 ± 18

[1] Calf circumference
[2] A: High Calorie Supplementation; B: Control
[3] Mean ± S.D.; Diet B Higher than Diet A at 25 Wk.

Table 5
Antibody levels (%) in relation to nutritional status[1] and diet[2]

Nutritional Status	Diet	Antibody Levels[3] against *E. coli* 10			
		Time Postpartum (Wk)			
		5	10	20	25
Thin	A	48 ± 22	41 ± 18	35 ± 19	37 ± 18
Thin	B	43 ± 26	36 ± 24	33 ± 22	41 ± 22
Heavy	A	55 ± 19	46 ± 12	50 ± 18	53 ± 23
Heavy	B	56 ± 30	42 ± 13	41 ± 21	42 ± 25

[1]Calf circumference; [2]A: High Calorie Supplementation; B: Control; [3]Mean ± S.D.

Table 6
Repeated measures analysis of variance*

Source	Probability			
	Antibody Levels		Antibody Avidity	
	E. coli 06	*E. coli* 10	*E. coli* 06	*E. coli* 10
Between Subjects				
Calf Circumference	NS	NS	NS	NS
Diet	NS	NS	NS	NS
Calf Cir x Diet	NS	NS	NS	NS
Initial Values	0.0001	0 0001	0.0001	0.0107
Within Subjects				
Week	0.0001	0 0344	0.0003	0.0001
Week x Calf cir	NS	NS	NS	NS
Week x Diet	0.0168	NS	NS	0.0009
Week x Diet x Calf	0.0844	NS	NS	NS
Week x Initial Value	0.0002	0.0005	0.0007	0.0001

* Probability, Pr > F

DISCUSSION

Undernourished children are restricted in their capacity to mount an appropriate mucosal IgA immune response to measles and polio vaccines (24). The information currently available, however, indicates that maternal under-nutrition does not have a deleterious effect on the capacity of mothers to provide their offspring with high levels of IgA antibodies via breast milk (15-18). Here, we have analyzed the impact on milk IgA antibody levels of caloric supplementation, during 20 weeks of lactation, of initially undernourished women. The results do not show any positive effect of caloric supplementation on the levels of the specific antibodies tested.

It is of interest to note that the behaviour during the 20-week study period of levels of the antibodies tested were dependent on initial values in the mothers, implying that whatever mechanisms are important in determining the content of specific antibodies in breast milk, are functional from the beginning of lactation and keep playing an important role throughout its duration. Nevertheless, and as we have previously shown (25), there are fluctuations in antibody titers during lactation. On this occasion, we noted the phenomenon among unsupplemented women whose levels of anti-*E. coli 06* antibodies increased by week 25 postpartum. Infections in the mother may be responsible for these changes in antibody milk content.

The lack of association between maternal nutritional status and milk IgA specific antibody content found in this analysis may be related to a preferential selection of IgA-committed lymphocytes from the circulation into the mammary gland, a process which may be operational even in undernourished subjects, at the expense of other mucosal sites such as the nasopharynx, where IgA immune responses have been reported to be impaired during under-nutrition (24).

In terms of antibody avidity, our results do not show an unequivocal beneficial effect of caloric supplementation. In the case of the antibodies directed against *E. coli 06*, where we observed changes in antibody levels among the unsupplemented mothers, avidity also changed in time but initial values were a determining factor of subsequent ones. Caloric supplementation did not have any effect on the anti-*E coli 06* antibody avidity. In contrast, the nutritional intervention was associated with higher avidities of anti-*E. coli 10* antibodies at week 20 and 25 postpartum. The difference was due to a significant decrease in the avidity of anti-*E. coli 10* antibody avidity among unsupplemented lactating women, suggesting that, in this case, the caloric supplement prevented the deterioration seen among controls.

The reported fluctuations in milk antibody levels (25), as well as the changes in antibody avidity presented here and by Roberton and colleagues (19), suggest that the mammary immune system is a very dynamic one, and that nutritional status is only one of the multiple factors to be considered as playing an important role in its expression. Antigenic exposure has to be taken into consideration and although infections, as monitored by morbidity, were not included in our analyses, we observed multiple patterns of relationships between the levels and avidities of specific IgA antibodies. There were cases among whom increases in antibody levels were accompanied by either increases or decreases in their avidity; in other instances, a decrease in the level of IgA specific antibody was followed by an increase in its avidity. More in-depth studies have to be carried out in order to explore the potential determinants of the human IgA response in the mammary gland. The value of nutritional treatment of undernourished women, especially during lactation, must be underscored and promoted, as a means to contribute to their general wellbeing.

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REFERENCES

1. Goldman AS, Ham Pong AJ, Goldblum RM. Host defenses: Development and maternal contributions. *Adv Pediatr* 1985; 32:71.
2. Cravioto A. Presencia de factores especificos en leche materna contra cepas de *Escherichia coli* causante de diarrea en humanos *Gaceta Med Mex* 1990; 126:35.
3. Butte N, Goldblum RM, Fehl LM, Loftin K, Smith CEO, Garza, AS Goldman. Daily Ingestion of Immunologic components in human milk during in first four months of life. *Acta Paediatr Scand* 1984; 73 296.
4. Otnaes A-B, Orstavik I. The effect of human milk fractions on rotavirus in relation to the secretory IgA content. *Acta Path Microbiol Scand* 1980; (Sect C) 88:15.
5. Johnson DF, France GL, Marmer DJ, Steel RW. Bactericidal mechanisms of human breast milk leukocytes. *Infect Immun* 1980; 28 314.
6. Holmgren J, Svennnerholm A-M, Lindblad M. Receptor-like compounds in human milk that inhibit classical and el tor *Vibrio cholerae* cell adherence (hemmaglutination) *Infect Immun* 1983; 39:147.
7. Ho PC, Lawton JWM. Human colostral cells: Phagocytosis and killing of *E. coli* and *C. albicans*. *J Pediatr* 1978; 93:910.
8. Nagy LK, Mackenzie T. The effect of colostrum or past colibacillosis on the adhesion of *Escherichia coli* to the small intestine of the pig. *Res Vet Med* 1976; 21:303.
9. Cleary TG, Chambers JP, Pickering LK. Protection of suckling mice from the heat-stable enterotoxin of *Escherichia coli* by human milk. *J Infect Dis* 1983; 148:1114.
10. Newburg DS, Pickering LK, McCluer RH, Cleary TG. Fucosylated Oligosaccharides of human milk protect suckling mice from heat-stable enterotoxin of *Escherichia coli*. *J Infect Dis* 1990; 162:1075.
11. Glass RI, Svennerholm A-M, Stoll BJ, Khan MR, Belayet Hossain KM, Imdadul Huq M, Holmgren J. Protection against cholera in breast-fed children by antibodies in breast milk. *New Engl J Med* 1983; 308:1389.
12. Cruz JR, Gil L, Cano F, Caceres P, Pareja G. Breast milk anti-*Escherichia coli* heat-labile toxin IgA antibodies protect against toxin-induced infantile diarrhea. *Acta Paediatr Scand* 1988; 77:658.
13. Machtinger S, Moss R. Cow's milk allergy in breast-fed infants: The role of allergen and maternal secretory IgA antibody. *Allergy Clin Immunol* 1986; 77:341.
14. Miranda R, Saravia NG, Ackerman R. Effect of maternal nutritional status on immunological substances in human colostrum and milk. *Am J Clin Nutr* 1983; 37.632.
15. Prentice A, Prentice AM, Cole TJ, Whitehead RG. Determinants of variations in breast milk protective factor concentrations in rural Gambian mothers. *Arch Dis Child* 1983; 58:518.
16. Reddy V, Bhaskaram C, Raghuvamulu N, Jagadeesan V. Antimicrobial factors in human milk. *Acta Paediatr Scand* 1977; 66:229.

17. Cruz JR, Carlsson B, Garcia B, Urrutia JJ, Hanson LA. Secretory IgA quantity and antibody levels against *Escherichia coli* in colostrum and milk from underprivileged and privileged mothers. *Ped Res* 1982; 16:272.
18. Cruz JR, Hanson LA. Specific milk immune response of rural and urban Guatemalan women to oral immunization with a food protein. *J Pediatr Gastroenterol Nutr* 1986; 5:450.
19. Roberton DM, Carlsson B, Coffman K, Hahn-Zoric M, Jalil F, Jones C, Hanson LA. Avidity of IgA antibody to *Escherichia coli* polysaccharide and diphtheria toxin in breast milk from Swedish ans Pakistani mothers. *Scand J Immunol* 1988; 28:783.
20. Gonzalez-Cossio T, Habitch JP, Delgado H, Rassmussen L. In manuscript.
21. Pullen GR, Fitzgerald MG, Hosking CS. Antibody avidity determination by ELISA using thiocyanate elution. *J Immunol Methods* 1986; 86:83.
22. Neter J, Wasserman W, Kutner MH. Applied linear statistical models. 3rd Edition. Homewood, Illinois: Irwin, 1990.
23. SAS Institute Inc. 1987. SAS/SAT. Guide for personal computers. 6th Edition. Cary, North Carolina.
24. Chandra RK. Reduced secretory antibody response in live attenuated measles and poliovirus vaccines in malnourished children. *Br Med J* 1975; 2:583.
25. Cruz JR, Arevalo C. Fluctuation of specific IgA antibodies in human milk. *Acta Paediatr Scand* 1986; 74:897.