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Oligosaccharides in Colostrum of Italian and Burkinabe Women

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ABSTRACT
Human milk contains a large number of compounds to provide nutrition and defense for the newborn. Among these, oligosaccharides are present in concentrations up to 12 g/L, and their composition varies during lactation. Colostrum from 53 Burkinabe women were collected at the maternity department of St Camille Medical Centre in Ouagadougou (Burkina Faso, West Africa). Colostrum from 50 Italian women were collected at the maternity department of St Bambino Hospital in Catania (Catania, East Sicily, Italy). All mothers spontaneously delivered at term. Italian mothers received an injection of the ergot derivative ergotamine after delivery. Ergotamine, notoriously, delays breastfeeding initiation up to 2 to 3 days. Chromatographic separation of colostrum from both Burkinabe and Italian women showed a progressive appearance of oligosaccharides in the first 3 days. Burkinabe women showed high concentrations of 2-fucosyllactose and lower concentrations of lacto-N-fucopentaose I. By contrast, Italian women showed inverted behaviour. A comparable percentage of the secretor genotype for the Lewis blood group phenotype in both Burkinabe and Italian women was found. According to the different ethnicity, different milk oligosaccharide profiles were documented in the present study. 2-Fucosyllactose in milk should be biologically significant for Burkinabe infants because of the high levels found in their mothers' colostrum after the second day of lactation. ***JPGN 43:372–378, 2006.***
Key Words: Burkinabe women—Colostrum—Italian women—Oligosaccharides.

INTRODUCTION

Human milk contains a large number of compounds that defend the newborn. Their appearance is regulated by neuroendocrine and immune factors modulated by the biological watch (1–3). Oligosaccharides, found in concentrations up to 12 g/L, are the third largest constituent in human milk. However, oligosaccharide total concentrations vary over the lactation course. In fact, their mean concentration at 1 year is about half of that in the first weeks postpartum (4,5). The colostrum (1–3 days postpartum) contains a 2-fold concentration of the total amount of oligosaccharides found in mature milk (6) and their initial concentration decreases in the first 120 days of lactation (5). Moreover, the different oligosaccharide distribution (fucosyl-oligosaccharides, sialyl-oligosaccharides and sialyl-fucosyl-oligosaccharides) dynamically varies during the different lactation phases (7,8).

Oligosaccharides are synthesized by sequential addition of monosaccharides to the lactose molecule by the specific enzyme glycosyltransferase (9). A strong relationship between the milk oligosaccharide phenotype and the Lewis blood group phenotype exists and implies a common genetic control. Polymorphisms of the Lewis and secretor genes, underlying Lewis blood group phenotypes, contribute to the concentration variations of 2- to 4-linked fucosyl-oligosaccharides in human milk (8). In fact, milk from Le^{a-b+} mothers contains more lacto-N-fucopentaose (LNFP) II (Le^a) and 3-fucosyllactose (FL) (Le^x) (oligosaccharides whose fucose is exclusively α1,3- or α1,4-linked). Milk from Le^{a-b-} mothers contains more LNFP I (H-1) and 2-FL (H-2), whose fucose is exclusively α1,2-linked. Milk with high α1,2/ α1,3 ratios are produced primarily by Le^{a-b-} mothers. Those with lower ratios are exclusively produced by Le^{a-b+} mothers (Tables 1 and 2).

The α1,2-linked-fucose-oligosaccharides/α1,2-linked devoid-fucose-oligosaccharides ratio changes during the first year of lactation from 5:1 to 1:1 (7).

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Milk oligosaccharides (4) also represent significant components in an innate immune system, by which the lactating mother protects her nursing infant from environmental pathogens, especially in the early months of life. At that time, the nursing infant is more susceptible to infections because of the immaturity of the immune system and to the permeability of the intestinal epithelial barrier. In fact, viruses, bacteria and toxins, after adhesion to the receptor located on the surface of the intestinal epithelial cells, become pathogenic.

The oligosaccharide structure is able to mimic the carbohydrate portion of glycoproteins and glycolipids of the membrane of the intestinal epithelial cells. Therefore, oligosaccharides may prevent pathogens from developing their full pathogenic potential (10,11). Newburg et al. (8) demonstrated that 2-linked fucosyl-oligosaccharides in human milk are significantly associated with a lesser disease risk in breastfed infants.

Moreover, the high content of oligosaccharides in breast milk may also modulate the monocyte activity in this biological liquid (12,13) because of the presence of the mannosyl-fucosyl receptor on macrophage surfaces (14). The colostrum's protective function depends on both the mother's genetic characteristic (Lewis blood group phenotypes) and breast milk oligosaccharide structures. Therefore, the study of milk oligosaccharides, lipids, hormones, cytokines and growth factors in different ethnic groups and in different lactation phases is crucial to obtain a better understanding of the lactation ontogenesis in humans (15–17).

PATIENTS AND METHODS

Subjects and Study Area

Colostrum from 53 consecutive Burkinabe women, who spontaneously delivered at term, were collected between July and October 2002 at the maternity department of St Camille Medical Centre (SCMC) in Ouagadougou (Burkina Faso, West Africa). Women who delivered by cesarean section were excluded from the study. Burkina Faso (formerly known as Upper Volta) was once a French colony; it gained its independence in 1960 and is currently one of the poorest countries in the West Burkinabe region. Its ethnic composition (about 1.5×10^6 population) is heterogeneous and includes Mossi, Peuhul, Gurunsi, Bobo and other ethnicities. They are primarily shepherds or nonnomadic farmers and live in sod and thatch huts in small rural villages. The socioeconomic status is poor and their hygienic-sanitary conditions are defective, for example, lack of fresh water supply in most homes. As a consequence, the oral transmission of infectious diseases is common, even as early as the first few days of life.

Colostrum from 50 consecutive Italian women, who spontaneously delivered at term, were collected at the maternity department of St Bambino Hospital in Catania (East Sicily, Italy). The women who delivered by cesarean section were excluded from the study.

Italian women who participated in this study received an injection of a powerful vasoconstrictor such as the ergot derivative ergotamine immediately after delivery. The Burkinabe women did not receive any pharmaceutical treatment after delivery. The ethical approval for the study was obtained from the institutional review board at the SCMC and from the St Bambino Hospital.

TABLE 1. *Thirteen human milk oligosaccharide standards and their retention times*

Oligosaccharide standard	Retention time (min)
TFLNH	7.95
DFLNH I	11.30
3-FL	12.05
LNFP II	13.35
Lactose	15.80
2-FL	18.20
LNFP I	22.70
MFLNH	24.00
LNT	26.60
LSTc	35.00
6-SL	36.80
LSTa	37.00
DSLNT	47.40

DSLNH indicates difucosyllacto-N-hexaose; DSLNT, disialyllacto-N-tetraose; LNFP, lacto-N-fucopentaose; LNT, lacto-N-tetraose; MFLNH, monofucosyllacto-N-hexaose; 6-SL, 6-sialyllactose; TFLNH, trifucosyllacto-N-hexaose.

TABLE 2. *Classification of mother according to the pattern of excreted oligosaccharides (secretor/Lewis) in breast milk*

Pattern	A	A1	B	C
Secretory ABH/Lewis	a−b+	a−b−	a+b−	"Poor"/ Lewis
Alpha-1,2-fucosyl-transferase	+	+	−	−
Alpha-1,4-fucosyl-transferase	+	−	+	−
Fucosylated oligosaccharides	+	+	+	−
2-FL	+	+	−	−
LNFP I	+	+	−	−
LNFP II	+	−	+	−
DFLNH	+	−	+	−
TFLNH	+	−	−	−

Clinical-demographic Features

Data on personal characteristics (age, number of pregnancies and gestational age) and clinical findings (history, symptoms and body temperature) were collected from all of the participants in the study and summarized in Table 3. The mother's food intake reflects the traditional eating habits of her country, that is, millet, vegetables, fruit and a little beef for Burkinabe women; whereas it consisted of grain, vegetables, fruit, fish and beef for Italians.

Milk Sample Collection

Mothers for colostrum donation were chosen in order of presentation at the maternity department. Exclusion criteria

TABLE 3. *Characteristics of mothers who gave their breast milk in the first 3 days after delivery*

Mother	No.	Age (yr)	No. of deliveries	Day 1	Day 2	Day 3
African	53	26 (17–40)	4 (1–9)	6 ± 0.5*	8 ± 0.5*	10 ± 0.5*
Italian	50	27 (20–30)	2 (1–3)	2 ± 0.1*	4 ± 0.1*	6 ± 0.1*

* $P < 0.0001$ (breast milk collected in 10 minutes).
Values are presented as median (range) or mL.

included HIV infection, sexually transmitted diseases and mastitis. All participating donors signed informed consent forms before starting the study. Both Burkinabe and Italian mothers started breastfeeding 6 hours after delivery and continued at intervals of 3 hours for 7 or more times per day. Colostrum for this study were collected at 24, 48 and 72 hours postpartum.

Milk samples were collected by the same medical team in Sicily and Burkina Faso: in the morning, 24 hours postpartum and before breastfeeding. Breast milk was extracted after a standardized procedure with a vacuum pump, collected into a graduated sterile polystyrene tube and then immediately refrigerated at 4°C. The extraction procedure lasted 10 minutes and was repeated for 3 consecutive days. After the collection, all of the mothers continued to breastfeed their babies ad libitum at SCMC for 3 days before going home.

Colostrum volumes were measured and samples were fractionated into 2-mL polystyrene tubes. They were transported on ice to the local laboratory and stored at −20°C. All of the samples were subsequently sent on dry ice to the Laboratory for the Diagnosis and Prevention of Metabolic Disease, Paediatric Department, University of Ancona, Ancona, Italy.

Oligosaccharide Determination

One milliliter of milk from each tube was added to 1 mL of acetonitrile. After stirring, samples were centrifuged at 4000*g* for 15 minutes to remove proteins and lipids. Supernatants

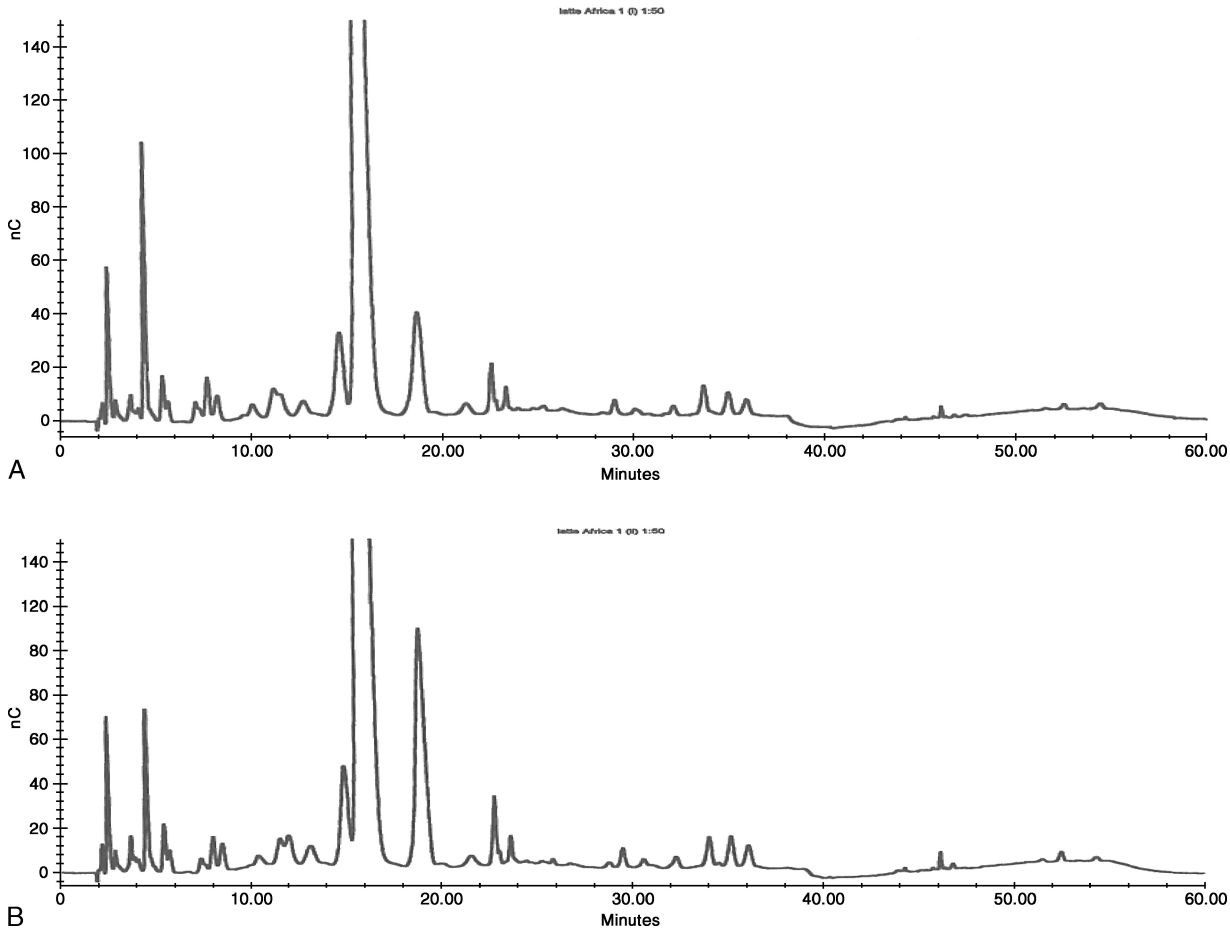

FIG. 1. Pattern of oligosaccharides in the colostrum from Burkinabe women on days 1 (A), 2 (B) and 3 (C). Pattern of oligosaccharides in the colostrum from Italian women on day 3 (D).

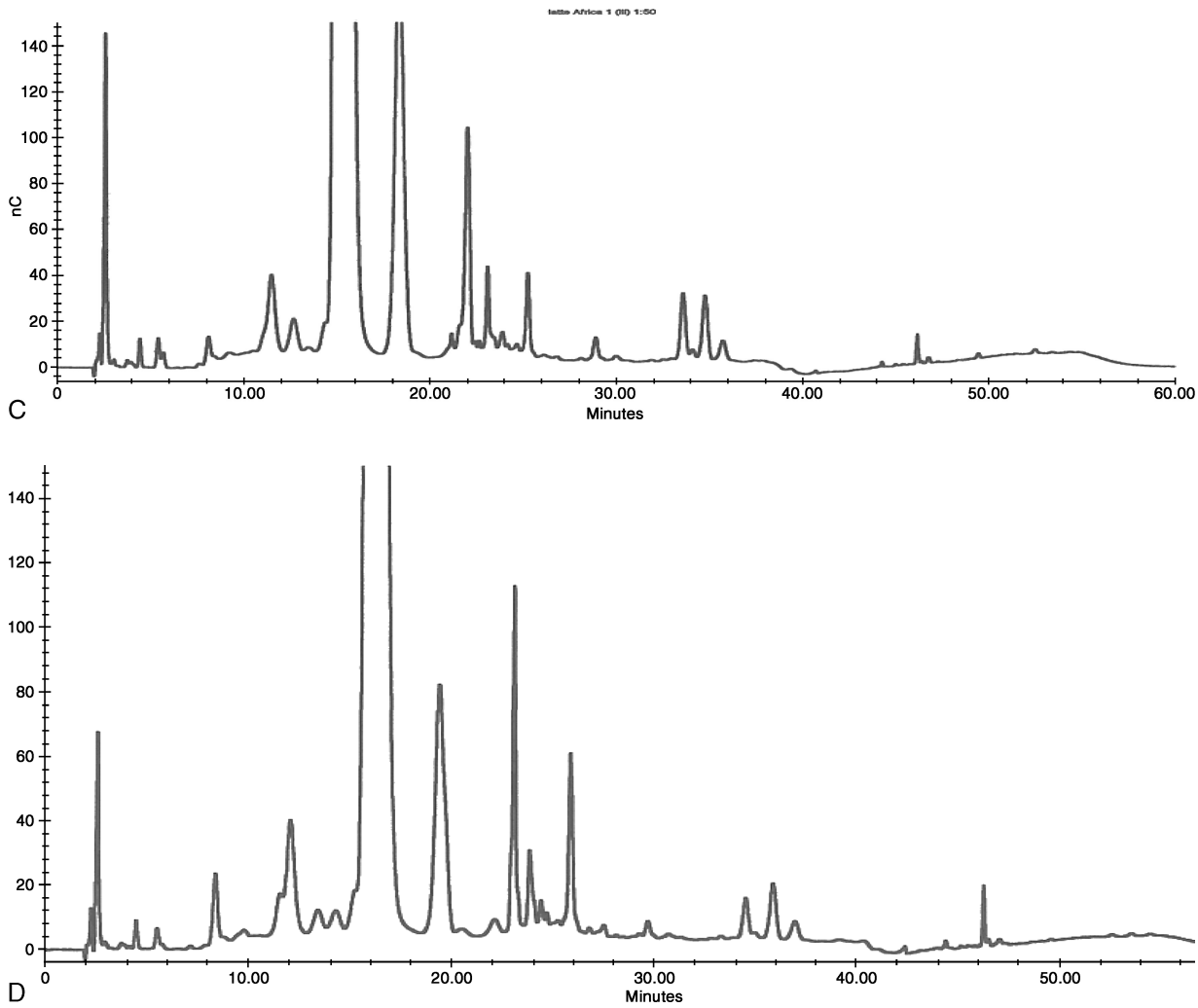

FIG. 1. (*continued*)

were diluted 1/24 (vol/vol) with deionized water for high-performance liquid chromatography and filtered through a 0.22-μm nylon membrane (Millipore, Billerica, MA). The final dilution was 1/48 (vol/vol).

Analytical Method

A 25-μL milk sample was injected by autosampler (AS40 Dionex, Sunnyvale, CA) into a Dionex high-performance anion-exchange chromatography DX-500 system consisting of a CarboPac PA-1 (4 × 250 mm) analytical column, a CarboPac PA (3 × 25 mm) precolumn, an electrochemical detector (ED 40) based on integrated and pulsed amperometric detection with a gold electrode and a GP 50 gradient pump.

Separation was obtained according to the method of Townsend et al. (18), modified as follows: eluent 1, aqueous 100 mmol/L NaOH and eluent 2, 1 mol/L sodium acetate in aqueous 100 mmol/L NaOH.

TABLE 4. *Levels of 2-FL and LNFP I in colostrum from Burkinabe and Italian women in the first 3 days after delivery*

Day no.	Burkinabe 2-FL (n = 53)	Italian 2-FL (n = 50)	Burkinabe LNFP I (n = 53)	Italian LNFP I (n = 50)
1	1.8 ± 1.6*	1.0 ± 0.8	0.8 ± 0.5**	1.5 ± 1.3
2	4.5 ± 3.2***	2.1 ± 1.4	1.1 ± 0.8***	2.5 ± 1.9
3	8.4 ± 2.6***	4.2 ± 1.2	4.4 ± 1.3*	5.0 ± 1.0

Burkinabe→Italian, Student *t* test *$P < 0.01$; **$P < 0.001$; ***$P < 0.0001$.
Values are presented as mg/mL.

The system was eluted at 0.7 mL/min flow rate through a gradient pump module using the following elution program: isocratic at 100% eluent 1 for 10 minutes; linear gradient to 5% eluent 2 in 8 minutes; linear gradient to 10% eluent 2 in 12 minutes; isocratic 10% eluent 2 for 7 minutes; linear gradient to 50% eluent 2 in 10 minutes; isocratic at 50% eluent 2 for 5 minutes and column equilibration 20 minutes to 100% eluent 1. The oligosaccharide characterization was performed using the following standards (Sigma, St Louis, MO; BioCarb, Lund, Sweden; and Dextra, Covington, LA) listed in order of increasing elution time (Table 1): lacto-N-difucohexaose II, trifucosyllacto-N-hexaose, difucosyllacto-N-hexaose b (DFLNHb), DFLNH, DFLNH I, 3-FL, LNFP II, lactose, 2′-FL, LNFP I, monofucosyllactose-N-hexaose II, lacto-N-neotetraose, lacto-N-neohexaose, lacto-N-tetraose, lacto-N-hexaose, monofucosylmonosialyllacto-N-neohexaose, sialyllacto-N-tetraose c (LSTc), 6′-sialyllactose, LSTa, disialyllacto-N-tetraose. The oligosaccharide quantifications were made using pulsed amperometric detection, corrected for the response factors of each individual oligosaccharide.

Oligosaccharide Classification

A classification according to the pattern of excreted oligosaccharides (secretor/Lewis) was made: A pattern (secretor ABH/Lewis a−b+), presence of all fucosyltransferase and fucosylated oligosaccharides; A1 pattern (secretor ABH/Lewis a−b−), absence of α1,4-fucosyltransferase and of trifucosyllacto-N-hexaose, DFLNH, LNFP II; B pattern (nonsecretor/Lewis a+b−), absence of α1,2-fucosyltransferase, 2′-FL and LNFP I; and C pattern (nonsecretor "poor"/Lewis a−b−), absence of α1,2- and α1,4- fucosyltransferase, that is, the absence of all α1,2- and α1,4-fucosylated oligosaccharides (Table 2).

Statistical Analysis

Demographic and clinical profiles were recorded in a computer file and analyzed by a standard software (SPSS-10; SPSS, Inc., Chicago, IL). Data were presented as a mean ± standard deviation. Statistical comparisons were performed using paired and unpaired Student *t* test or Mann-Whitney *U* test when appropriate, considering $P < 0.05$ as statistically significant.

RESULTS

The 2 mothers' groups show a comparable mean age. Burkinabe mothers had a high average number of deliveries, with as many as 9 being reported in 1 mother. In contrast, none of the Italian mothers had more than 3 deliveries. The drawn colostrum volumes increased in the following 3 days, being the Italian colostrum mean volume always lower than the African analogue (Table 3). Colostrum chromatographic analyses from both Burkinabe and Italian women, belonging to A pattern, showed a progressive appearance of oligosaccharides during the first 3 days (Figs. 1A–C). In the colostrum of Burkinabe women, 2′-FL was the most abundant oligosaccharide followed by LNFP I + 3′-FL + LNT + LSTc + 6-sialyllactose (Figs. 1A–C).

The 2′-FL concentration observed at day 2 in Burkinabe women's colostrum was reached only at day 3 by Italian women (Fig. 1D, Table 4), who always showed a higher LNFP I concentration with respect to 2′-FL.

No significant intersample variation was observed and the chromatographic A pattern profile of Burkinabe and Italian women remained constant during the 3 days. In Burkinabe and Italians classified as B pattern, no peaks corresponding to 2′-FL or LNFP I were observed.

Single oligosaccharide analyses confirm that 71.7% (38/53) Burkinabe colostrum samples could be classified as A pattern (secretor genotype/Lewis a−b+), 3.8% (2/53) as A1 pattern (secretor genotype/Lewis a−b−) and 24.5% (13/53) as B pattern (nonsecretor genotype a+b−). In Italian mothers' colostrum, percentages of A (73.3%, 22/30), A1 (3.3%, 1/30) and B (23.3%, 7/30) patterns were comparable to those found in Burkinabe mothers' colostrum.

No oligosaccharide migrating contaminations were found in the chromatographic profiles. Moreover, the comparable retention times, presently observed in all chromatograms, confirm the good reproducibility of the method used.

DISCUSSION

The drawn colostrum mean volume in Italian mothers was smaller with respect to that of Burkinabes. This datum is not unexpected because in many European and American hospitals, it is a standard practice, after delivery, to inject women with ergonovine, a powerful vasoconstrictor that reduces serum prolactin levels, thus inhibiting breastfeeding initiation by 2 to 3 days (19). This fact helps to rationalize the reduced colostrum volume. For ethical reasons, it was not possible to use a control group without ergotamine injections. Nevertheless, literature data already demonstrated the vasoconstrictor effect of ergotamine on lactation (20,21). Women who delivered at SCMC did not receive uterine stimulants, which could reduce postpartum milk secretion.

On a qualitative level, the analysis of colostrum from Burkinabe and Italian mothers indicates a comparable oligosaccharide composition in secretor ABH/Lewis a−b+ women and suggests that the oligosaccharide secretion is maintained in ontogenesis.

However, some oligosaccharide concentrations clearly differentiate colostrum from Burkinabe and Italian women. The 2′-FL is the first and prevalent oligosaccharide in Burkinabe colostrum followed by LNFP I. Therefore, Burkinabe newborns received more 2′-FL from breast milk than Italian newborns, once colostrum volumes are taken into account.

The reason for a delay in the appearance of 2′-FL in Italian mothers is not clear. This difference could be

caused entirely by the ergotamine treatment or to some other differences between these populations. However, others have found differences between populations that were based on genetic differences. In fact, results obtained by Chaturvedi et al. (7) for secretor ABH/Lewis a−b+ Mexican women, in whom the predominant oligosaccharide during the first few months of lactation was 2′-FL followed by LNFP I, support the hypothesis that this delay may be attributed to racial factors. The highest concentration of 2′-FL (3 g/L) and LNFP I (2 g/L) observed during the first 3 months declined by 1 year postpartum up to 1.2 g/L and 250 mg/L, respectively. In contrast, 3-FL increased over the course of lactation from an initial concentration of 300 mg/L to 1.1 g/L. Therefore, these 3 oligosaccharides accounted for most of the modifications in the first year of lactation (7).

On the other hand, colostrum from secretor Japanese mothers shows first LNFP-1 as the predominant oligosaccharide followed by 2′-FL + lacto-N-difuco-tetraose, LNFP II + lacto-N-difucohexaose II and 3-FL (22).

In addition, there are concentration differences and temporal changes for each oligosaccharide that characterize breast milk in different geographic and ethnic areas (23). Erney et al. (24) studied the fucosyl-oligosaccharides in human milk for different populations and found that 100% of Mexican and 46% of Philippine samples contained 2′-FL. This may be explained by a nonuniform distribution of genetically determined traits.

However, in our study, we found that all Burkinabe and Italian women show the same secretor pattern (Le^{a-b+}; 72%–73%) and nonsecretor pattern distribution (Le^{a+b-}; 23%–24%), regardless of ethnicity. Therefore, both extracted colostrum volume and 2′-FL concentration differences could be caused by the ergotamine treatment. Moreover, other environmental or cultural differences between these 2 populations cannot be neglected.

Interestingly, Newburg et al. (8) found that the variable expression of α1,2-linked fucosyl-oligosaccharides in Mexican women's milk was significantly linked to the diarrheal disease incidence among breastfed infants. This means that human milk protection against diarrheal diseases may depend on both fucosyl-oligosaccharide secretion and different 2′-FL contents. In fact, Coppa et al. (25) recently demonstrated that fucosyl-oligosaccharides of human milk inhibit the adhesion of *Listeria monocytogenes* to the adenocarcinoma cell line of the human colon by means of a selective receptor-like mechanism, mimicking intestinal receptors for pathogens (26).

In the aboriginal Mestizo Mexicans, the low prevalence (1% or less) of nonsecretor mothers (24–27) could be because of the fact that most infants receive breast milk that contains less protective α1,2-linked fucosylated-oligosaccharides. In fact, individuals of the O blood group type show a greater susceptibility to cholera (28,29) and to other virus strains (30), thus suggesting a basis for selecting a genotype that provides the highest protection against pathogens.

Therefore, the oligosaccharide function relates to competition with epithelial ligands on the intestinal mucosa for bacterial binding and prevention of their intestinal attachment (31) and to the mannose-fucose receptors on the macrophage cell surface that are also present in colostrum (16). Because several ligands of mannose-fucose receptors modulate the respiratory burst response of macrophages (32), α1,2-linked fucosylated-oligosaccharides contained in human colostrum may add a novel function to the cascade of events following the appearance of lactation.

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