Lead Article

NUTRITION REVIEWS

# 2′-fucosyllactose: an abundant, genetically determined soluble glycan present in human milk

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*Lactose is the preeminent soluble glycan in milk and a significant source of energy for most newborn mammals. Elongation of lactose with additional monosaccharides gives rise to a varied repertoire of free soluble glycans such as 2′-fucosyllactose (2′-FL), which is the most abundant oligosaccharide in human milk. In infants, 2′-FL is resistant to digestion and reaches the colon where it is partially fermented, behaving as soluble prebiotic fiber. Evidence also suggests that portions of small soluble milk glycans, including 2′-FL, are absorbed, thus raising the possibility of systemic biological effects. 2′-FL bears an epitope of the Secretor histo-blood group system; approximately 70–80% of all milk samples contain 2′-FL, since its synthesis depends on a fucosyltransferase that is not uniformly expressed. The fact that some infants are not exposed to 2′-FL has helped researchers to retrospectively probe for biological activities of this glycan. This review summarizes the attributes of 2′-FL in terms of its occurrence in mammalian phylogeny, its postulated biological activities, and its variability in human milk.*



## INTRODUCTION

Mother's milk is, for a period of time, the sole source of nutrients for newborn and developing mammals. The proportions of milk macronutrients, proteins, lipids and carbohydrates differ from one species to the other[1,2] and it is generally recognized that milk from each species is particularly suited for the development and well-being of its own offspring.[3,4] In the particular case of human milk, individual proteins and lipids (such as fatty acids) have been studied not only in terms of their contributions as energy, carbon skeletons or amino acid sources, but also as bioactive substances associated with diverse biological functions such as cognitive development[5,6] and immunity.[7] In milk from most species, lactose, which is a digestible disaccharide found only in milk, is the dominant soluble glycan. Its primary role is to provide readily available energy for newborn mammals. In humans, lactose is present at concentrations that average 70 g/L,[8,9] thus contributing 280 Kcal/L, which is 81% of the caloric contribution of 38 g of fat found in the same volume of milk. As lactose enters the human small intestine, it is hydrolyzed into its monosaccharide building blocks glucose (Glc) and galactose (Gal), which are then absorbed. While the role of digestible glycans as energy sources has long been recognized, dietary fiber was the last macronutrient to be systematically studied as an important component of the human diet. Similarly, indigestible soluble glycans in milk, which are built by adding one or more monosaccharides to lactose, act as fiber, and their roles in human and non-human animal health are still being elucidated. Free soluble glycans in human milk are customarily referred to as human milk oligosaccharides (HMOs) even though some of these carbohydrate structures are also present in milk of other mammals and are, therefore, not exclusive to human milk. The present review focuses on a particular soluble milk glycan (SMG), the trisaccharide 2′-fucosyllactose (2′-FL); the aim is to summarize different aspects of this glycan, which is the most abundant in human milk with

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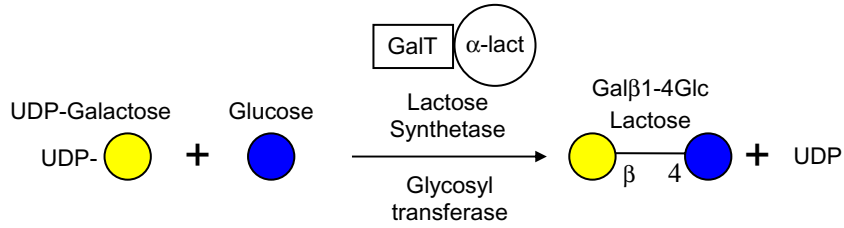


*Figure 1* **Biosynthesis of lactose.** Lactose is synthesized in the Golgi of mammary gland cells during lactogenesis. The monosaccharide galactose (Gal ●) is transferred from a sugar nucleotide donor; Uridine-diphospho galactose to glucose (Glc ●) that functions as an acceptor. In mammals, synthesis of milk saccharides is driven by glycosyltransferases – in this case, the lactose synthase complex constituted by galactosyltransferase and α-lactalbumin.

the exception of lactose.[10] As the body of literature on milk oligosaccharides has grown dramatically in the last decade, it is important to initiate a new stage of research in which individual soluble glycan structures are studied and their effects distinguished from those ascribed to the complex mixture of human SMGs.

One interesting aspect of 2′-FL is that it is not present in milk produced by all women and its concentration varies significantly during lactation and among mothers.[10–12] In addition 2′-FL is one of the most studied oligosaccharides in regards to postulated biological activities associated with its presence in human milk and the genetics of its expression in humans.[13,14] At a minimum, this soluble glycan acts as one of the primordial prebiotics that supports the colonization of the human large intestine with bacteria that may be beneficial for the breastfed infant.[15,16] Another intriguing aspect of 2′-FL is its presence in milk of particular non-human animals that range from monotremes to certain old-world primates, thus providing evolutionary clues as to its function and an ancient natural history of this molecule amongst mammals.[17] Perhaps one of the most appealing characteristics of 2′-FL is its simplicity, which, in turn, facilitates its synthesis through chemical, enzymatic, and biotechnological processes. Lacto-*N*-neotetraose, which has been tested in clinical settings,[18] and 2′-FL were the first milk oligosaccharides synthesized reliably in biological systems[19] and tested for biological activity.[20]

## Structure and synthesis of milk oligosaccharides

SMGs are present in the milk of several species,[17] although human milk appears to contain the highest diversity of these structures and perhaps the highest concentration (5–15 g/L).[10,13] SMGs in human milk may encompass between three and five of the following monosaccharides: Glc, Gal, N-acetylglucosamine (GlcNAc), L-fucose (Fuc), and the sialic acid N-acetylneuraminic acid (Neu5Ac). Lactose (Galβ1-4Glc) is found at the reducing end of all milk oligosaccharides described so far. The simple addition of one of the monosaccharides Fuc or Neu5Ac to lactose renders the resulting trisaccharides resistant to digestion by human enzymes,[21,22] which is also true for larger and more complex soluble glycan structures. Undigested oligosaccharides gain access to the human colon where they can be fermented by resident bacteria or simply exit the body in the feces,[23,24] both of which are functions and behaviors of dietary fiber. Thus, mammals that provide oligosaccharides in their milk to their newborn and developing offspring use lactose to build molecules with expanded functions akin to those found in carbohydrate macronutrients of the human diet. As stated above, lactose is the basis of most milk carbohydrate systems and it is unique to milk; it is synthesized by the action of the enzyme UDP-Gal:N-acetylglucosamine β1-4-galactosyltransferase (Gal-T) (EC 2.4.1.38) (Figure 1). Regularly, this enzyme transfers UDP-Gal to GlcNAc moieties in glycoconjugates; however, affinity for free glucose is increased during lactation. This change in specificity occurs when the enzyme binds to α-lactalbumin, which is synthesized in response to the hormone prolactin; the complex comprised of Gal-T and α-lactalbumin is referred to as "lactose synthase" or "lactose synthetase" (Figure 1).[25] Although the expression of lactose requires the presence of an enzyme that is necessary for proper embryogenesis, growth, and development,[26] that is not the case for the synthesis of fucosylated oligosaccharides such as 2′-FL. The enzymes necessary for the synthesis of these structures are primary gene products that are not uniformly distributed amongst humans; therefore, milk glycans are secondary gene products that may or may not be present in milk depending on the genotype of the mother.[27]

To better understand the distribution of 2′-FL, it is pertinent to revisit the pathways involved in the synthesis of small soluble glycans present in human milk. The synthesis of these molecules follows a similar path to that described in Figure 1; however, in contrast with Gal-T, other known glycosyltransferases do not seem to require modifiers such as α-lactalbumin and are not universally

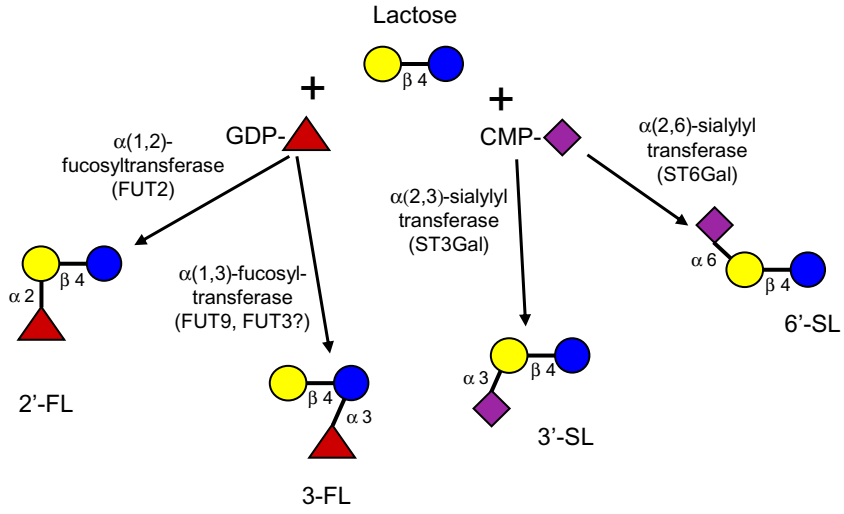


*Figure 2* **Biosynthesis of small soluble milk glycans.** Small soluble milk glycans are synthesized by decorating lactose with either the neutral monosaccharide fucose (Fuc ▲) or the acidic N-acetylneuraminic acid (Neu5Ac ◆). Fucosylated oligosaccharides such as 2′-fucosyllactose (2′-FL) and 3-fucosyllactose (3-FL) require the presence of the sugar nucleotide guanosine diphospho-fucose (GDP-Fuc GDP ▲), which acts as a donor of fucose residues. Several enzymes can affect these transfers; 2′-FL requires the presence of a particular fucosyltransferase that transfers fucose to the 2 position of β-Gal residues (FUT2), while 3-FL may be synthesized by a variety of fucosyltransferases. The synthesis of the acidic oligosaccharides 3′-sialyllactose (3′-SL) and 6′-sialyllactose (6′-SL) is catalyzed by the two different sialic acid transferases termed ST3Gal and ST6, respectively, and the sugar donor is citidine monophosphate-N-acetylneuraminic acid (CMP-Neu5Ac, CMP ◆).

expressed. In humans, the simplest oligosaccharides are synthesized as described in Figure 2. There are larger soluble glycans in human and non-human milk that result from the sequential elongation and decoration of simple structures. These include more than one hundred well-characterized linear, branched, fucosylated, and/or sialylated structures identified so far, which use primarily lacto-*N*-tetraose or lacto-*N*-neotetraose as core structures.[28] When human SMGs are compared with those of other species (such as goat or cow), three salient features can be noted: 1) the ratio of fucosylated to non-fucosylated glycans is relatively high in human milk, 2) neutral structures are present in human milk at higher concentrations than acidic ones, and 3) Type I (Galβ1-3GlcNAcβ1-3. . .) motifs are predominant in human milk while other mammals have a predominance of Type II (Galβ1-4GlcNAcβ1-3. . .) structures.[17,29] As can be surmised from Table 1,[11,13,28] Fucα1-2 residues are commonly found attached to the terminal galactose moiety of Type I structures, generating the Fucα1-2=Galβ1-3GlcNAcβ1-R motif. 2′-FL is an unusual SMG in which the fucose residue is attached to Galβ1-4 joined to a reducing glucose, as opposed to a GlcNAc residue in a β1-3 linkage. In the strict sense, 2′-FL is not a Type II structure, since the ring of the glucose residue of lactose oscillates between the α and β anomeric configurations, thus allowing for different presentations of the nearby non-reducing end. These presentations or conformations are not available in larger structures in which the reducing terminus is unable to influence binding or recognition at the other side of the molecule due to the fact that it is separated by at least two additional monosaccharide units.

## Influence of the Secretor histo-blood group system status on the synthesis of 2′-FL

Figure 2 illustrates the synthesis pathways of small molecular weight SMGs. As with lactose, the synthesis of oligosaccharides requires sugar nucleotides, such as guanosine-5′-diphospho-α-L-fucose (GDP-Fuc) for fucosyloligosaccharides, and a glycosyltransferase, which in the case of 2′-FL is fucosyltransferase 2 (FUT2). Variations in fucosylated human milk glycans among individuals are primarily due to differential expression of fucosyltransferases that are responsible for the synthesis of immunodominant epitopes of histo-blood group systems. The diversity and extent of fucosylation of glycans in a given human milk sample depend on the status of the donor in regards to two histo-blood group systems: Lewis and Secretor (Figure 3).[27,30] Only the Secretor histo-blood group system is relevant to the synthesis of 2′-FL in the lactating mammary gland. There are two phenotypes in this system, as described in Figure 3: the *Secretor* (*Se*) gene encodes the α1-2 fucosyltransferase FUT2; the expression

*Table 1* **Structures of the main small soluble milk glycans present in human milk.**

| Name | Abbreviation | Structure | Antigenic determinant |
|---|---|---|---|
| 2′-fucosyllactose | 2′-FL | Fucα1-2Galβ1-4Glc | *Se* |
| 3-fucosyllactose | 3-FL | Galβ1-4(Fucα1-3)Glc | ND |
| Difucosyllactose | DFL | Fucα1-2Galβ1-4(Fucα1-3)Glc | $Le^y$ |
| 3′-sialylactose | 3′-SL | NeuAcα2-3Galβ1-4Glc | ND |
| 6′-sialyllactose | 6′-SL | NeuAcα2-6Galβ1-4Glc | ND |
| 3′-sialyl-3-fucosyllactose | $sLe^x$ | NeuAcα2-3Galβ1-4(Fucα1-3)Glc | Sialyl $Le^x$ |
| Lacto-*N*-tetraose | LNT | *Galβ1-3GlcNAcβ1-3Galβ1-4Glc | ND |
| Lacto-*N*-neotetraose | LNnT | **Galβ1-4GlcNAcβ1-3Galβ1-4Glc | ND |
| Lacto-*N*-fucopentaose I | LNFP I | *Fucα1-2Gal β1-3GlcNAcβ1-3Galβ1-4Glc | ND |
| Lacto-*N*-fucopentaose II | LNFP II | *Gal β1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc | $Le^a$ |
| Lacto-*N*-fucopentaose III | LNFP III | **Gal β1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc | $Le^x$ |
| Lacto-*N*-fucopentaose V | LNFP V | *Gal β1-3GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc | ND |
| Lacto-*N*-hexaose | LNH | *Gal β1-3GlcNAcβ1-3[Gal β1-4GlcNacβ1-6]Galβ1-4Glc | ND |
| Lacto-*N*-neohexaose | LNnH | **Gal β1-4GlcNAcβ1-3[Gal β1-4GlcNacβ1-6]Galβ1-4Glc | ND |
| Lacto-*N*-difucohexaose I | LNDFH I | *Fucα1-2Gal β1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc | $Le^b$ |
| Lacto-*N*-difucohexaose II | LNDFH II | *Gal β1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc | $Le^a$ |
| Difucosylated lacto-*N*-hexaose a | DFLNH a | *Fucα1-2Gal β1-3GlcNAcβ1-3[Gal β1-4(Fucα1-3)GlcNacβ1-6] Galβ1-4Glc | $Le^x$ |
| Difucosylated lacto-*N*-hexaose c | DFLNH c | *Fucα1-2Gal β1-3(Fucα1-4)GlcNAcβ1-3[Gal β1-4GlcNacβ1-6] Galβ1-4Glc | $Le^b$ |

Adapted from Erney et al.,[11] Kobata,[28] and Kunz et al.[13]
* Type I chain structures Galβ1-3GlcNAc-R.
** Type II structures Galβ1-4GlcNAc-R.
*Abbreviations*: ND, not determined - no antigenic determinants were defined for these structures.

of this enzyme is a dominant trait, while non-Secretors are homozygous for mutated copies of the gene that do not encode an active transferase. Secretor phenotypes are thus defined by the presence of α1-2 fucosylated epitopes, such as 2′-FL, in their secretions, including saliva, tears, and milk.[31] Non-Secretors lack 2′-FL and other milk oligosaccharides and glycoconjugates with Fucα1-2 epitopes in their secretions. In blood, the Fucα1-2 structural motif is synthesized in certain glycoconjugates by a different enzyme (FUT1) that synthesizes the H antigen. This immunodominant epitope is present in the majority of the population. Once the H antigen is synthesized, it can remain unaltered (O blood group); it can be further elongated by a N-acetylgalactosamine (blood group A) or by a galactose (blood group B).[32] FUT1 and FUT2 are related but independent enzymes and only FUT2 is relevant to the synthesis of 2′-FL (Figure 4). This clarification is important because sometimes oligosaccharides that contain

**Secretor and H Immunodominant epitopes**

| Site of antigenic determinant expression | Phenotypes (Genotypes) | Primary gene product | Secondary Gene Product |
|---|---|---|---|
| Milk Oligosaccharides | Secretor *(SeSe/Sese)* | FUT-2 | 2′-FL and others  |
| | Non secretor *(sese)* | Not active protein | none |
| Erythrocyte glycoproteins (and glycolipids) | "O" *(HH/Hh)* | FUT-1 | ""O" or "H" antigen (Fucα1-2 Gal)  |
| | Bombay *(hh)* | Not active protein | None, very rare, so called *Bombay* blood group; when "H" ("O") antigen is absent, A or B antigens can not be synthesized (person lacks A,B,AB or O antigens) |

*Figure 3* **Illustration of how Secretor and H(O) antigens are synthesized by different enzymes with different specificities and in different tissues.**

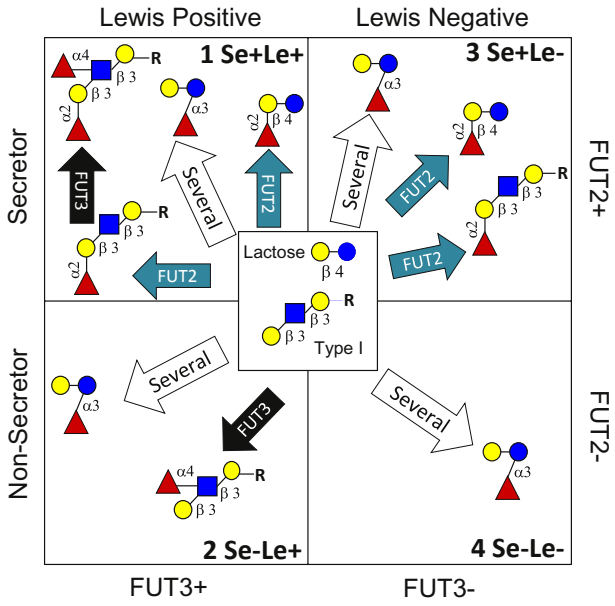


*Figure 4* **Genetic determination of the SMG profile.** SMG fucosylation depends on the Secretor and Lewis blood group. The *Secretor* gene encodes the α1-2 fucosyltransferase FUT2, which transfers a Fuc residue to a terminal Gal in α1-2 linkage. The *Lewis* gene encodes the α1-3/4 fucosyltransferase FUT3, which catalyzes the addition of Fuc in α1-3 or α1-4 linkage to subterminal GlcNAc. Based on the expression of these fucosyltransferases, four milk groups can be distinguished. If both FUT2 and FUT3 are expressed, milk contains SMG with $Le^b$ antigens (group 1). If only FUT3 is expressed, milk contains SMGs with $Le^a$ antigens (group 2). If FUT3 is not expressed, neither $Le^b$ nor $Le^a$ epitopes are present, but the SMG profile is different depending on whether FUT2 is expressed (groups 3 and 4). The milk of Secretor women (groups 1 and 3) is characterized by the presence of α1-2 fucosylated SMGs, such as 2′-FL and LNFP I.

Fucα1-2 motifs are referred to as "H oligosaccharides" even if their synthesis is not dependent on the expression of FUT1. An isomer of 2′-FL present in human milk is 3-fucosyllactose (see Figure 2). There are various fucosyltransferases catalyzing α1-3 fucosylation to lactose to produce 3-FL[33] and its expression has been found to be independent of known histo-blood group system status.[11,34] In one study, samples with more than 4 g/L of 2′-FL were found and the percentage of samples with 2′-FL matched the 70–78% determinations of Secretor status from saliva immunoassays.[11] Genes of the Secretor and Lewis histo-blood group systems are highly polymorphic and their inheritance is believed to play an evolutionary role in affecting survival of humans during pathogen outbreaks.[35] Milk glycans are a reflection of surface carbohydrates on epithelial cells that are used as adhesion targets by pathogens and toxins. For this reason, it is believed that the different phenotypes, Secretors and non-Secretors, may represent different degrees of susceptibility to certain infectious diseases.[36] For example, the non-Secretor phenotype has been associated with an increased risk of urinary tract infections[37] and vaginal candidiasis,[38] as well as an increased susceptibility to infection by *Haemophilus influenzae*,[39] *Neisseria meningitidis*, and *Streptococcus pneumoniae*.[40] Similarly, non-Secretor status has been genetically associated with increased risk of Crohn's disease,[41] susceptibility to type I diabetes,[42] and sepsis in premature offspring; low Secretor phenotype was associated with necrotizing enterocolitis.[43] Several advantages have been associated with non-Secretor status. For example, non-Secretors seem to have a degree of protection against *Helicobacter pylori* infection[44] and a reduced risk for diarrhea caused by certain genotypes of norovirus.[45] Evidence also suggests that non-Secretor individuals may be at lower risk of being infected by HIV-1 and those who are infected seem to have a slower progression of related disease, as compared with Secretors.[46] One study reported Secretors may have higher susceptibility to influenza viruses, rhinoviruses, respiratory syncytial virus, and echoviruses.[47] In contrast, Secretor-dependent mucosal glycosylation modulates innate immune responses in Rhesus monkeys[48] and may confer protection against *Campylobacter* infection in infants.[49] Secretor status seems to be influential in determining the composition of the bifidobacterial population in the intestinal microbiota in adults.[50] It is important to emphasize that in the context of early nutrition, a Secretor mother supplies 2′-FL in her milk independent of the status (Secretor, non-Secretor) of her progeny. In this regard 2′-FL could function as a decoy for pathogens that target Fucα1-2 epitopes in epithelial cells of Secretor infants.

## Analytical history of 2′-FL

The structural diversity and complexity of milk glycans represent a challenge for their analysis. Over the years, numerous strategies for HMO analysis have been developed[51,52]; from the beginning of these efforts, 2′-FL has played an important role due to its relative abundance when compared with other soluble glycans. In 1954 Polonowski, Lespagnol, and Montreuil applied two-dimensional paper chromatography and identified the fucosyllactoses, 2′-FL and 3-FL.[53] In 1967, Grollman and Ginsburg proved that 2′-FL was not detectable in milk samples from non-Secretor women.[54] Expanding on this observation, Kobata, in 1969, developed a new method to determine the oligosaccharide patterns using small amounts of milk.[55] The method combined gel filtration and paper chromatography, allowing the simultaneous resolution of 14 oligosaccharides using 10 mL of milk.

They detected three different soluble glycan patterns: 80% of the samples showed 14 spots; 15% of the samples, corresponding to non-Secretors, lacked 2′-FL and three other α1-2 fucosyloligosaccharides (DFL, LNFP I, LNDFH I, see Table 1).[56–58] New sensitive analytical techniques were later established in order to elucidate novel milk oligosaccharides. Methods such as labeling with tritiated sodium borohydride enabled the resolution of 2′-FL and other glycans.[59] In the 1980s, Egge et al.[60] applied classic chromatographic separation methods together with high-performance thin-layer liquid chromatography (HPLC), mass spectrometry (MS), and $^{1}$H-NMR spectroscopy to analyze fucose-containing HMOs. This method was highly reliable, sensitive, and specific, and allowed the characterization of fucosylated oligosaccharides. Egge observed the high variability of carbohydrate structures and recommended analyzing the milk from single donors to reduce the number of isomers present in pooled milk. Significant progress in NMR techniques allowed advances in the conformational analysis of glycans and was widely used for a period of time, allowing the identification of 2′-FL in several milk samples.[61–63] More recently, high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC) achieved accurate separation of several oligosaccharides isomers, 2′-FL and 3-FL among them.[11,12,64] The use of MS significantly improved HMO detection and quantitation of milk glycans and MS profiling using MALDI-TOF was first described in 1994 by Stahl et al.[65]

In the new century, methods such as HPLC-chip/TOF MS provided effective separation and improved sensitivity and reproducibility. It allows rapid and accurate identification of HMOs with unambiguous differentiation of isomers.[66] This technique has been used to investigate temporal and individual variations in SMG.[67,68] Ruhaak and Lebrilla[52] recently introduced nLC-PGC-chip-TOF MS for HMO analysis. This method is excellent for separating isomers and oligomers, combining good separation with efficient identification. It allowed rapid simultaneous profiling and quantitation of >100 SMG structures.[69] A recent publication in 2013 described a graphitic carbon high-performance liquid chromatography-mass spectrometry method, designed to resolve and quantify neutral oligosaccharides simultaneously.[70] The technique provides sensitive, precise, and accurate quantitation and is suitable for the analysis of large numbers of milk samples; 2′-FL is well resolved in this method. However, it is worth mentioning that techniques used in many laboratories include several preparative steps that may involve partial loss of the sample. Therefore, data regarding "relative quantities" of HMOs should be interpreted with caution. Ideally, a method reducing or quantifying the losses would be most advisable.

## PRESENCE OF 2′-FL IN NON-HUMAN MAMMALS

Some SMGs present in human milk are also found in milk of other mammals; for example, the sialyllactoses 3′-SL and 6′-SL are also present in murine and bovine milk and have been detected in bovine milk-based infant formula.[71] In contrast, 2′-FL is either absent or present in low amounts in bovine and murine milk but appears early in mammalian phylogeny in monotremes. Table 2[11,12,19,61–63,69,72–102] shows a summary of small molecular weight fucosylated oligosaccharides found in milk from different subclasses of mammals. At least three factors have to be taken into account when considering the data in this table: 1) in most cases only a few samples of animal milk per species or subspecies have been analyzed; 2) it is unknown if the oligosaccharide repertoires in non-human animals are as susceptible to individual variation as oligosaccharide profiles in human milk; and 3) different analytical methods have been used to determine oligosaccharide content in non-human milk. Although the purpose of this review is to explore the potential biological roles of 2′-FL in human health, it is pertinent to review its natural occurrence to have a sense of its prevalence and perhaps its importance through mammalian evolution.

### Monotremes (subclass Prototheria) and marsupials (subclass Metatheria)

Monotremes are regarded as primitive mammals because they lay eggs and possess other reptile-like features. Their milk has been the focus of interest precisely because of the unique transitional nature of these animals; their soluble glycans can be viewed as a record of milk composition from the time in which mammals initiated their differentiation from egg-laying species. Monotreme milk differs from that of marsupials and placental mammals, mainly because of its high content of fucosylated structures. Free lactose was found to be a minor component of both echidna and platypus milk.[72] The major oligosaccharides of echidna milk are 2′-FL and difucosyllactose (DFL), while DFL is abundant in platypus milk. Fucosylated oligosaccharides usually predominate over lactose during early to mid-lactation.[73] Milk samples from several marsupials have been analyzed and no fucosylated oligosaccharides have been detected.[76,103]

### Placental mammals (subclass Eutheria)

The literature reports several studies on SMGs of eutherian species, although the emphasis has been on the analysis of milk of domestic animals. Infants have historically been fed either milk of domestic herbivorous animals or formulas made with components of these animals'

*Table 2* **Distribution of the small fucosylated SMGs in milk.**

| Subclass | Order | Family | Common name | 2′-FL[a] | 3-FL[a] | DFL[a] | Reference no. |
|---|---|---|---|---|---|---|---|
| Prototheria | Monotremes | Tachyglossidae | Echidna | ✓ | | ✓ | 72 |
| | | Ornithorhynchidae | Platypus | | | ✓ | 72–74 |
| Metatheria | Marsupials | Macropodiae | Kangaroo | | | | 75 |
| | | | Wallaby | | | | 76–78 |
| | | Phalangeridae | Possum | | | | 79 |
| Eutheria | Artiodactyla | Bovidae | Domestic cow | | | | 80 |
| | | | Domestic goat | ✓ | | | 81–83 |
| | | Suidae | Domestic pig | ✓ | | ✓ | 84 |
| | | Camelidae | Bactrian camel | | ✓ | | 85 |
| | Carnivora | Ursidae | Ezo brown bear | ✓ | | | 86 |
| | | | Japanese black bear | ✓ | | | 62 |
| | | | Polar bear | ✓ | | | 63 |
| | | Procyonidae | White-nosed coati | ✓ | | | 87 |
| | | Canidae | Dog | ✓ | | | 88 |
| | | Mustelidae | Mink | | | | 89 |
| | | Phocidae | Hooded seal | ✓ | | | 90 |
| | | | Artic harbor seal | ✓ | | | 91 |
| | | | Bearded seal | ✓ | | | 92 |
| | | | Crabeater seal | ✓ | | | 61 |
| | | Felidae | African lion | ✓ | | | 93 |
| | | Hyaenidae | Spotted hyena | ✓ | | | 94 |
| | Rodentia | Muridae | Mouse | | ✓ | | 19 |
| | Proboscidea | Elephantidae | Asian elephant | | ✓ | | 95,96 |
| | Cetacea | Balaenopteridae | Minke whale | ✓ | | | 97 |
| | Primates | Daubentonidae | Aye Aye | | ✓ | ✓ | 98 |
| | | Lemuridae | Madagascar lemur | | ✓ | | 98 |
| | | Cebidae | Tufted capuchin | | ✓ | | 99,100 |
| | | Cercopithecidae | Rhesus macaque | | ✓ | | 99,101 |
| | | | Toque macaque | | ✓ | | 99 |
| | | | Baboon | | ✓ | | 99 |
| | | Hominidae | Chimpanzee | ✓ | ✓ | | 101,102 |
| | | | Bonobo | ✓ | ✓ | | 102 |
| | | | Orangutan | ✓ | | | 101,102 |
| | | | Human | ✓ | ✓ | ✓ | 11,12,69 |

[a] Blank cells indicate has not been detected.
*Symbol*: ✓, present.

milk. Oligosaccharides in the milk of domestic commercial animals are present in low concentrations and are less complex in structure than those of other mammals. Linkages to fucose are usually rare, and sialyloligosaccharides are dominant.[104] For example, no fucosylated oligosaccharides have been found in bovine milk, with the exception of a recent report that reveals the presence of trace amounts of 2′-FL.[105] In contrast, 70% of bovine milk oligosaccharides are sialylated, which is also true for ovine milk.[106] Similar to bovine milk, porcine SMGs are mainly sialylated oligosaccharides, although six fucosylated structures have been identified, including 2′-FL.[84] While it has been previously reported that 2′-FL is present in goat's milk and colostrum,[81,82] a recent report indicates that no fucosyloligosaccharides are detectable using fast atom bombardment mass spectrometry.[83] As discussed above, differences could be due to the analytical techniques used or individual variations amongst specimens. A recent study that characterized soluble glycans in camel milk, found that colostrum has a particularly high concentration of oligosaccharides and 3-FL was detected.[85] 2′-FL has also been detected in milk from several bear species,[62,63,86] and the dominant oligosaccharide in coati milk is precisely 2′-FL.[87] Fucosylated soluble glycans such as 3-FL, LNFP I, and DFLNH II (Table 1) were found in elephant milk,[95] but 2′-FL was absent.

Primate milk generally contains lower amounts of soluble glycans other than lactose and their oligosaccharide structures are less complex than their human milk counterparts. Compared to the more than 200 different soluble glycans that have been detected in human milk,[66] around 130 structures have been identified in chimpanzee and 50 in gorilla,[101] while only 30–40 have been found in porcine and bovine milk.[80,84] Fucosylation varies greatly among primates; 50% of chimpanzee milk structures are fucosylated, being the closest to human

milk in this regard. Some authors speculate about the importance of fucosylation in primate lactation and propose that a correlation between SMG patterns and social interactions exists. Primates such as chimpanzees, who live in large social groups, would have a strong pathogenic pressure that would favor immunological adaptations, including an important role for oligosaccharides with anti-infective and prebiotic functions.[101] In studies on milk oligosaccharides in apes, performed by Urashima et al.,[102] 2′-FL was detected in chimpanzee, bonobo, and gorilla milk, as well as gorilla colostrum, while 3-FL was found in chimpanzee, bonobo, and orangutan milk. Apparently, contradicting data were later published by Tao et al.,[101] indicating that neither 2′-FL nor 3-FL was detected in chimpanzee, gorilla, or rhesus milk. Since inter-individual variation in milk oligosaccharides has been observed in humans,[27] and some histo-blood group polymorphisms in humans seem to be inherited from great apes,[107] it is possible that some primates also reflect their genetically determined glycosyltransferase repertoire in their milk oligosaccharide profile.

## PRESENCE OF 2′-FL IN HUMAN MILK

Developments in instrumentation and analytical techniques have resulted in accurate quantification of many oligosaccharides, facilitating the study of variation across the population and during lactation.[30,67] While the following discussion on variability encompasses HMO in general, emphasis is given to fucosylated oligosaccharides and to other structures that vary concomitantly or antagonistically with 2′-FL.

The major source of variation in HMOs among individuals is the Secretor and Lewis status of milk donors.[10,27,30] Distribution of these phenotypes varies among populations[11,12] and expression changes over the course of lactation.[10,108] Recently, HMO monitoring of individual samples using high-throughput methods has confirmed that the proportion of different oligosaccharide structures varies among individual milk samples. Variations found in MALDI-TOF-MS profile spectra confirmed that each lactating woman expresses an individual HMO pattern, even though it is possible to assign the majority of the HMO profiles to the different Lewis histo-blood system groups.[30]

Determining variations is relevant in pediatric nutrition if the different biological functions associated with particular HMO structures are taken into account.[20,109]

## VARIABILITY AMONG GEOGRAPHIC POPULATIONS

A number of studies over the past 14 years have been performed to gain insight into the variability of HMOs in different populations. More than 400 human milk samples from women living in 10 different countries (Chile, China, France, Germany, Italy, Mexico, the Philippines, Singapore, Sweden, and the United States) were analyzed by Erney et al.[11] Donors were grouped based on their national origins, and general variation trends were dependent on the donor's geographical origin. The presence of 2′-FL in milk provides a good basis to assign Secretor status. 2′-FL was found in 100% of the samples from Mexico, but only in 46% of the samples from the Philippines (Figure 5A). Based on the presence of 2′-FL in milk, 85% of the study population could be classified as Secretors. With regard to 3-FL, there were no major differences among the samples from different countries, with 3-FL being present in more than 96% of the analyzed specimens.[11] When taking into account averages by continent, the most abundant sugar in all samples was 2′-FL (2.38 g/L average); in samples where 2′-FL was absent, 3-FL was present in the highest concentration. 3-FL showed the greatest interregional variability, with averages ranging from 1.84 g/L in the samples from the United States, to 0.76 g/L in the Latin American samples (Figure 5B).[11] This finding is consistent with a synthesis process in which enzymes compete for the same substrates and the absence of one enzyme results in the availability of substrate for the other with the concomitant synthesis of distinctive structures. It should be noted that glycan synthesis not only has the absolute requirement of glycosyltransferases but that these enzymes have a discrete intracellular localization with simultaneous or sequential access to pools of transient saccharides that move from the endoplasmic reticulum to the Golgi apparatus on their way to the external cell membrane.[110] Results from Erney's study reconfirm that the *Se* gene is more prevalent in some geographical regions than others (Figure 6).[11,12,111–117] Musumeci et al.[111] undertook a study on the colostrum of 53 Burkinabe women and 50 Italian women. Both groups were found to have a comparable percentage of the Secretor genotype and 2′-FL was found to be the most abundant and prevalent oligosaccharide in the colostrum of Burkinabe women. This pattern was inverted in Italian women, in whom 2′-FL showed a delayed increase in concentration. Thurl et al.[12] analyzed 175 milk samples from 30 German women during the first 3 months of lactation and classified them into four groups according to their Lewis (Le) and Secretor (Se) status and the concomitant SMG profiles (Figure 4). 2′-FL was found to be the major oligosaccharide in the samples of milk groups 1 (Le+Se+) and 3 (Le-Se+) and the highest concentration of 2′-FL was found in group 3, which corroborates earlier reports.[10,11,108,109] The concentration of 2′-FL in Secretor milk decreased during the study period to roughly half of the initial concentration.[12] Non-Secretors from milk group 2 (Le+Se-) synthesized

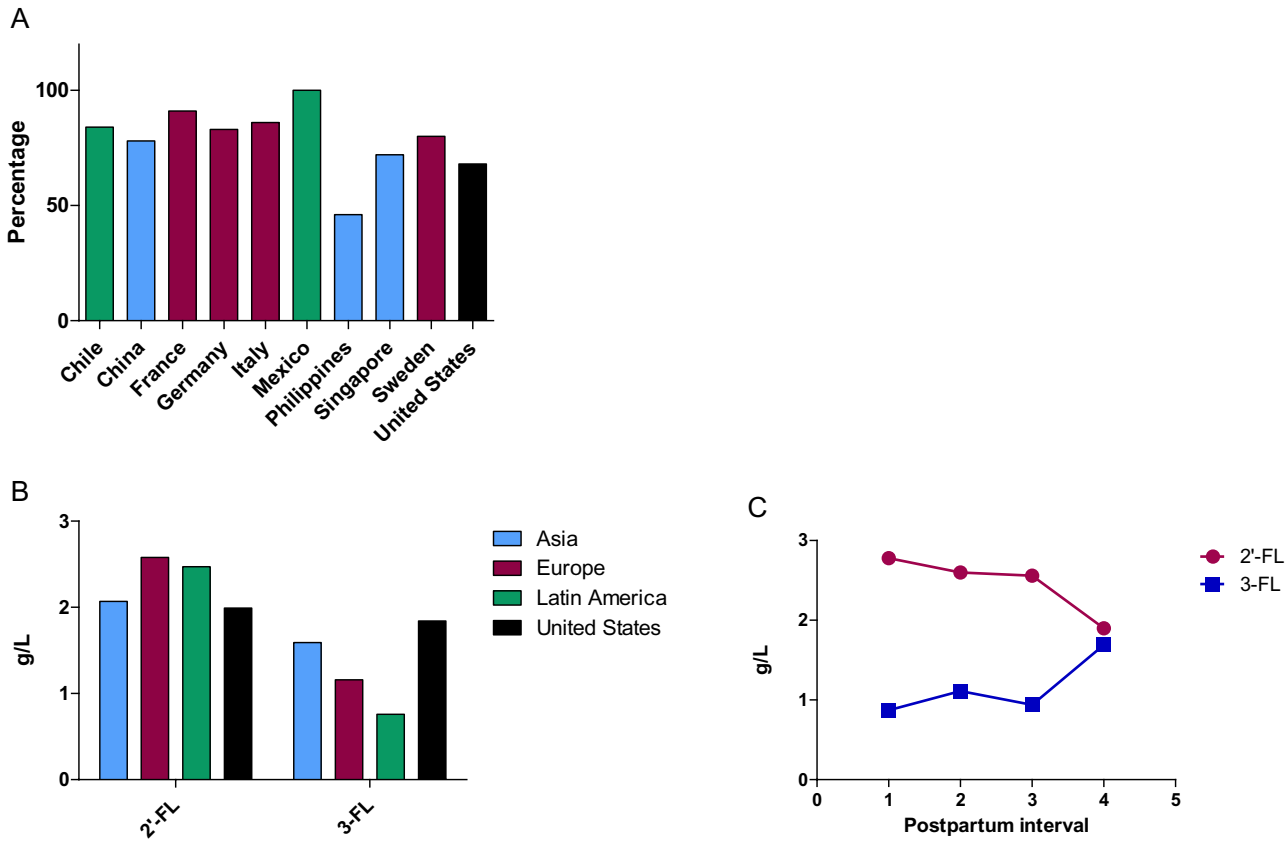


*Figure 5* **Concentration of 2′-FL in human milk by region and postpartum interval. A)** Percentage of donors per country producing 2′-FL. B) Mean concentration of 2′-FL and 3-FL by region. (Latin America: Chile and Mexico; Europe: France, Germany, and Italy; Asia: China, Philippines, and Singapore). C) Mean concentration of 2′-FL and 3-FL by postpartum interval (1: 0–2 days; 2: 3–10 days; 3: 11–30 days; 4: 31–452 days).
Adapted from Erney et al.[11]

3-FL predominantly.[12] None of the 30 women in the study had an oligosaccharide profile without α1-2- and α1-4-linked fucosyloligosaccharides, as would correspond to Le-Se- individuals, categorized as group 4 (Figure 4). This is consistent with the low prevalence (1%) of the Le-Se- genotype in Caucasian women.[118] Studies on the milk of Japanese women by Sumiyoshi et al.[112] found LNFP I to be the most abundant oligosaccharide, followed by 2′-FL and DFL, although no histoblood group testing was performed to corroborate this finding. Conversely, milk from Samoan women had particularly low concentrations of 2′-FL;[113] previous publications showed that "weak" Secretor individuals, expressing both Lewis a ($Le^a$) and Lewis b ($Le^b$) antigens, were common in Polynesia. There is a high frequency of non-Secretors in Europe (20%) and a low frequency of non-Secretors in the Mexican Mestizo population (1%).[11,109,111,118] Thurl et al.[12] speculate that the high incidence of Secretors evolved among the Mexican population as a result of pathogenic *Escherichia coli* species, since babies fed with milk from Secretor mothers have a lower risk of infection by stable-toxin enterotoxigenic *E. coli*.[109] In other countries or regions with different evolutionary pressures from pathogens, Secretors would not provide advantages to their newborns, thus leading to a higher prevalence of non-Secretors. There are significant differences in the concentration values obtained by different research groups for several milk oligosaccharides, i.e., 2′-FL and 3-FL (Table 3).[29,70] A plausible explanation for the variations in the quantitative results may be the different analytical methods used in the studies.[29] Nevertheless, the evidence indicates that milk from different mothers may differ both qualitatively and quantitatively in its oligosaccharide content,[10–12] and 2′-FL is not an exception.

## VARIABILITY OVER THE COURSE OF LACTATION

Patterns of milk oligosaccharides also change during the course of lactation, as several studies have shown.[10,108] 2′-FL was found to be the most abundant oligosaccharide early in lactation, maintaining stable levels in the first

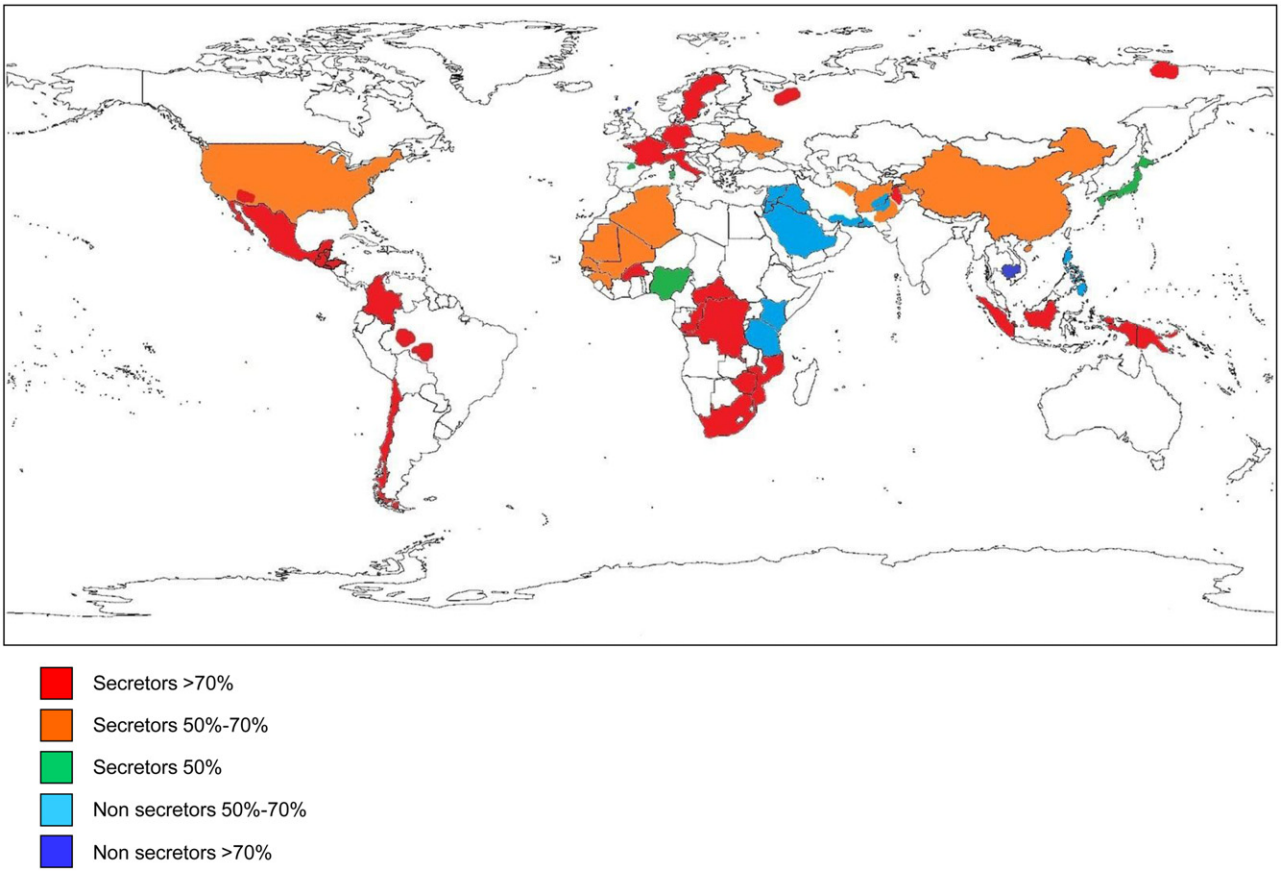


*Figure 6* **Geographical distribution of Secretor and non-Secretor phenotypes.** Each color represents a range of frequencies of the regions with the most common phenotype. Data was compiled from a variety of studies on oligosaccharides in milk from women in different populations,[11,12,111–115] and from studies on *FUT2* polymorphisms.[116,117] Note that different patterns may appear in isolated populations.

month postpartum.[11] Thereafter, there was a reduction in its concentration, which was more pronounced by 1 year of lactation. 3-FL increased over the same period of time (Figure 5C).[10,11] The decrease in 2′-FL during lactation was also observed in subsequent studies.[12,112,114] When monitoring hundreds of structures, little variation in the total oligosaccharide content during the first week of lactation was found. For each individual, however, small variations appeared in HMOs during the first 2–3 months. While each donor was internally consistent, differences among donors were apparent.[67] Changes in oligosaccharide content during the second 6 months of lactation were also observed. In Secretor donors, the ratio of α1-2- and α1-3/4-linked fucosyloligosaccharides approached parity by 1 year. In one case, a putative non-Secretor donor produced milk initially devoid of α1-2-linked fucosyloligosaccharides, but synthesized some structures late in lactation.[10] Analytical techniques continue to improve and provide more accurate measurement of HMOs. Bao et al.[70] have developed a liquid chromatography-mass spectrometry method that allows measurement of individual differences in HMO patterns, as well as variations during lactation with an exceptional degree of precision and accuracy. This will provide a useful tool to study variation in larger human cohorts in the future.

## Preterm milk

Milk obtained from mothers after preterm delivery differs from milk produced by mothers after term delivery with regard to protein, fat, lactose, and mineral content as well as concentrations of other molecules. Differences prevail for around 8 weeks after birth, suggesting adjustments in lactation related to earlier delivery.[120] Early studies on preterm milk composition suggested that colostrum contained a higher amount of SMG than term milk, with the total amount decreasing over the course of lactation.[121] Later, Gabrielli et al.[115] studied differences in oligosaccharide composition according to histo-blood group status in preterm milk and corroborated these observations. It has been postulated that HMOs reduce

*Table 3* **Mean concentrations (g/L) of small SMGs identified in different studies.**

| Reference | Days of lactation | 2′-FL | 3-FL | DFL |
|---|---|---|---|---|
| Kunz et al.[95a] | ND | 0.45 ± 0.43 | 0.07 ± 0.08 | |
| Coppa et al.[108a] | d 4 | 3.93 ± 1.11 | 0.34 ± 0.06 | |
| Coppa et al.[108a] | d 60 | 1.84 ± 0.39 | 0.71 ± 0.07 | |
| Erney et al.[11b] | d 3–10[d] | 2.6 | 1.11 | 0.59 |
| Erney et al.[11b] | d 31–450[d] | 1.9 | 1.69 | 0.47 |
| Chaturvedi et al.[10a] | ND | 2.43 ± 0.26 | 0.86 ± 0.10 | 0.43 ± 0.04 |
| Sumiyoshi et al.[112c] | d 10 | 0–3.45[g] | 0.003–0.766 | |
| Musumeci et al.[111] | d 2 | 4.5 ± 3.2[e]<br>2.1 ± 1.4[f] | | |
| Asakuma et al.[114a] | d 2 | 2.01 ± 1.07 | 0.28 ± 0.26 | 0.28 ± 0.30 |
| Thurl et al.[12b] | d 8 | 3.37 | 0.26 | 0.33 |
| Thurl et al.[12b] | d 30 | 2.96 | 0.42 | 0.37 |
| Leo et al.[113a] | d 5–10 | 0.22 ± 0.37 | 1.67 ± 0.82 | 0.07 ± 0.06 |
| Bao et al.[70c] | d 3–29 | 0.006–1.36 | 0.03–1.34 | |

*Abbreviation*: ND, not determined – reference did not specify the time of sample collection.
[a] Values from these studies are mean values ± SD.
[b] Indicates data expressed as mean concentration values.
[c] Data are represented as ranges.
[d] Mean concentration values of the different regions studied in that specific post-partum interval.
[e] Values corresponding to Burkinabe women.
[f] Values corresponding to Italian women.
[g] Values correspond to 2′-FL and DFL together.
Adapted from Bao et al.[70] and Urashima et al.[29]

the risk of necrotizing enterocolitis, either promoting the growth and metabolism of beneficial bacteria, blocking the adhesion of pathogens to intestinal cells, and/or modulating the inflammatory response.[122] This would highlight the importance of breastfeeding for preterm infants, who are at higher risk of contracting infective illnesses due to an underdeveloped immune system. It has even been suggested that pooled milk be fed to preterm infants, rather than milk from a single donor, as this could increase the probability of providing bioactive structures to these infants.[115] In contrast with previous accounts, a recent publication by De Leoz et al.[68] suggests that preterm milk samples have a less diverse SMG repertoire than term milk. Moreover, these researchers observed fluctuations in fucosylation in preterm samples, and found a trend of relatively low content of 2′-FL. They concluded that fucosylation is not well regulated in women delivering preterm and speculated about the implications for the premature infant.[68]

## FUNCTIONS AND PROPERTIES OF 2′-FL

During the last decade, HMOs have attracted interest as several research groups have probed their nutritional properties and their roles as bioactive substances. Consequently, an increasing amount of evidence supporting the potential beneficial effects of HMOs has been reported[14,122] and several hypotheses for mechanisms of action have been postulated. These mechanisms may be separated into two different groups: direct effect on the infant by interacting with cells and tissues and indirect effects by interacting with beneficial microbiota and pathogens.

### Absorption and distribution data

Direct interaction of SMG with human cells can occur in the lumen of the gastrointestinal tract or systemically after absorption and/or translocation into the circulation. It has been demonstrated that fructooligosaccharides (FOS) composed of three and four monosaccharides can be found in the urine of infants fed formula containing 3 g/L of non-reducing FOS[18]; this implies that a fraction of these gained access to the plasma prior to urinary excretion. Intact SMG, including 2′-FL and 3-FL, have been detected in the urine of breastfed infants,[123] while these glycans are absent in the urine of formula-fed infants. This evidence strongly suggests that SMG are absorbed and reach systemic circulation. It has been hypothesized that soluble glycans from human milk found in the urine of breastfed infants could prevent infections in the urinary tract, as reported by Martin-Sosa et al.[124] Although the presence of 2′-FL in urine was first described by Lundbland et al.,[125] a recent report by Rudloff et al.[126] conclusively demonstrates that some HMOs reach the systemic circulation. These researchers designed a set of in vivo studies to investigate the fate of milk oligosaccharides by providing a lactating mother with a bolus of $^{13}$C-Gal and measuring the appearance

of $^{13}C$- labeled HMO in the urine of her breastfed infant.[123,127] There are limited data on the absorption, distribution, metabolism, and excretion of non-digestible soluble glycans, although a published study demonstrates that a trisaccharide is quickly cleared from the plasma into a depot compartment and that the bulk is cleared precisely through the urine.[128] This was true for intravenous and oral administration of the synthetic soluble glycan globotriose. The prebiotic effect attributed to milk oligosaccharides is consistent with the fact that these glycans can be found in the feces of breastfed infants, thus demonstrating that they are not digestible in vivo.[21,22] This was verified by in vitro studies that showed that 2′-FL is resistant to the action of enzyme preparations of human and porcine brush border membranes and pancreas.[21,22] Soluble glycan profiles from feces of breastfed infants resemble those of their mother's milk; for example, 2′-FL and 3-FL have been detected in feces of breastfed babies whose mother's milk contained these fucosylated glycans.[23,129,130] Conversely, oligosaccharides found in feces of formula-fed infants were only present in small amounts and were structurally different from their human milk counterparts. It is speculated that these oligosaccharides originated from bacteria or are the result of remodeling of intestinal glycoconjugates.[131,132] More recent studies with capillary electrophoresis, laser-induced fluorescence detection, and mass spectrometry have confirmed those previous observations. FL was reported to be the most abundant glycan in both fecal extracts and breast milk.[23] In this study, 3-FL and 2′-FL were identified as a group since both isomers were not resolved. Two main mechanisms have been proposed for the exertion of beneficial effects of SMG through their interactions with microbes: HMOs can act as soluble decoys that emulate cell surface glycans preventing bacterial binding to intestinal cells,[20] and they could also function as prebiotics by promoting the growth of beneficial microbiota in the gut.[133] To exert such effects, it is required that HMOs remain undigested in the upper intestinal tract, and gain access to the lower parts of the intestine, where they may be metabolized by the intestinal microbiota or excreted in the feces.[21,22] Taken together, results from fecal analyses of breastfed infants are consistent with the availability of 2′-FL to support and maintain colonization with beneficial bacteria. Albrecht et al.[24,134] went further by monitoring oligosaccharide patterns in breastfed babies together with the respective breast milk structures up to 6 months after birth. They proposed several stages for HMO processing and degradation, depending on infant age, blood group, and feeding regimen. In addition, 1–2% has been reported to be excreted with the infant's urine,[123,126] which implies either absorption or passive systemic translocation of several hundred milligrams of oligosaccharides per day.

## Anti-infective properties

Infections by bacteria, viruses, and protozoa still remain the most common causes of infant mortality. Many of these pathogens need to attach to specific glycans on the cell surface as a first step to infect the host and cause disease. Pathogens usually use carbohydrate-binding proteins called adhesins or microbial lectins to bind to cell surface glycans. HMOs are either identical to or resemble host cell surface glycans and may act as soluble decoy receptors preventing pathogen binding and reducing the risk of infection.[13,14] Some of the HMOs that inhibit pathogen binding include fucosylated glycans. A low content of fucosylated HMOs is associated with increased risk of diarrhea in breastfed infants.[49] The most extensive documentation on the potential role of HMOs as anti-adhesive antimicrobials pertains to studies on *Campylobacter jejuni* infections,[20,49] one of the most common causes of bacterial diarrhea and infant mortality. The first step for *Campylobacter* infection is binding to human intestinal epithelium by recognizing the H-2 antigen Fucα1-2Galβ1-4GlcNAc-R. In vitro, ex vivo, and in vivo experiments performed by Ruiz-Palacios et al.[20] led to the conclusion that α1-2-fucosylated HMOs, and 2′-FL in particular, can inhibit or prevent *Campylobacter* infection or colonization. These results were further supported by a study with 93 breastfeeding mother-infant pairs among whom the incidence of diarrhea was lower in infants who received 2′-FL from their mother's milk.[49] It should be noted that there are other glycans in milk that contain the Fucα1-2 epitope besides 2′-FL. At a minimum, there are glycoproteins that can be detected by *Ulex europaeus* agglutinin 1.[34] The authors were not able to distinguish if the observed effects were attributed to an SMG or other types of glycoconjugates. Cell surface carbohydrates not only function as targets for pathogen adhesion, they are also targets for microbial toxins.[135,136] Stable-toxin from enterotoxigenic *E. coli* is also inhibited by 2-linked fucosylated oligosaccharides.[109]

Some microorganisms are decorated with glycans that bind to host lectins, thus providing an additional mechanism to bind to their target cells. This is particularly relevant in light of the prevalence of lectins in human tissues.[137] Such is the case of the envelope glycoprotein gp120, which is involved in the binding of human immunodeficiency virus (HIV) to DC-SIGN (dendritic cell-specific ICAM3-grabbing non-integrin) on human dendritic cells.[138] DC-SIGN has a high affinity for the glycans with Lewis antigens.[139] Therefore, Lewis antigen-containing HMOs could prevent HIV from binding to DC-SIGN in the breastfed infant, as they do in vitro.[140] This may explain why mother-to-child transmission through breastfeeding is quite inefficient, with the majority of infants not acquiring HIV, despite being

exposed to the virus in their mother's milk.[141] Bode et al.[142] have found that higher concentrations of SMG in the mother's milk are associated with protection against postnatal HIV transmission. Antimicrobial effects of HMOs may not only be relevant to enteric infections; it has also been shown that breastfed infants are less likely to develop otitis media caused by *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, or *Haemophilus influenzae*.[143,144] 2′-FL and other fucosylated HMOs may prevent certain pathogens from binding to their host cell receptors, thereby protecting the infant not only from intestinal, but also from upper respiratory and urinary tract infections. In some cases, preclinical data strongly suggest a correlation between the presence of this oligosaccharide and protection from disease.

## Immunity

As stated above, the presence of HMOs (including 2′-FL) in the urine of breastfed infants is indirect evidence of their presence in the systemic circulation. The ability of HMOs to exert direct effects on the immune system was tested by Eiwegger et al.,[145] who found that sialylated HMOs stimulated T cells toward increased production of IFN-γ (Th1), and decreased production of IL-4 (Th2) cytokines. There are different ways in which HMOs may influence immune responses; for example, pooled acidic HMOs are able to reduce selectin-mediated cell-cell interactions.[146,147] This suggests that specific HMOs may have anti-inflammatory roles, which contribute to reducing the incidence of inflammatory diseases in breastfed infants. Several reports on 2′-FL and its potential interactions with the immune system have been submitted for publication, but the literature is currently sparse in this regard.

## Prebiotics

Among the broad range of functions attributed to SMGs, probably the best known and most referenced is their role as prebiotics to promote intestinal colonization by a healthy gut microbiota.[13,122,133,148,149] Since HMOs are resistant to digestion in the infant gastrointestinal tract,[21,22] the vast majority reach the distal small intestine and colon intact. Once there, they may contribute to the establishment of a protective microbiota by favoring the growth of beneficial microorganisms, therefore behaving as typical prebiotics.[150] It is worth taking into account that the vast majority of data regarding the prebiotic effect of HMOs derives from in vitro fermentation studies, with bacteria isolated from infant feces. Evidence indicates that only certain bacterial species and strains have developed strategies for sustaining growth and promoting metabolic activity by fermenting soluble glycans of human milk.[151,152] Among the bacteria tested in vitro, only some *Bifidobacterium* and *Bacteroides* species were able to consume HMOs as the sole carbon source to reach high cell densities.[153,154] At least two bacterial adaptations are necessary to effectively use the soluble glycans present in human milk: transport systems to import intact glycans for internal use and/or glycosidases to hydrolyze glycans into its constituent monosaccharides. To hydrolyze milk oligosaccharides, four types of glycosidases are needed: α-fucosydase, α-sialidase, β-galactosidase, and β-N-acetylhexosaminidase.[155] In addition, some bacterial species preferentially import intact low-molecular-weight HMOs, while others export glycosidases for external hydrolysis of soluble glycans.[154,156] Genomes from various strains may harbor a diverse number of glycosidase-related genes.[153] For example, sequencing the genome of *Bifidobacterium longum* subsp. *infantis* revealed a 43-kb gene cluster controlling the expression of glycosidases but also sugar transporters and glycan-binding proteins likely linked to HMO metabolism. This particular cluster is not found in other bifidobacteria species and explains why this subspecies is unusually efficient at metabolizing human SMG.[149,156] Consistent with the presence of the above-mentioned gene cluster, several groups have shown that *B. longum* subsp. *infantis*, a common member of the intestinal microbiota of breastfed infants, grows well with SMG as the sole carbohydrate source.[153,157] This strain shows a preference for short-chain soluble glycans, including 2′-FL and 3-FL. All human SMGs disappear from the external milieu to an equal extent, which suggests that these are transported into the bacteria in their intact forms.[157] *B. bifidum* grows slightly slower on SMG, hydrolyzing mainly 2′-FL and lacto-*N*-tetraose while leaving behind monosaccharides that can be shared with other bacteria in a symbiotic relationship.[157] *B. longum* subsp. *longum* and *B. breve* hardly grow on HMOs and only metabolize lacto-*N*-tetraose.[157] There is a preferential usage of type I HMOs by bifidobacteria, except for *B. longum* subsp. *infantis*, which incorporates both type I and type II HMOs.[157] The predominance of *B. longum* subsp. *infantis* may contribute to lower the numbers of pathogenic bacteria simply by preferentially consuming HMOs and removing them from the intestinal milieu. In addition *B. longum* subsp. *infantis* and other infant-associated bacteria produce short-chain fatty acids and other metabolites that create an environment favoring the growth of beneficial bacteria over potential pathogens.[158] A recent study identified 2′-FL, DFL, and 3-FL as the major components consumed by *B. longum* subsp. *infantis* in vitro. Their catabolism produced lactate and short-chain fatty acids, which resulted in significant pH reduction. This, in turn, reduced the growth of putative pathogenic bacteria, such as *E. coli* and *Clostridium perfringens*, therefore confirming the prebiotic effect of these glycans.[16]

## CONSIDERATIONS

For more than two decades, reports on the biological activities and properties of soluble glycans present in human milk have suggested that their biosynthesis, which implies energy expenditure for a lactating mother, is biologically justified by the benefits that they conferred to breastfed infants. The present literature review emerged from the need to study and account for the properties and postulated effects of single free, soluble glycans of human milk, as opposed to the entire molecular class constituted by human milk oligosaccharides. By discerning the roles of individual molecular species, it may be possible to understand how infants are affected by the presence or absence of particular structures and explore potential uses in health applications outside of the realm of pediatric nutrition. Some aspects of 2′-FL biochemistry and biology are intriguing. Human milk is recognized as the gold standard of infant nutrition and its most abundant soluble glycan is absent in the milk of more than 20% of mothers. Not all human milk is equivalent with respect to glycan content, which suggests that there are different gold standards, as human milk is not a homogeneous biological fluid. If variability of 2′-FL content among milk samples from different women is intriguing, the fact that some animals have it in their milk and others don't is equally interesting.

Soluble glycan analysis of milk from monotremes revealed that fucosyllactoses are the dominant structures in these primordial mammals. If this was true at the onset of mammalian differentiation, then fucosyllactoses could have been the first free soluble glycans in milk of any species. Since intestinal fucosidases that would be able to digest fucosylated glycans have not been found, it is conceivable that these glycans had biological activities that were different from being energy sources for the suckling offspring. It is also fascinating that 2′-FL is present in the milk of some species and ostensibly absent from others. It could be argued that both the expression of FUT2 (or its equivalent in other species) and its cellular topology affect the synthesis of soluble glycans. If this was true, one could detect α1-2 fucosylated glycoproteins in Western blots of milk proteins even in the absence of 2′-FL. However, in our laboratory 2′-FL has only been found in milk that also contains glycoproteins decorated with Fucα1-2 glycans.[19,34] This has been true for samples from human, mouse, rabbit, and cow milk and suggests that the expression of a fucosyltransferase is sufficient to generate free soluble glycans and milk glycoproteins. In humans, when 2′-FL is present, it is the most abundant milk glycan. Non-Secretor mothers lack 2′-FL, but they also lack the concomitant fucosylation of milk glycoproteins. From the evidence analyzed in the present review, the role of 2′-FL as a *sui generis* prebiotic seems to be a plausible one; denying the readily available energy of lactose, by making it indigestible, and the additional energy investment of adding an additional monosaccharide (fucose)- would make sense if that energy were to be available for symbiotic species that protect the breastfed infant. Perhaps milk from ruminants better supports the needs of calves, which require a very different microflora and microfauna to digest cellulose, while monogastric animals require promoters of microflora consistent with ancestral diet patterns. Another particular trait of 2′-FL is the diversity of biological activities ascribed to it.

## CONCLUSION

Healthy skepticism is inevitable considering that several groups have found dissimilar biological activities for 2′-FL. It is speculated that this oligosaccharide is unique in terms of its degrees of freedom when compared to other Fucα1-2 glycans. The fact that lactose is used as substrate of FUT2 when its preferred substrates are type I structures would support this notion. As larger amounts of 2′-FL become available, the collegial efforts of researchers will allow them to discern whether some of the observed effects are robust and reproducible.

It is now possible to synthesize oligosaccharides on a large scale,[18,19,159,160] which will allow this oligosaccharide to be tested in clinical trials and in extensive animal and in vitro studies. This is expected to corroborate and provide support to the previously described roles of 2′-FL as soluble decoys and a prebiotic and may shed light on new functions yet to be described. Perhaps 2′-FL will later be viewed as the tip of the scientific iceberg that motivated researchers to further probe the nature of human milk glycans; in the near future, it may also provide incentive to explore the natural roles and design applications of human milk glycans to promote human health.

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## REFERENCES

1. Jensen RG. *Handbook of Milk Composition*. San Diego, CA: Academic Press; 1995.

2. Jenness R. The composition of human milk. Semin Perinatol. 1979;3:225–239.
3. Coveney J, Darnton-Hill I. Goat's milk and infant feeding. Med J Aust. 1985;143:508–510.
4. Jirillo F, Jirillo E, Magrone T. Donkey's and goat's milk consumption and benefits to human health with special reference to the inflammatory status. Curr Pharm Des. 2010;16:859–863.
5. McJarrow P, Schnell N, Jumpsen J, et al. Influence of dietary gangliosides on neonatal brain development. Nutr Rev. 2009;67:451–463.
6. Willatts P, Forsyth JS. The role of long-chain polyunsaturated fatty acids in infant cognitive development. Prostaglandins Leukot Essent Fatty Acids. 2000;63:95–100.
7. Niers L, Stasse-Wolthuis M, Rombouts FM, et al. Nutritional support for the infant's immune system. Nutr Rev. 2007;65:347–360.
8. Newburg DS, Neubauer SH. Carbohydrates in milks: analysis, quantities and significance. In: Jensen RG, ed. *Handbook of Milk Composition*. San Diego, CA: Academic Press; 1995:273–349.
9. Fox FP, McSweeney PLH. *Dairy Chemistry and Biochemistry*. New York: Kluwer Academic/Plenum Publishers; 1998.
10. Chaturvedi P, Warren CD, Altaye M, et al. Fucosylated human milk oligosaccharides vary between individuals and over the course of lactation. Glycobiology. 2001;11:365–372.
11. Erney RM, Malone WT, Skelding MB, et al. Variability of human milk neutral oligosaccharides in a diverse population. J Pediatr Gastroenterol Nutr. 2000;30:181–192.
12. Thurl S, Munzert M, Henker J, et al. Variation of human milk oligosaccharides in relation to milk groups and lactational periods. Br J Nutr. 2010;104:1261–1271.
13. Kunz C, Rudloff S, Baier W, et al. Oligosaccharides in human milk: structural, functional, and metabolic aspects. Annu Rev Nutr. 2000;20:699–722.
14. Newburg DS, Ruiz-Palacios GM, Morrow AL. Human milk glycans protect infants against enteric pathogens. Annu Rev Nutr. 2005;25:37–58.
15. Ruiz-Moyano S, Totten SM, Garrido D, et al. Variation in consumption of human milk oligosaccharides by infant-gut associated strains of *Bifidobacterium breve*. Appl Environ Microbiol. 2013;79:6040–6049.
16. Yu ZT, Chen C, Kling DE, et al. The principal fucosylated oligosaccharides of human milk exhibit prebiotic properties on cultured infant microbiota. Glycobiology. 2013;23:169–177.
17. Urashima T, Saito T, Nakamura T, et al. Oligosaccharides of milk and colostrum in non-human mammals. Glycoconj J. 2001;18:357–371.
18. Prieto PA. In vitro and clinical experiences with a human milk oligosaccharide, Lacto-N-*neo*Tretraose, and fructooligosaccharides. FFI J Jpn. 2005;210:1018–1030.
19. Prieto PA, Mukerji P, Kelder B, et al. Remodeling of mouse milk glycoconjugates by transgenic expression of a human glycosyltransferase. J Biol Chem. 1995;270:29515–29519.
20. Ruiz-Palacios GM, Cervantes LE, Ramos P, et al. *Campylobacter jejuni* binds intestinal H(O) antigen (Fuc alpha 1, 2Gal beta 1, 4GlcNAc), and fucosyloligosaccharides of human milk inhibit its binding and infection. J Biol Chem. 2003;278:14112–14120.
21. Engfer MB, Stahl B, Finke B, et al. Human milk oligosaccharides are resistant to enzymatic hydrolysis in the upper gastrointestinal tract. Am J Clin Nutr. 2000;71:1589–1596.
22. Gnoth MJ, Kunz C, Kinne-Saffran E, et al. Human milk oligosaccharides are minimally digested in vitro. J Nutr. 2000;130:3014–3020.
23. Albrecht S, Schols HA, van den Heuvel EG, et al. CE-LIF-MS$^n$ profiling of oligosaccharides in human milk and feces of breast-fed babies. Electrophoresis. 2010;31:1264–1273.
24. Albrecht S, Schols HA, van den Heuvel EG, et al. Occurrence of oligosaccharides in feces of breast-fed babies in their first six months of life and the corresponding breast milk. Carbohydr Res. 2011;346:2540–2550.
25. Kuhn NJ, Carrick DT, Wilde CJ. Lactose synthesis: the possibilities of regulation. J Dairy Sci. 1980;63:328–336.
26. Asano M, Furukawa K, Kido M, et al. Growth retardation and early death of beta-1,4-galactosyltransferase knockout mice with augmented proliferation and abnormal differentiation of epithelial cells. EMBO J. 1997;16:1850–1857.
27. Thurl S, Henker J, Siegel M, et al. Detection of four human milk groups with respect to Lewis blood group dependent oligosaccharides. Glycoconj J. 1997;14:795–799.
28. Kobata A. Structures and application of oligosaccharides in human milk. Proc Jpn Acad Ser B Phys Biol Sci. 2010;86:731–747.
29. Urashima T, Asakuma S, Leo F, et al. The predominance of type I oligosaccharides is a feature specific to human breast milk. Adv Nutr. 2012;3:473S–482S.
30. Blank D, Gebhardt S, Maass K, et al. High-throughput mass finger printing and Lewis blood group assignment of human milk oligosaccharides. Anal Bioanal Chem. 2011;401:2495–2510.
31. Viverge D, Grimmonprez L, Cassanas G, et al. Discriminant carbohydrate components of human milk according to donor secretor types. J Pediatr Gastroenterol Nutr. 1990;11:365–370.
32. Clausen H, Hakomori S. ABH and related histo-blood group antigens; immunochemical differences in carrier isotypes and their distribution. Vox Sang. 1989;56:1–20.
33. Ma B, Simala-Grant JL, Taylor DE. Fucosylation in prokaryotes and eukaryotes. Glycobiology. 2006;16:158R–184R.
34. Prieto PA. Profiles of human milk oligosaccharides and production of some human milk oligosaccharides in transgenic animals. Adv Nutr. 2012;3(Suppl):S456–S464.
35. Anstee DJ. The relationship between blood groups and disease. Blood. 2010;115:4635–4643.
36. Le Pendu J. Histo-blood group antigen and human milk oligosaccharides: genetic polymorphism and risk of infectious diseases. Adv Exp Med Biol. 2004;554:135–143.
37. Kinane DF, Blackwell CC, Brettle RP, et al. ABO blood group, secretor state, and susceptibility to recurrent urinary tract infection in women. Br Med J (Clin Res Ed). 1982;285:7–9.
38. Thom SM, Blackwell CC, MacCallum CJ, et al. Non-secretion of blood group antigens and susceptibility to infection by *Candida* species. FEMS Microbiol Immunol. 1989;1:401–405.
39. Blackwell CC, Jonsdottir K, Hanson MF, et al. Non-secretion of ABO blood group antigens predisposing to infection by *Haemophilus influenzae*. Lancet. 1986;2:687.
40. Blackwell CC, Jonsdottir K, Hanson M, et al. Non-secretion of ABO antigens predisposing to infection by *Neisseria meningitidis* and *Streptococcus pneumoniae*. Lancet. 1986;2:284–285.
41. McGovern DP, Jones MR, Taylor KD, et al. Fucosyltransferase 2 (FUT2) non-secretor status is associated with Crohn's disease. Hum Mol Genet. 2010;19:3468–3476.
42. Smyth DJ, Cooper JD, Howson JM, et al. FUT2 nonsecretor status links type 1 diabetes susceptibility and resistance to infection. Diabetes. 2011;60:3081–3084.
43. Morrow AL, Meinzen-Derr J, Huang P, et al. Fucosyltransferase 2 non-secretor and low secretor status predicts severe outcomes in premature infants. J Pediatr. 2011;158:745–751.
44. Ikehara Y, Nishihara S, Yasutomi H, et al. Polymorphisms of two *fucosyltransferase* genes (*Lewis* and *Secretor* genes) involving type I Lewis antigens are associated with the presence of anti-*Helicobacter pylori* IgG antibody. Cancer Epidemiol Biomarkers Prev. 2001;10:971–977.
45. Marionneau S, Airaud F, Bovin NV, et al. Influence of the combined ABO, FUT2, and FUT3 polymorphism on susceptibility to Norwalk virus attachment. J Infect Dis. 2005;192:1071–1077.
46. Kindberg E, Hejdeman B, Bratt G, et al. A nonsense mutation (428G–>A) in the fucosyltransferase FUT2 gene affects the progression of HIV-1 infection. AIDS. 2006;20:685–689.
47. Raza MW, Blackwell CC, Molyneaux P, et al. Association between secretor status and respiratory viral illness. BMJ. 1991;303:815–818.
48. Linden S, Mahdavi J, Semino-Mora C, et al. Role of ABO secretor status in mucosal innate immunity and *H. pylori* infection. PLoS Pathog. 2008;4:e2.
49. Morrow AL, Ruiz-Palacios GM, Altaye M, et al. Human milk oligosaccharides are associated with protection against diarrhea in breast-fed infants. J Pediatr. 2004;145:297–303.
50. Wacklin P, Makivuokko H, Alakulppi N, et al. Secretor genotype (*FUT2* gene) is strongly associated with the composition of *Bifidobacteria* in the human intestine. PLoS ONE. 2011;6:e20113.
51. Ruhaak LR, Lebrilla CB. Analysis and role of oligosaccharides in milk. BMB Rep. 2012;45:442–451.
52. Ruhaak LR, Lebrilla CB. Advances in analysis of human milk oligosaccharides. Adv Nutr. 2012;3(Suppl):S406–S414.
53. Polonowski M, Montreuil J. Étude chromatographique des polyosides du lait de femme. C R Hebd Seances Acad Sci. 1954;238:2263–2264.
54. Grollman EF, Ginsburg V. Correlation between secretor status and the occurrence of 2′-fucosyllactose in human milk. Biochem Biophys Res Commun. 1967;28:50–53.
55. Kobata A. Possible application of milk oligosaccharides for drug development. Chang Gung Med J. 2003;26:621–636.
56. Yamashita K, Kobata A. Oligosaccharides of human milk: V. Isolation and characterization of a new trisaccharide, 6′galactosyllactose. Arch Biochem Biophys. 1974;161:164–170.
57. Kobata A, Ginsburg V. Oligosaccharides of human milk. IV. Isolation and characterization of a new hexasaccharide, lacto-N-neohexaose. Arch Biochem Biophys. 1972;150:273–281.
58. Kobata A, Ginsburg V. Oligosaccharides of human milk. 3. Isolation and characterization of a new hexasaccharide, lacto-N-hexaose. J Biol Chem. 1972;247:1525–1529.
59. Takasaki S, Kobata A. Microdetermination of individual neutral and amino sugars and N-acetylneuraminic acid in complex saccharides. J Biochem. 1974;76:783–789.

60. Egge H, Dell A, Von Nicolai H. Fucose containing oligosaccharides from human milk. I. Separation and identification of new constituents. Arch Biochem Biophys. 1983;224:235–253.
61. Urashima T, Hiramatsu Y, Murata S, et al. Identification of 2′-fucosyllactose in milk of the crabeater seal (*Lobodon carcinophagus*). Comp Biochem Physiol B Biochem Mol Biol. 1997;116:311–314.
62. Urashima T, Sumiyoshi W, Nakamura T, et al. Chemical characterization of milk oligosaccharides of the Japanese black bear, *Ursus thibetanus japonicus*. Biochim Biophys Acta. 1999;1472:290–306.
63. Urashima T, Yamashita T, Nakamura T, et al. Chemical characterization of milk oligosaccharides of the polar bear, *Ursus maritimus*. Biochim Biophys Acta. 2000;1475:395–408.
64. Thurl S, Muller-Werner B, Sawatzki G. Quantification of individual oligosaccharide compounds from human milk using high-pH anion-exchange chromatography. Anal Biochem. 1996;235:202–206.
65. Stahl B, Thurl S, Zeng J, et al. Oligosaccharides from human milk as revealed by matrix-assisted laser desorption/ionization mass spectrometry. Anal Biochem. 1994;223:218–226.
66. Ninonuevo MR, Park Y, Yin H, et al. A strategy for annotating the human milk glycome. J Agric Food Chem. 2006;54:7471–7480.
67. Ninonuevo MR, Perkins PD, Francis J, et al. Daily variations in oligosaccharides of human milk determined by microfluidic chips and mass spectrometry. J Agric Food Chem. 2008;56:618–626.
68. De Leoz ML, Gaerlan SC, Strum JS, et al. Lacto-N-tetraose, fucosylation, and secretor status are highly variable in human milk oligosaccharides from women delivering preterm. J Proteome Res. 2012;11:4662–4672.
69. Wu S, Tao N, German JB, et al. Development of an annotated library of neutral human milk oligosaccharides. J Proteome Res. 2010;9:4138–4151.
70. Bao Y, Chen C, Newburg DS. Quantification of neutral human milk oligosaccharides by graphitic carbon high-performance liquid chromatography with tandem mass spectrometry. Anal Biochem. 2013;433:28–35.
71. Martin-Sosa S, Martin MJ, Garcia-Pardo LA, et al. Sialyloligosaccharides in human and bovine milk and in infant formulas: variations with the progression of lactation. J Dairy Sci. 2003;86:52–59.
72. Messer M, Kerry KR. Milk carbohydrates of the echidna and the platypus. Science. 1973;180:201–203.
73. Amano J, Messer M, Kobata A. Structures of the oligosaccharides isolated from milk of the platypus. Glycoconj J. 1985;2:121–135.
74. Messer M, Gadiel PA, Ralston GB, et al. Carbohydrates of the milk of the platypus. Aust J Biol Sci. 1983;36:129–137.
75. Anraku T, Fukuda K, Saito T, et al. Chemical characterization of acidic oligosaccharides in milk of the red kangaroo (*Macropus rufus*). Glycoconj J. 2012;29:147–156.
76. Messer M, Trifonoff E, Stern W, et al. Structure of a marsupial-mild trisaccharide. Carbohydr Res. 1980;83:327–334.
77. Messer M, Trifonoff E. Structure of a branched tetrasaccharide from marsupial milk. Carbohydr Res. 1982;102:316–320.
78. Merchant J, Green B, Messer M, et al. Milk composition in the red-necked wallaby, *Macropus rufogriseus banksianus* (Marsupialia). Comp Biochem Physiol A Comp Physiol. 1989;93:483–488.
79. Crisp EA, Cowan PE, Messer M. Changes in milk carbohydrates during lactation in the common brushtail possum, *Trichosurus vulpecula* (Marsupialia: Phalangeridae). Reprod Fertil Dev. 1989;1:309–314.
80. Tao N, DePeters EJ, Freeman S, et al. Bovine milk glycome. J Dairy Sci. 2008;91:3768–3778.
81. Nakamura T, Urashima T. The milk oligosaccharides of domestic farm animals. Trends Glycosci Glycotechnol. 2004;16:135–142.
82. Urashima T, Bubb WA, Messer M, et al. Studies of the neutral trisaccharides of goat (*Capra hircus*) colostrum and of the one- and two-dimensional 1H and 13C NMR spectra of 6′-N-acetylglucosaminyllactose. Carbohydr Res. 1994;262:173–184.
83. Martinez-Ferez A, Rudloff S, Guadix A, et al. Goat's milk as a natural source of lactose-derived oligosaccharides: isolation by membrane technology. Int Dairy J. 2006;16:173–181.
84. Tao N, Ochonicky KL, German JB, et al. Structural determination and daily variations of porcine milk oligosaccharides. J Agric Food Chem. 2010;58:4653–4659.
85. Fukuda K, Yamamoto A, Ganzorig K, et al. Chemical characterization of the oligosaccharides in Bactrian camel (*Camelus bactrianus*) milk and colostrum. J Dairy Sci. 2010;93:5572–5587.
86. Urashima T, Kusaka Y, Nakamura T, et al. Chemical characterization of milk oligosaccharides of the brown bear, *Ursus arctos yesoensis*. Biochim Biophys Acta. 1997;1334:247–255.
87. Urashima T, Yamamoto M, Nakamura T, et al. Chemical characterisation of the oligosaccharides in a sample of milk of a white-nosed coati, *Nasua narica* (Procyonidae: Carnivora). Comp Biochem Physiol A Mol Integr Physiol. 1999;123:187–193.
88. Bubb WA, Urashima T, Kohso K, et al. Occurrence of an unusual lactose sulfate in dog milk. Carbohydr Res. 1999;318:123–128.
89. Urashima T, Nakamura T, Ikeda A, et al. Characterization of oligosaccharides in milk of a mink, *Mustela vison*. Comp Biochem Physiol A Mol Integr Physiol. 2005;142:461–471.
90. Urashima T, Arita M, Yoshida M, et al. Chemical characterisation of the oligosaccharides in hooded seal (*Cystophora cristata*) and Australian fur seal (*Arctocephalus pusillus doriferus*) milk. Comp Biochem Physiol B Biochem Mol Biol. 2001;128:307–323.
91. Urashima T, Nakamura T, Yamaguchi K, et al. Chemical characterization of the oligosaccharides in milk of high Arctic harbour seal (*Phoca vitulina vitulina*). Comp Biochem Physiol A Mol Integr Physiol. 2003;135:549–563.
92. Urashima T, Nakamura T, Nakagawa D, et al. Characterization of oligosaccharides in milk of bearded seal (*Erignathus barbatus*). Comp Biochem Physiol B Biochem Mol Biol. 2004;138:1–18.
93. Senda A, Hatakeyama E, Kobayashi R, et al. Chemical characterization of milk oligosaccharides of an African lion (*Panthera leo*) and a clouded leopard (*Neofelis nebulosa*). Anim Sci J. 2010;81:687–693.
94. Uemura Y, Takahashi S, Senda A, et al. Chemical characterization of milk oligosaccharides of a spotted hyena (*Crocuta crocuta*). Comp Biochem Physiol A Mol Integr Physiol. 2009;152:158–161.
95. Kunz C, Rudloff S, Schad W, et al. Lactose-derived oligosaccharides in the milk of elephants: comparison with human milk. Br J Nutr. 1999;82:391–399.
96. Uemura Y, Asakuma S, Yon L, et al. Structural determination of the oligosaccharides in the milk of an Asian elephant (*Elephas maximus*). Comp Biochem Physiol A Mol Integr Physiol. 2006;145:468–478.
97. Urashima T, Sato H, Munakata J, et al. Chemical characterization of the oligosaccharides in beluga (*Delphinapterus leucas*) and Minke whale (*Balaenoptera acutorostrata*) milk. Comp Biochem Physiol B Biochem Mol Biol. 2002;132:611–624.
98. Taufik E, Fukuda K, Senda A, et al. Structural characterization of neutral and acidic oligosaccharides in the milks of strepsirrhine primates: greater galago, aye-aye, Coquerel's sifaka and mongoose lemur. Glycoconj J. 2012;29:119–134.
99. Goto K, Fukuda K, Senda A, et al. Chemical characterization of oligosaccharides in the milk of six species of New and Old World monkeys. Glycoconj J. 2010;27:703–715.
100. Urashima T, Kawai Y, Nakamura T, et al. Chemical characterisation of six oligosaccharides in a sample of colostrum of the brown capuchin, *Cebus apella* (Cebidae: primates). Comp Biochem Physiol C Pharmacol Toxicol Endocrinol. 1999;124:295–300.
101. Tao N, Wu S, Kim J, et al. Evolutionary glycomics: characterization of milk oligosaccharides in primates. J Proteome Res. 2011;10:1548–1557.
102. Urashima T, Odaka G, Asakuma S, et al. Chemical characterization of oligosaccharides in chimpanzee, bonobo, gorilla, orangutan, and siamang milk or colostrum. Glycobiology. 2009;19:499–508.
103. Bradbury JH, Collins JG, Jenkins GA, et al. 13C-NMR study of the structures of two branched oligosaccharides from marsupial milk. Carbohydr Res. 1983;122:327–331.
104. Zivkovic AM, Barile D. Bovine milk as a source of functional oligosaccharides for improving human health. Adv Nutr. 2011;2:284–289.
105. Aldredge DL, Geronimo MR, Hua S, et al. Annotation and structural elucidation of bovine milk oligosaccharides and determination of novel fucosylated structures. Glycobiology. 2013;23:664–676.
106. Nakamura T, Urashima T, Nakagawa M, et al. Sialyllactose occurs as free lactones in ovine colostrum. Biochim Biophys Acta. 1998;1381:286–292.
107. Segurel L, Thompson EE, Flutre T, et al. The ABO blood group is a trans-species polymorphism in primates. Proc Natl Acad Sci U S A. 2012;109:18493–18498.
108. Coppa GV, Pierani P, Zampini L, et al. Oligosaccharides in human milk during different phases of lactation. Acta Paediatr Suppl. 1999;88:89–94.
109. Newburg DS, Ruiz-Palacios GM, Altaye M, et al. Innate protection conferred by fucosylated oligosaccharides of human milk against diarrhea in breastfed infants. Glycobiology. 2004;14:253–263.
110. Yan A, Lennarz WJ. Unraveling the mechanism of protein N-glycosylation. J Biol Chem. 2005;280:3121–3124.
111. Musumeci M, Simpore J, D'Agata A, et al. Oligosaccharides in colostrum of Italian and Burkinabe women. J Pediatr Gastroenterol Nutr. 2006;43:372–378.
112. Sumiyoshi W, Urashima T, Nakamura T, et al. Determination of each neutral oligosaccharide in the milk of Japanese women during the course of lactation. Br J Nutr. 2003;89:61–69.
113. Leo F, Asakuma S, Fukuda K, et al. Determination of sialyl and neutral oligosaccharide levels in transition and mature milks of Samoan women, using anthranilic derivatization followed by reverse phase high performance liquid chromatography. Biosci Biotechnol Biochem. 2010;74:298–303.
114. Asakuma S, Urashima T, Akahori M, et al. Variation of major neutral oligosaccharides levels in human colostrum. Eur J Clin Nutr. 2008;62:488–494.
115. Gabrielli O, Zampini L, Galeazzi T, et al. Preterm milk oligosaccharides during the first month of lactation. Pediatrics. 2011;128:e1520–e1531.
116. Ferrer-Admetlla A, Sikora M, Laayouni H, et al. A natural history of FUT2 polymorphism in humans. Mol Biol Evol. 2009;26:1993–2003.

117. Fumagalli M, Cagliani R, Pozzoli U, et al. Widespread balancing selection and pathogen-driven selection at blood group antigen genes. Genome Res. 2009;19:199–212.
118. Oriol R, Le Pendu J, Mollicone R. Genetics of ABO, H, Lewis, X and related antigens. Vox Sang. 1986;51:161–171.
119. Henry S, Oriol R, Samuelsson B. Lewis histo-blood group system and associated secretory phenotypes. Vox Sang. 1995;69:166–182.
120. Bauer J, Gerss J. Longitudinal analysis of macronutrients and minerals in human milk produced by mothers of preterm infants. Clin Nutr. 2011;30: 215–220.
121. Coppa GV, Pierani P, Zampini L, et al. Lactose, oligosaccharide and monosaccharide content of milk from mothers delivering preterm newborns over the first month of lactation. Minerva Pediatr. 1997;49:471–475.
122. Bode L. Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology. 2012;22:1147–1162.
123. Rudloff S, Obermeier S, Borsch C, et al. Incorporation of orally applied (13)C-galactose into milk lactose and oligosaccharides. Glycobiology. 2006;16:477–487.
124. Martin-Sosa S, Martin MJ, Hueso P. The sialylated fraction of milk oligosaccharides is partially responsible for binding to enterotoxigenic and uropathogenic *Escherichia coli* human strains. J Nutr. 2002;132:3067–3072.
125. Rudloff S, Kunz C. Milk oligosaccharides and metabolism in infants. Adv Nutr. 2012;3(Suppl):S398–S405.
126. Rudloff S, Pohlentz G, Borsch C, et al. Urinary excretion of in vivo (1)(3)C-labelled milk oligosaccharides in breastfed infants. Br J Nutr. 2012;107: 957–963.
127. Obermeier S, Rudloff S, Pohlentz G, et al. Secretion of 13C-labelled oligosaccharides into human milk and infant's urine after an oral [13C]galactose load. Isotopes Environ Health Stud. 1999;35:119–125.
128. Leach JL, Garber SA, Marcon AA, et al. In vitro and in vivo effects of soluble, monovalent globotriose on bacterial attachment and colonization. Antimicrob Agents Chemother. 2005;49:3842–3846.
129. Coppa GV, Pierani P, Zampini L, et al. Characterization of oligosaccharides in milk and feces of breast-fed infants by high-performance anion-exchange chromatography. Adv Exp Med Biol. 2001;501:307–314.
130. De Leoz ML, Wu S, Strum JS, et al. A quantitative and comprehensive method to analyze human milk oligosaccharide structures in the urine and feces of infants. Anal Bioanal Chem. 2013;405:4089–4105.
131. Chaturvedi P, Warren CD, Buescher CR, et al. Survival of human milk oligosaccharides in the intestine of infants. Adv Exp Med Biol. 2001;501: 315–323.
132. Marcobal A, Barboza M, Sonnenburg ED, et al. Bacteroides in the infant gut consume milk oligosaccharides via mucus-utilization pathways. Cell Host Microbe. 2011;10:507–514.
133. German JB, Freeman SL, Lebrilla CB, et al. Human milk oligosaccharides: evolution, structures and bioselectivity as substrates for intestinal bacteria. Nestle Nutr Workshop Ser Pediatr Program. 2008;62:205–218; discussion 218–222.
134. Albrecht S, Schols HA, van Zoeren D, et al. Oligosaccharides in feces of breast- and formula-fed babies. Carbohydr Res. 2011;346:2173–2181.
135. Coppa GV, Zampini L, Galeazzi T, et al. Human milk oligosaccharides inhibit the adhesion to Caco-2 cells of diarrheal pathogens: *Escherichia coli*, *Vibrio cholerae*, and *Salmonella fyris*. Pediatr Res. 2006;59:377–382.
136. Dallas SD, Rolfe RD. Binding of *Clostridium difficile* toxin A to human milk secretory component. J Med Microbiol. 1998;47:879–888.
137. Vasta GR. Roles of galectins in infection. Nat Rev Microbiol. 2009;7:424–438.
138. van Kooyk Y, Geijtenbeek TB. DC-SIGN: escape mechanism for pathogens. Nat Rev Immunol. 2003;3:697–709.
139. Naarding MA, Ludwig IS, Groot F, et al. Lewis X component in human milk binds DC-SIGN and inhibits HIV-1 transfer to CD4+ T lymphocytes. J Clin Invest. 2005;115:3256–3264.
140. Hong P, Ninonuevo MR, Lee B, et al. Human milk oligosaccharides reduce HIV-1-gp120 binding to dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN). Br J Nutr. 2009;101:482–486.
141. Coutsoudis A, Dabis F, Fawzi W, et al. Late postnatal transmission of HIV-1 in breast-fed children: an individual patient data meta-analysis. J Infect Dis. 2004;189:2154–2166.
142. Bode L, Kuhn L, Kim HY, et al. Human milk oligosaccharide concentration and risk of postnatal transmission of HIV through breastfeeding. Am J Clin Nutr. 2012;96:831–839.
143. Abrahams SW, Labbok MH. Breastfeeding and otitis media: a review of recent evidence. Curr Allergy Asthma Rep. 2011;11:508–512.
144. Andersson B, Porras O, Hanson LA, et al. Inhibition of attachment of *Streptococcus pneumoniae* and *Haemophilus influenzae* by human milk and receptor oligosaccharides. J Infect Dis. 1986;153:232–237.
145. Eiwegger T, Stahl B, Schmitt J, et al. Human milk-derived oligosaccharides and plant-derived oligosaccharides stimulate cytokine production of cord blood T-cells in vitro. Pediatr Res. 2004;56:536–540.
146. Bode L, Kunz C, Muhly-Reinholz M, et al. Inhibition of monocyte, lymphocyte, and neutrophil adhesion to endothelial cells by human milk oligosaccharides. Thromb Haemost. 2004;92:1402–1410.
147. Bode L, Rudloff S, Kunz C, et al. Human milk oligosaccharides reduce platelet-neutrophil complex formation leading to a decrease in neutrophil beta 2 integrin expression. J Leukoc Biol. 2004;76:820–826.
148. Marcobal A, Sonnenburg JL. Human milk oligosaccharide consumption by intestinal microbiota. Clin Microbiol Infect. 2012;18(Suppl 4):S12–S15.
149. Zivkovic AM, German JB, Lebrilla CB, et al. Human milk glycobiome and its impact on the infant gastrointestinal microbiota. Proc Natl Acad Sci U S A. 2011;108(Suppl 1):S4653–S4658.
150. Roberfroid M. Prebiotics: the concept revisited. J Nutr. 2007;137(Suppl):S830–S837.
151. LoCascio RG, Ninonuevo MR, Freeman SL, et al. Glycoprofiling of bifidobacterial consumption of human milk oligosaccharides demonstrates strain specific, preferential consumption of small chain glycans secreted in early human lactation. J Agric Food Chem. 2007;55:8914–8919.
152. LoCascio RG, Ninonuevo MR, Kronewitter SR, et al. A versatile and scalable strategy for glycoprofiling bifidobacterial consumption of human milk oligosaccharides. Microb Biotechnol. 2009;2:333–342.
153. Marcobal A, Barboza M, Froehlich JW, et al. Consumption of human milk oligosaccharides by gut-related microbes. J Agric Food Chem. 2010;58:5334–5340.
154. Ward RE, Ninonuevo M, Mills DA, et al. In vitro fermentability of human milk oligosaccharides by several strains of bifidobacteria. Mol Nutr Food Res. 2007;51:1398–1405.
155. Kitaoka M. Bifidobacterial enzymes involved in the metabolism of human milk oligosaccharides. Adv Nutr. 2012;3:422S–429S.
156. Sela DA, Chapman J, Adeuya A, et al. The genome sequence of *Bifidobacterium longum* subsp. infantis reveals adaptations for milk utilization within the infant microbiome. Proc Natl Acad Sci U S A. 2008;105:18964–18969.
157. Asakuma S, Hatakeyama E, Urashima T, et al. Physiology of consumption of human milk oligosaccharides by infant gut-associated bifidobacteria. J Biol Chem. 2011;286:34583–34592.
158. Lievin V, Peiffer I, Hudault S, et al. *Bifidobacterium* strains from resident infant human gastrointestinal microflora exert antimicrobial activity. Gut. 2000;47:646–652.
159. Lee WH, Pathanibul P, Quarterman J, et al. Whole cell biosynthesis of a functional oligosaccharide, 2′-fucosyllactose, using engineered *Escherichia coli*. Microb Cell Fact. 2012;11:48–56.
160. Baumgartner F, Seitz L, Sprenger GA, et al. Construction of *Escherichia coli* strains with chromosomally integrated expression cassettes for the synthesis of 2[prime]-fucosyllactose. Microb Cell Fact. 2013;12:40–52.