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REVIEW

Oligosaccharides of milk and colostrum in non-human mammals

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Mammalian milk or colostrum usually contains, in addition to lactose, a variety of neutral and acidic oligosaccharides. Although the oligosaccharides of human milk have been reviewed in several recent publications, those of non-human mammals have received much less attention. This paper reviews the chemical structures and the variety of milk oligosaccharides in species other than humans, including placental mammals (e.g. primates, domestic herbivores, bears and other carnivores, the rat and the elephant) as well as monotremes (platypus and echidna) and marsupials (e.g. wallaby). The gastrointestinal digestion and absorption and the possible biological functions of these oligosaccharides are also discussed.

Keywords: **milk oligosaccharide, non-human mammal, milk, colostrum**

The dominant saccharide in mammalian milk or colostrum is generally the disaccharide lactose (Gal(β1-4)Glc), which is synthesized in lactating mammary glands from UDP-Gal (donor) and Glc (acceptor) by a transgalactosylation catalysed by a β4galactosyltransferase (β4GT). In other tissues this enzyme transfers Gal from UDP-Gal to non reducing GlcNAc residues in glycoconjugates to synthesize N-acetyllactosamine (Gal(β1-4)GlcNAc) units, but in the lactating mammary gland it is recruited for a second biosythetic function, the production of lactose [1]. In this tissue it is lactose rather than N-acetyllactosamine which is produced because of the modifying action of α-lactalbumin, the effect of which is to change β4GT's acceptor from GlcNAc to Glc.

Mammalian milk or colostrum usually contains, in addition to lactose, a variety of other saccharides [2]. For example, human milk or colostrum has been reported to contain more than 100 oligosaccharides, i.e. saccharides containing three or more monosaccharide residues, and the chemical structures of at least 80 of these have been described [3]. The oligosaccharide content of mature human milk is from 12 to 14 g per litre while that of colostrum is about 21 to 24 g per litre [3]. Almost all of the human milk oligosaccharides have a lactose unit at their reducing ends, and they can be divided into 12 groups based on their core structures (Figure 1) [4]. The synthesis of human milk oligosaccharides within the lactating mammary gland is catalysed by a variety of specific glycosyltransferases which transfer galactosyl, sialyl, N-acetylglucosaminyl and fucosyl residues from nucleotide sugars to a non-reducing residue of these core structures. The actions of these glycosyltransferases are not dependent on α-lactalbumin. Most of these transferases are probably identical with those that participate in the synthesis of glycoprotein and glycolipid cell surface components [3].

Numerous oligosaccharides, last reviewed in 1995 [3], have been detected in the milk or colostrum of many mammalian species other than humans [2]. In this paper we describe the chemical structures of the oligosaccharides of milk and colostrum of non-human placental mammals, with special emphasis on recent studies. In addition we review, in more detail than hitherto, studies on the milk oligosaccharides of monotremes and marsupials and discuss the metabolic fate and possible biological functions of milk and colostrum oligosaccharides in mammals.

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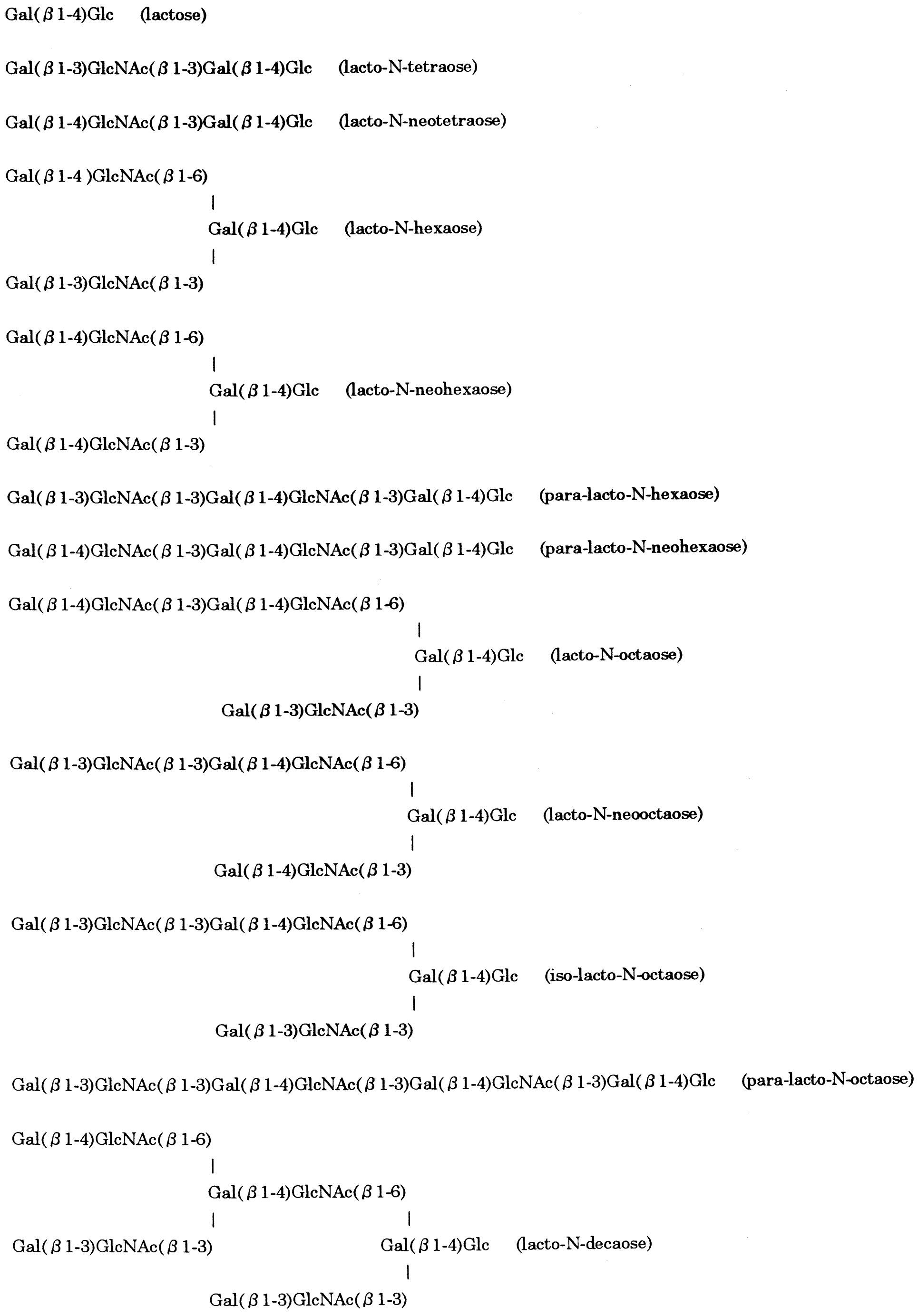


Figure 1. List of the 12 'core' oligosaccharides found in human milk.

Chemical structures of milk oligosaccharides

Placental mammals (Infraclass Eutheria)

Non-human primates

The only non-human primates whose milk sugars have been studied appear to be the rhesus monkey and the brown capuchin (*Cebus apella*). Rhesus monkey milk has been reported to contain sialylated and fucosylated oligosaccharides [5]. Brown capuchin colostrum contains at least six oligosaccharides (Table 1), five of which are also found in human milk [6]. The exception is lacto-N-novopentaose I (Gal(β1-3)[Gal(β1-4)GlcNAc(β1-6)]Gal(β1-4)Glc), which is also found in bovine [7] and equine [8] colostrum and is a prominent constituent of tammar wallaby milk sugars [9] (see below). These findings are based on only one relatively small

Table 1. Milk Oligosaccharides in several mammals

Oligosaccharides	*Reference*
Brown capuchin colostrum	
3-fucosyllactose	
Gal(β1-4)[Fuc(α1-3)]Glc	6
β3′-galactosyllactose	
Gal(β1-3)Gal(β1-4)Glc	6
β6′-galactosyllactose	
Gal(β1-6)Gal(β1-4)Glc	6
lacto-N-novopentaose I	
Gal(β1-4)GlcNAc(β1-6) | Gal(β1-4)Glc | Gal(β1-3)	6
lacto-N-neohexaose	
Gal(β1-4)GlcNAc(β1-6) | Gal(β1-4)Glc | Gal(β1-4)GlcNAc(β1-3)	6
3′-N-acetylneuraminyllactose	
Neu5Ac(α2-3)Gal(β1-4)Glc	6
Bovine colostrum or milk	
N-acetylgalactosaminylglucose	
GalNAc(β1-4)Glc	11
N-acetyllactosamine	
Gal(β1-4)GlcNAc	11
3-fucosyllactosamine	
Gal(β1-4)[Fuc(α1-3)]GlcNAc	12
α3′-N-acetylgalactosaminyllactose	
GalNAc(α1-3) Gal(β1-4)Glc	7
α3′-galactosyllactose	
Gal(α1-3)Gal(β1-4)Glc	7,13
β3′-galactosyllactose	
Gal(β1-3)Gal(β1-4)Glc	12,13
β4′-galactosyllactose	
Gal(β1-4)Gal(β1-4)Glc	13
β6′-galactosyllactose	
Gal(β1-6)Gal(β1-4)Glc	12,13
lacto-N-novopentaose I	
Gal(β1-4)GlcNAc(β1-6) | Gal(β1-4)Glc | Gal(β1-3)	7
lactose-3′-O-phosphate	
Gal(β1-4)Glc-3′-PO_4	35
3-N-acetylneuraminylgalactose	
Neu5Ac(α2-3)Gal	14
3′-N-acetylneuraminyllactose	
Neu5Ac(α2-3)Gal(β1-4)Glc	15,16,34
6′-N-acetylneuraminyllactose	
Neu5Ac(α2-6)Gal(β1-4)Glc	14,15,16,34
3′-N-glycolylneuraminyllactose	
Neu5Gc(α2-3)Gal(β1-4)Glc	14,16,34
6′-N-glycolylneuraminyllactose	
Neu5Gc(α2-6)Gal(β1-4)Glc	16
6′-N-acetylneuraminyllactosamine	
Neu5Ac(α2-6)Gal(β1-4)GlcNAc	14,16,34
6′-N-glycolylneuraminyllactosamine	
Neu5Gc(α2-6)Gal(β1-4)GlcNAc	16
3″-N-acetylneuraminyl-3′-galactosyllactose	
Neu5Ac(α2-3)Gall-3Gal1–4Glc	34
di-N-acetylneuraminyllactose	
Neu5Ac(α2-8)Neu5Ac(α2-3)Gal(β1-4)Glc	14,16,34
6′-N-acetylneuraminyllactose-1-O-phosphate	
Neu5Ac(α2-6)Gal(β1-4)GlcNAc-1-PO_4	34
6′-N-acetylneuraminyllactose-6-O-phosphate	
Neu5Ac(α2-6)Gal(β1-4)GlcNAc-6-PO_4	33,34
Buffalo colostrum	
di-N-acetylneuraminyllactose	
Neu5Ac(α2-8) Neu5Ac(α2-3) Gal(β1-4)Glc	36
Equine colostrum	
β3′-galactosyllactose	
Gal(β1-3)Gal(β1-4)Glc	8
β6′-galactosyllactose	
Gal(β1-6)Gal(β1-4)Glc	8
lacto-N-neotetraose	
Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc	24
iso-lacto-N-neotetraose	
Gal(β1-4)GlcNAc(β1-6)Gal(β1-4)Glc	24
lacto-N-novopentaose I	
Gal(β1-4)GlcNAc(β1-6) | Gal(β1-4)Glc | Gal(β1-3)	8
lacto-N-neohexaose	
Gal(β1-4)GlcNAc(β1-6) | Gal(β1-4)Glc | Gal(β1-4)GlcNAc(β1-3)	24
N-acetyllactosamine-1-O-phosphate	
Gal(β1-4)GlcNAc-1-phosphate	25
3′-N-acetylneuraminyllactose	
Neu5Ac(α2-3)Gal(β1-4)Glc	25
Caprine colostrum or milk	
2′-fucosyllactose	
Fuc(α1-2)Gal(β1-4)Glc	21
α3′-galactosyllactose	
Gal(α1-3)Gal(β1-4)Glc	21
β3′-galactosyllactose	
Gal(β1-3)Gal(β1-4)Glc	21
β6′-galactosyllactose	
Gal(β1-6)Gal(β1-4)Glc	21
3′-N-acetylneuraminyllactose	
Neu5Ac(α2-3)Gal(β1-4)Glc	22,23
6′-N-acetylneuraminyllactose	
Neu5Ac(α2-6)Gal(β1-4)Glc	22,23

Table 1. (*continued*)

Oligosaccharides	*Reference*
6′-N-glycolylneuraminyllactose Neu5Gc(α2-6)Gal(β1-4)Glc	22
6′-N-acetylneuraminyllactosamine Neu5Ac(α2-6)Gal(β1-4)GlcNAc	22
3′-N-acetylneuraminyl-β6′-galactosyllactose Gal(β1-6)Gal(β1-4)Glc | Neu5Ac(α2-3)	23
6′-N-acetylneuraminyl-β3′-galactosyllactose Gal(β1-3)Gal(β1-4)Glc | Neu5Ac(α2-6)	23
Ovine colostrum	
α3′-galactosyllactose Gal(α1-3)Gal(β1-4)Glc	19
β3′-galactosyllactose Gal(β1-3)Gal(β1-4)Glc	19
β6′-galactosyllactose Gal(β1-6)Gal(β1-4)Glc	19
3′-N-acetylneuraminyllactose Neu5Ac(α2-3)Gal(β1-4)Glc	20
3′-N-glycolylneuraminyllactose Neu5Gc(α2-3)Gal(β1-4)Glc	20
6′-N-glycolylneuraminyllactose Neu5Gc(α2-6)Gal(β1-4)Glc	20
Ezo brown bear milk	
2′-fucosyllactose Fuc(α1-2)Gal(β1-4)Glc	38
α3′-galactosyllactose Gal(α1-3)Gal(β1-4)Glc	38
lacto-N-difucohexaose III Fuc(α1-2)Gal(β1-4)GlcNAc(β1-3)-Gal(β1-4)Glc | Fuc(α1-3)	38
galactosyl lacto-N-fucopentaose III Gal(α1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc | Fuc(α1-3)	38
tetrafucosyl lacto-N-neohexaose Fuc(α1-3) | Fuc(α1-2)Gal(β1-4)GlcNAc(β1-6) | Gal(β1-4)Glc | Fuc(α1-2)Gal(β1-4)GlcNAc(β1-3) | Fuc(α1-3)	38
digalactosyl difucosyl lacto-N-neohexaose Fuc(α1-3) | Gal(α1-3)Gal(β1-4)GlcNAc(β1-6) | Gal(β1-4)Glc | Gal(α1-3)Gal(β1-4)GlcNAc(β1-3) | Fuc(α1-3)	38
Japanese black bear milk	
2′-fucosyllactose Fuc(α1-2)Gal(β1-4)Glc	39
α3′-galactosyllactose Gal(α1-3)Gal(β1-4)Glc	39
α3′-galactosyl-3-fucosyllactose Gal(α1-3)Gal(β1-4)Glc | Fuc(α1-3)	39
B-tetrasaccharide Gal(α1-3)Gal(β1-4)Glc | Fuc(α1-2)	39
B-pentasaccharide Gal(α1-3)Gal(β1-4)Glc | | Fuc(α1-2) Fuc(α1-3)	39
galactosyl lacto-N-fucopentaose III Gal(α1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc | Fuc(α1-3)	39
B-heptasaccharide-type II chain Gal(α1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc | | Fuc(α1-2) Fuc(α1-3)	39
galactosyl difucosyl lacto-N-neotetraose Gal(α1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc | | Fuc(α1-3) Fuc(α1-3)	39
digalactosyl difucosyl lacto-N-neohexaose Fuc(α1-3) | Gal(α1-3)Gal(β1-4)GlcNAc(β1-6) | Gal(β1-4)Glc | Gal(α1-3)Gal(β1-4)GlcNAc(β1-3) | Fuc(α1-3)	39

Table 1. (*continued*)

Oligosaccharides	*Reference*
digalactosyl trifucosyl lacto-N-neohexaose a Fuc(α1-2) Fuc(α1-3) | | Gal(α1-3)Gal(β1-4)GlcNAc(β1-6) | Gal(β1-4)Glc | Gal(α1-3)Gal(β1-4)GlcNAc(β1-3) | Fuc(α1-3)	39
digalactosyl trifucosyl lacto-N-neohexaose b Fuc(α1-3) | Gal(α1-3)Gal(β1-4)GlcNAc(β1-6) | Gal(β1-4)Glc | Gal(α1-3)Gal(β1-4)GlcNAc(β1-3) | | Fuc(α1-2) Fuc(α1-3)	39
Polar bear milk	
α3′-galactosyllatose Gal(α1-3)Gal(β1-4)Glc	40
2′-fucosyllactose Fuc(α1-2)Gal(β1-4)Glc	40
B-tetrasaccharide Gal(α1-3)Gal(β1-4)Glc | Fuc(α1-2)	40
α3′-galactosyl-3-fucosyllactose Gal(α1-3)Gal(β1-4)Glc | Fuc(α1-3)	40
A-tetrasaccharide GalNAc(α1-3)Gal(β1-4)Glc | Fuc(α1-2)	40
A-pentasaccharide GalNAc(α1-3)Gal(β1-4)Glc | | Fuc(α1-2) Fuc(α1-3)	40
galactosyl lacto-N-neotetraose Gal(α1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc	40
B-hexasaccharide- type II chain Gal(α1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc | Fuc(α1-2)	40
galactosyl lacto-N-fucopentaose III Gal(α1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc | Fuc(α1-3)	40
galactosyl difucosyl lacto-N-neotetraose Gal(α1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc | | Fuc(α1-3) Fuc(α1-3)	40
digalactosyl lacto-N-neohexaose Gal(α1-3)Gal(β1-4)GlcNAc(β1-6) | Gal(β1-4)Glc | Gal(α1-3)Gal(β1-4)GlcNAc(β1-3)	40
digalactosyl difucosyl lacto-N-neohexaose Fuc(α1-3) | Gal(α1-3)Gal(β1-4)GlcNAc(β1-6) | Gal(β1-4)Glc | Gal(α1-3)Gal(β1-4)GlcNAc(β1-3) | Fuc(α1-3)	40
White nosed coati milk	
2′-fucosyllactose Fuc(α1-2)Gal(β1-4)Glc	41
α3′-galactosyllactose Gal(α1-3)Gal(β1-4)Glc	41
lacto-N-neotetraose Gal(β1-4)GlcNAc(β1-3) Gal(β1-4)Glc	41
lacto-N-fucopentaose IV Fuc(α1-2)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc	41
galactosyl lacto-N-neotetraose Gal(α1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc	41
galactosyl monofucosyl lacto-N-neohexaose Fuc(α1–2) { Gal(β1-4)GlcNAc(β1-6) Gal(α1–3) { | Gal(β1–4)Glc | Gal(β1-4)GlcNAc(β1-3)	41
Crabeater seal milk	
2′-fucosyllactose Fuc(α1-2)Gal(β1-4)Glc	44
Hooded seal milk	
2′-fucosyllactose Fuc(α1-2)Gal(β1-4)Glc	42
lacto-N-neotetraose Gal(β1-4)GlcNAc(β1-3) Gal(β1-4)Glc	42
lacto-N-fucopentaose IV Fuc(α1-2)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc	42

Table 1. (*continued*)

Oligosaccharides	*Reference*
lacto-N-neohexaose Gal(β1-4)GlcNAc(β1-6) | Gal(β1-4)Glc | Gal(β1-4)GlcNAc(β1-3)	42
monofucosyl lacto-N-neohexaose a Fuc(α1-2)Gal(β1-4)GlcNAc(β1-6) | Gal(β1-4)Glc | Gal(β1-4)GlcNAc(β1-3)	42
monofucosyl lacto-N-neohexaose b Gal(β1-4)GlcNAc(β1-6) | Gal(β1-4)Glc | Fuc(α1-2)Gal(β1-4)GlcNAc(β1-3)	42
difucosyl lacto-N-neohexaose Fuc(α1-2)Gal(β1-4)GlcNAc(β1-6) | Gal(β1-4)Glc | Fuc(α1-2)Gal(β1-4)GlcNAc(β1-3)	42
para-lacto-N-neohexaose Gal(β1-4)GlcNAc(β1-3) Gal(β1-4)- GlcNAc(β1-3) Gal(β1-4)Glc	42
fucosyl para-lacto-N-neohexaose Fuc(α1-2)Gal(β1-4)GlcNAc(β1-3)- Gal(β1-4)GlcNAc(β1-3) Gal(β1-4)Glc	42
Elephant milk	
α3′-galactosyllactose Gal(α1-3)Gal(β1-4)Glc	49
2′-fucosyllactose Fuc(α1-2)Gal(β1-4)Glc	49
3-fucosyllactose Gal(β1-4)[Fuc(α1-3)]Glc	49
lacto-N-tetraose Gal(β1-3)GlcNAc(β1-3)Gal(β1-4)Glc	49
lacto-N-neohexaose Gal(β1-4)GlcNAc(β1-3) Gal(β1-4)Glc	49
lacto-N-fucopentaose I Fuc(α1-2)Gal(β1-3)GlcNAc(β1-3) Gal(β1-4)Glc	49
lacto-N-difucohexaose I Fuc(α1-2)Gal(β1-3) [Fuc(α1-4)] GlcNAc- (β1-3) Gal(β1-4)Glc	49
3′-N-acetylneuraminyllactose Neu5Ac(α2-3)Gal(β1-4)Glc	49
6′-N-acetylneuraminyllactose Neu5Ac(α2-6)Gal(β1-4)Glc	49
N-acetylneurarninyl lacto-N-tetraose a Neu5Ac(α2-3)Gal(β1-3) GlcNAc(β1-3)- Gal(β1-4)Glc	49
N-acetylneuraminyl lacto-N-neotetraose c Neu5Ac(α2-6)Gal(β1-4) GlcNAc(β1-3)- Gal(β1-4)Glc	49
Rat milk	
3′-N-acetylneuraminyllactose Neu5Ac(α2-3)Gal(β1-4)Glc	52
3′-N-acetylneuraminyllactose-6′-o-sulphate Neu5Ac(α2-3)Gal(β1-4)Glc-6′-SO_3	31,50
lactose-6′-O-sulphate Gal(β1-4)Glc-6′-SO_3	50,51
β6′galactosyl-myo-inositol Gal(β1-6)myo-inositol	54
Dog milk	
2′-fucosyllactose Fuc(α1-2)Gal(β1-4)Glc	53
lactose-3′-O-sulphate Gal(β1-4)Glc-3′-SO_3	53
Echidna milk	
2′-fucosyllactose Fuc(α1-2)Gal(β1-4)Glc	57,59
2′,3-difucosyllactose Fuc(α1-2)Gal(β1-4)Glc | Fuc(α1-3)	57,59
3′-4-O-acetyl N-acetylneuraminyllactose 4-OAc-Neu5Ac(α2-3)Gal(β1-4)Glc	57,61,62
Platypus milk	
2′,3-difucosyllactose Fuc(α1-2)Gal(β1-4)Glc | Fuc(α1-3)	57,59
lacto-N-fucopentaose III Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc | Fuc(α1-3)	60
lacto-N-difucohexaose III Fuc(α1-2)Gal(β1-4) GlcNAc(β1-3)Gal(β1-4)Glc | Fuc(α1-3)	60
difucosyl lacto-N-neotetraose Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc | | Fuc(α1-3) Fuc(α1-3)	60
lacto-N-difucohexaose I Fuc(α1-2)Gal(β1-3) GlcNAc(β1-3)Gal(β1-4)Glc | Fuc(α1-4)	60
Fuc(α1-2)Gal(β1-4) GlcNAc(β1-?)Gal(β1-4)Glc | | Fuc(α1-3) Fuc(α1-3)	60

Table 1. (*continued*)

<table>
<tr><th><i>Oligosaccharides</i></th><th><i>Reference</i></th></tr>
<tr><td>difucosyl lacto-N-neohexaose
Fuc(α1-3)
|
Gal(β1-4) GlcNAc(β1-6)
|
Gal(β1-4)Glc
|
Gal(β1-4)GlcNAc(β1-3)
|
Fuc(α1-3)</td><td>60</td></tr>
<tr><td>trifucosyl lacto-N-neohexaose a
Fuc(α1-3)
|
Fuc(α1-2)Gal(β1-4) GlcNAc(β1-6)
|
Gal(β1-4)Glc
|
Gal(β1-4) GlcNAc(β1-3)
|
Fuc(α1-3)</td><td>60</td></tr>
<tr><td>trifucosyl lacto-N-neohexaose b
Fuc(α1-3)
|
Gal(β1-4) GlcNAc(β1-6)
|
Gal(β1-4)Glc
|
Fuc(α1-2)Gal(β1-4) GlcNAc(β1-3)
|
Fuc(α1-3)</td><td>60</td></tr>
<tr><td><i>Tammar wallaby milk</i></td><td></td></tr>
<tr><td>β3′-galacosyllactose
Gal(β1-3)Gal(β1-4)Glc</td><td>67</td></tr>
<tr><td>digalactosyllactose
Gal(β1-3)Gal(β1-3)Gal(β1-4)Glc</td><td>68</td></tr>
<tr><td>trigalactosyllactose
Gal(β1-3)Gal(β1-3)Gal(β1-3)Gal(β1-4)Glc</td><td>68</td></tr>
<tr><td>tetragalactosyllactose
Gal(β1-3)Gal(β1-3)Gal(β1-3) Gal(β1-3)-Gal(β1-4)Glc</td><td>68</td></tr>
<tr><td>pentagalactosyllactose
Gal(β1-3)Gal(β1-3)Gal(β1-3) Gal(β1-3)-Gal(β1-3)Gal(β1-4)Glc</td><td>68</td></tr>
<tr><td>lacto-N-novotetraose
GlcNAc(β1-6)
|
Gal(β1-4)Glc
|
Gal(β1-3)</td><td>70</td></tr>
<tr><td>lacto-N-novopentaose I
Gal(β1-4)GlcNAc(β1-6)
|
Gal(β1-4)Glc
|
Gal(β1-3)</td><td>9</td></tr>
<tr><td>g alactosyl lacto-N-novopentaose I
Gal(β1-4) GlcNAc(β1-6)
|
Gal(β1-4)Glc
|
Gal(β1-3) Gal(β1-3)</td><td>9</td></tr>
<tr><td>Ra or Rb Ra or Rb
| |
Gal(β1-3)[Gal(β1-3)]n Gal(β1-4)Glc</td><td>72</td></tr>
<tr><td>Ra Ra
| |
Gal(β1-3)[Gal(β1-3)]n Gal(β1-4)Glc
Ra = Neu5Ac(α2-3/2–6)Gal(β1-4)-GlcNAc(β1-6)
Rb = Gal(β1-4)GlcNAc(β1-6)</td><td>72</td></tr>
</table>

Glc; D-glucose, Gal; D-galactose, Fuc; L-fucose, GlcNAc; N-acetylglucosamine, GalNAc; N-acetylgalactosamine, Neu5Ac; N-acetylneuraminic acid, Neu5Gc; N-glycolylneuraminic acid.

sample of colostrum and further studies on larger samples of colostrum or milk from a greater variety of non-human primates would be required before any convincing comparisons with human milk sugars can be drawn.

Domestic herbivorous mammals (cow, sheep, goat and horse)

Bovine milk normally contains 1–2 g/L of free saccharides other than lactose but larger amounts occur in colostrum [10]; the content for milk is significantly lower than that for human milk. Nine neutral oligosaccharides have been described in bovine colostrum (Table 1) [7,11,12], including four galactosyllactoses which are found in the mature milk as well [13]. Some of the neutral oligosaccharides have the following features which are not seen in human neutral oligosaccharides. (1) Two trisaccharides have a Gal(α1-3)Gal or GalNAc(α1-3)Gal unit at their non reducing ends. (2) Bovine colostrum contains lacto-N-novopentaose I and the disaccharide GalNAc(β1-4)Glc, which to date has not been reported in any other milk or colostrum. (3) Two of the neutral oligosaccharides have GlcNAc residues at their reducing ends.

Bovine colostrum contains ten sialyl oligosaccharides [14–16] (Table 1) in which two kinds of sialic acid, Neu5Ac and Neu5Gc, are found, in contrast to human milk or colostrum oligosaccharides which contain only Neu5Ac. Recent studies indicate that Neu5Gc is found in all mammals except humans and that this is due to a mutation in CMP-sialic acid hydroxylase which occurred in the hominid lineage subsequent to its divergence from the lineage of the great apes [17].

Bovine colostrum contains sialyl N-acetyllactosamine as well as sialyllactose, while human milk contains only sialyllactose. Kuhn and Brossmer [18] showed that the Neu5Ac in α2,3 N-acetylneuraminyllactose is O-acetylated, but O-acetylation of the Neu5Ac residue of this saccharide in bovine colostrum or milk has not to date been confirmed. The dominant neuramin lactose from human milk has the N-acetylneuraminic acid linked to galactose via an α2,6 bond whereas the dominant bovine neuramin lactose is linked 2,3.

Ovine [19,20], caprine [21–23] and equine [8,24,25] colostrum oligosaccharides are also shown in Table 1. Equine oligosaccharides include lacto-N-neotetraose and lacto-N-neohexaose, which are found in human milk, whereas Gal(β1–4)GlcNAc(β1–6)Gal(β1–4)Glc(iso-lacto-N-neotetraose) and lacto-N-novopentaose I are not. Lacto-N-novopentaose I has been identified in milk of the tammar wallaby [9] and the brown capuchin [6], and in bovine colostrum [7]. These differences between species with respect to GlcNAc-containing milk oligosaccharides can be assumed to be due to differences in the distributions of one or more βN-acetylglucosaminyltransferases, which transfer GlcNAc from UDP-GlcNAc to Gal, in their lactating mammary glands. Ovine and caprine sialyl oligosaccharides, like the bovine ones, contain Neu5Gc as well as Neu5Ac.

Bovine and caprine colostrum contain more N-acetylneuraminyllactose/lactosamine than N-glycolylneuraminyllactose/lactosamine, in contrast to ovine colostrum which contains more of the latter and equine colostrum in which oligosaccharides containing Neu5Gc have not been detected [14–16,20,22,23,25]. These difference must be due to differences in the activity of CMP-sialic acid hydroxylase in the lactating mammary glands of these species. Since equine erythrocytes contain Neu5Gc to the exclusion of Neu5Ac and bovine erythrocytes contain mainly Neu5Gc [26], the differences in the activity of this enzyme are tissue-specific as well as species-specific.

Sialyl lactose lactones

Sialyl oligosaccharides such as Neu5Ac/Gc(α2-3)Gal(β1-4)Glc or Neu5Ac/Gc (α2–6)Gal(β1-4)Glc, being acidic, have sucessfully been purified using anion exchange chromatography. Nakamura et al. [20], however, detected sialyl oligosaccharides from ovine colostrum in the unadsorbed or weakly adsorbed fraction during anion exchange chromatography. It was suspected that these oligosaccharides could be lactones of sialyllactose [27], in which the carboxyl group of sialic acid is blocked by dehydration via the OH group of the sialic acid residue at C-7 or of the galactose residue at either C-2 or C-4. The main saccharides in these fraction were indeed determined by NMR spectroscopy to be lactones of Neu5Gc(α2-3)Gal(β1-4)Glc. The results showed that ovine colostrum contains at least two such lactones, but their exact structures have not yet been fully elucidated.

Sialic oligosaccharides units such as Neu5Ac(α2-6/3)Gal(β1-)R in glycoconjugates are receptor sites for the binding of influenza virus to the bronchus or gut mucosa [28,29]. Influenza virus has a haemagglutinin, which is a lectin binding to sialic acid, as well as neuraminidase which releases non-reducing sialic acid from sialyl oligosaccharide units [28–30]. Influenza viruses infect the bronchus or gut through the ability of haemagglutinin to bind to sialyl oligosaccharide units in cell surface glycoconjugates. Free sialyl oligosaccharides, when ingested via colostrum or milk, should inhibit the attachment of influenza virus to the intestinal mucosa, but when their sialic acid is released by the action of virus neuraminidase, the non-sialylated product does not bind to virus haemagglutinin. The sialyllactose lactone of ovine colostrum, however, may be resistant to the action of virus neuraminidase even though it may bind to haemagglutinin, but this has not as been established. This hypothesis suggests that the sialyllactose lactones may be better inhibitors of the attachment of influenza virus to the gut mucosa than their parent sialyllactose, i.e. they may be significant as anti-infection substances for influenza virus.

Oligosaccharide derivatives with phosphate or sulphate

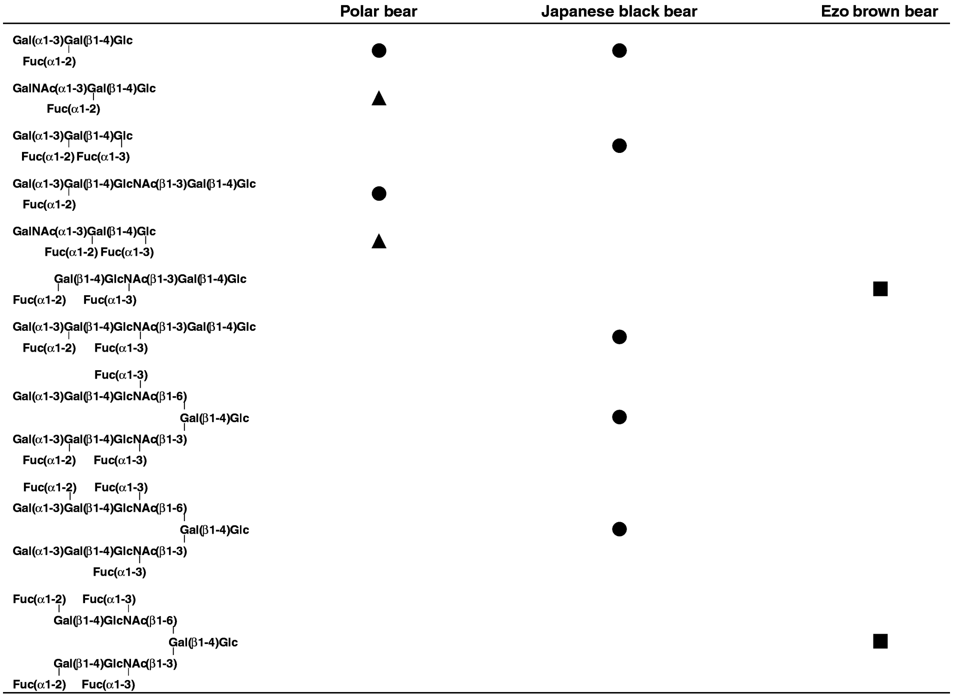
Human milk has been suggested, on the basis of Fab-MS spectra, to contain N-acetylneuraminyllactose sulfate, but its structure was not fully established [31]. In addition, human milk contains a number of sulfated fucosyl oligosaccharides [32]. Two phosphorylated sialyl oligosaccharides, Neu5Ac(α2-6) Gal(β1-4)GlcNAc-1-phosphate and Neu5Ac(α2-6)Gal(β1-4) GlcNAc-6-phosphate are present in bovine colostrum [33,34]. Two phosphorylated non-sialyl oligosaccharide, Gal(β1-4) Glc-3′-phosphate and Gal(β1-4)GlcNAc-1-phosphate have been reported in bovine [35] and equine [25] colostrum, respectively (Table 1).

Carnivorous mammals (*bears, coati and seal*)

Among eutherian mammals, bears appeared to be exceptional in that their milk was found to contain only small amounts of lactose relative to oligosaccharides [37]. None of these oligosaccharides had, however, been studied in detail until recently. The chemical structures of the milk oligosaccharides of the Ezo brown bear (*Ursus arctos yesoensis*) [38], Japanese black bear (*Ursus thibetanus japonicus*) [39] and the polar bear (*Ursus maritimus*) [40] have now been determined with the aid of NMR spectroscopy (Table 1).

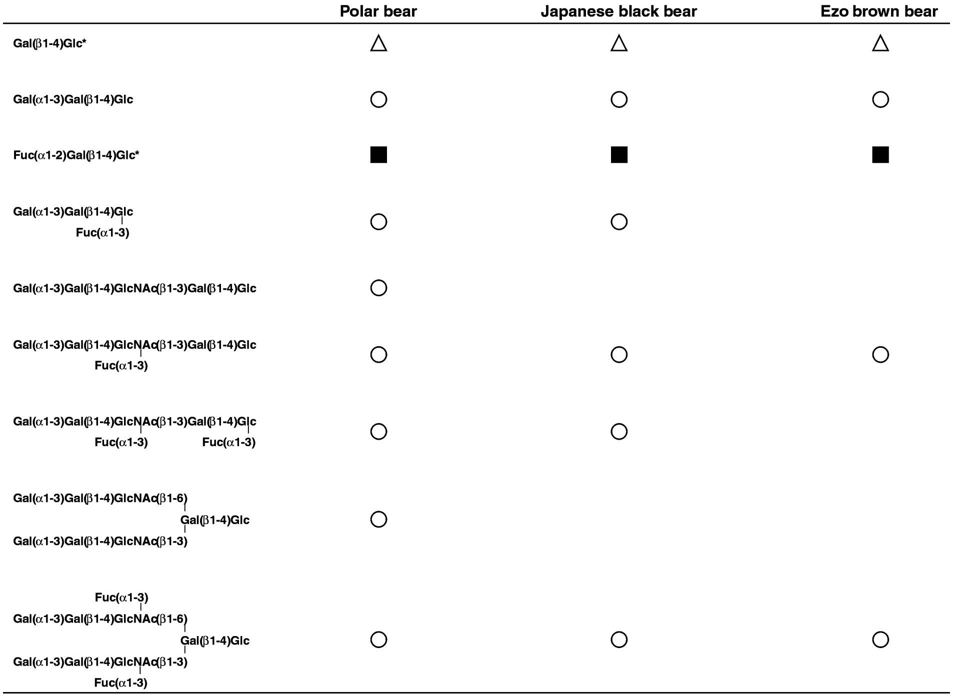
The dominant saccharide in Japanese black bear milk at 22 to 37 days post partum was found to be Gal(α1-3)[Fuc(α1-2)]Gal(β1-4)[Fuc(α1-3)]Glc (B-pentasaccharide), whereas Gal(α1-3)Gal(β1-4)Glc was the most prominent from 76 to 91 days post partum. Gal(α1-3)Gal(β1-4)Glc was the dominant saccharide in polar bear milk at both 4 and 27 months post partum. In Ezo brown bear milk at 71 days post partum, Fuc(α1-2)Gal(β1-4)Glc was the most dominant saccharide followed by Gal(α1-3)Gal(β1-4)Glc. Lactose was found to be only a very minor component in the milk of all three species of bears.

The chemical structures of milk oligosaccharides of the three species are compared in Figures 2 and 3. Figure 2 shows



	Polar bear	Japanese black bear	Ezo brown bear
Gal(α1-3)Gal(β1-4)Glc Fuc(α1-2)	●	●	
GalNAc(α1-3)Gal(β1-4)Glc Fuc(α1-2)	▲		
Gal(α1-3)Gal(β1-4)Glc Fuc(α1-2) Fuc(α1-3)		●	
Gal(α1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc Fuc(α1-2)	●		
GalNAc(α1-3)Gal(β1-4)Glc Fuc(α1-2) Fuc(α1-3)	▲		
Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc Fuc(α1-2) Fuc(α1-3)			■
Gal(α1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc Fuc(α1-2) Fuc(α1-3)		●	
Fuc(α1-3) Gal(α1-3)Gal(β1-4)GlcNAc(β1-6) Gal(β1-4)Glc Gal(α1-3)Gal(β1-4)GlcNAc(β1-3) Fuc(α1-2) Fuc(α1-3)		●	
Fuc(α1-2) Fuc(α1-3) Gal(α1-3)Gal(β1-4)GlcNAc(β1-6) Gal(β1-4)Glc Gal(α1-3)Gal(β1-4)GlcNAc(β1-3) Fuc(α1-3)		●	
Fuc(α1-2) Fuc(α1-3) Gal(β1-4)GlcNAc(β1-6) Gal(β1-4)Glc Gal(β1-4)GlcNAc(β1-3) Fuc(α1-2) Fuc(α1-3)			■

Figure 2. The milk oligosaccharides with A (▲), B (●) and H (■) antigens of the polar bear, the Japanese black bear and the Ezo brown bear.



	Polar bear	Japanese black bear	Ezo brown bear
Gal(β1-4)Glc*	△	△	△
Gal(α1-3)Gal(β1-4)Glc	○	○	○
Fuc(α1-2)Gal(β1-4)Glc*	■	■	■
Gal(α1-3)Gal(β1-4)Glc Fuc(α1-3)	○	○	
Gal(α1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc	○		
Gal(α1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc Fuc(α1-3)	○	○	○
Gal(α1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc Fuc(α1-3) Fuc(α1-3)	○	○	
Gal(α1-3)Gal(β1-4)GlcNAc(β1-6) Gal(β1-4)Glc Gal(α1-3)Gal(β1-4)GlcNAc(β1-3)	○		
Fuc(α1-3) Gal(α1-3)Gal(β1-4)GlcNAc(β1-6) Gal(β1-4)Glc Gal(α1-3)Gal(β1-4)GlcNAc(β1-3) Fuc(α1-3)	○	○	○

Figure 3. The milk oligosaccharides with the α-Gal epitope (○) of the polar bear, the Japanese black bear and the Ezo brown bear. *These saccharides do not contain the α-Gal epitope.

the structures of saccharides containing the human group A (GalNAc(α1-3)[Fuc(α1-2)]Galβ1-R), group B (Gal(α1-3)[Fuc(α1-2)]Galβ1-R) and group H (Fuc(α1-2)Galβ1-R) antigens, while Figure 3 shows saccharides containing the α-Gal epitope (Gal(α1-3)Gal(β1-4)GlcNAcβ1-R). Most of the bear milk oligosaccharides contain lactose, lacto-N-fucopentaose III (Gal(β1-4)[Fuc(α1-3)]GlcNAc(β1-3)Gal(β1-4)Glc) or difucosyl lacto-N-neohexaose (Gal(β1-4)[Fuc(α1-3)]GlcNAc(β1-3){Gal(β1-4)[Fuc(α1-3)]GlcNAc(β1-6)}Gal(β1-4)Glc) as core units from which the structures are built by the addition of ABH antigens or the α-Gal epitope. The milk of all three bear species contains oligosaccharides with the α-Gal epitope, but polar bear milk contains saccharides with the A and B antigens, Japanese black bear milk contains those with the A antigen, and Ezo brown bear milk contains those with the H antigen. It is still unknown whether this heterogeneity is species-specific because an insufficient number of samples have been analysed so far; Urashima et al. [38–40] used samples from two polar bears, four Japanese black bears and only one from an Ezo brown bear.

The oligosaccharide patterns of bear milk are very different from those of human milk or colostrum. Human milk oligosaccharides contain a series of 12 core units (Figure 1) to which Le^a, Le^b, Le^d or Le^x and/or N-acetylneuraminic acid are often attached at the non-reducing end. There are only a few oligosaccharides with A or B antigens in human milk, although these units are shown to occur in the glycoconjugates of human tissues, including red blood cells. Oligosaccharides with the α-Gal epitope have not been found among the free oligosaccharides of human milk or human tissue glycoconjugates. Human blood contains antibodies against the α-Gal epitope, showing that this epitope is foreign to humans, unlike the situation in bears.

Of the oligosaccharides with the α-Gal epitope the simplest, Gal(α1-3)Gal(β1-4)Glc, has been found in bovine [7], ovine [19], and caprine [21] colostrum and in white nosed coati milk [41]. The presence of higher oligosaccharides with the α-Gal epitope has not yet, however, been reported in milk of other species, except the white-nosed coati (see below). Although the α-Gal epitope has been extensively found in mammalian tissue glycoconjugates, it is a rarity among free oligosaccharides. Free oligosaccharides containing A and B antigens have so far not been found in any non-human milk and/or colostrum except bears. In view of these findings, the presence of free oligosaccharides containing the α-Gal epitope and group A and B antigens can be regarded as characteristic of bear milk.

Some heterogeneity of milk oligosaccharides was observed between two female polar bears (Figure 4). The milk of female (1) contained oligosaccharides with A or B antigens including A-tetra and B-tetrasaccharides, whereas that of female (2) contained only oligosaccharides with A antigen including A-tetra and A-pentasaccharide. No oligosaccharide with B antigen was found in the milk of female (2). The GlcNAc residues of the higher oligosaccharides, which contained a lacto-N-neotetraose or lacto-N-neohexaose unit, were not substituted by an α(1–3) linked Fuc residue. With the exception of this animal, the GlcNAc residues of the bear

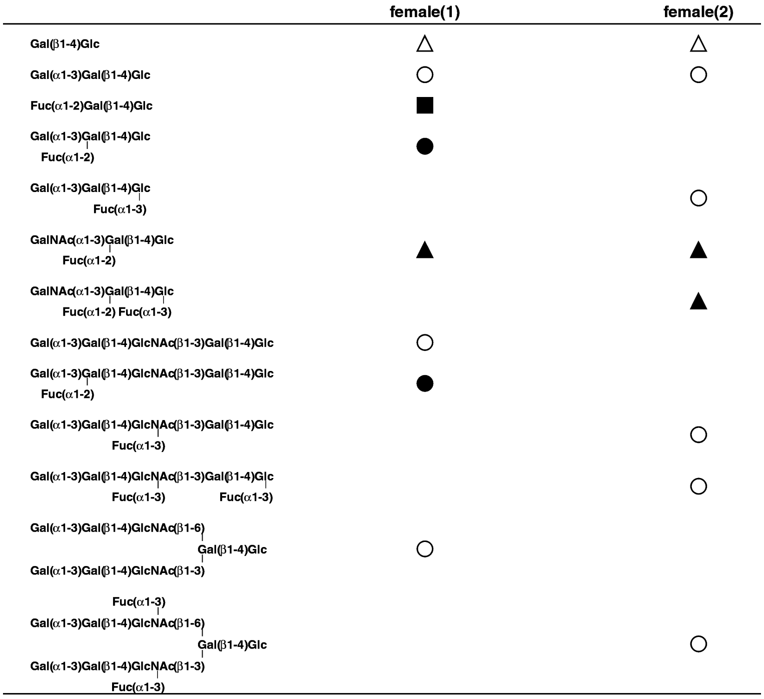


Figure 4. Oligosaccharides detected in polar bear milk from two animals.

milk oligosaccharides studied so far were always found to be substituted by an α(1–3) linked Fuc residue. The mammary glands of polar bear female (1), but not those of other bears, presumably lack the appropriate α(1–3)fucosyltransferase activity which transfers Fuc from GDP-Fuc to the GlcNAc residue of an N-acetyllactosaminyl unit (Gal(β1-4)GlcNAc-R). In contrast to the findings with polar bears, heterogeneity of milk oligosaccharides was not observed in four females of the Japanese black bear [39].

To clarify whether this unique pattern of milk oligosaccharides is also found in related species of the Order Carnivora, the milk of a white nosed coati (*Nasua narica*, Procyonidae) was studied [41]. It was found that the concentration of trisaccharides in the coati milk was similar to that of lactose and, in addition, the milk contained other oligosaccharides (Table 1). The ratio of oligosaccharides to lactose was lower in the coati milk than in bear milk. The dominant oligosaccharide was the trisaccharide Fuc(α1-2)Gal(β1-4)Glc. The higher oligosaccharides contained lacto-N-neotetraose or lacto-N-neohexaose as core units and also had the H antigen or the α-Gal epitope at their non-reducing ends. The oligosaccharide structures resembled those of the Ezo brown bear, except that the GlcNAc residues of coati oligosaccharides were not substituted by α(1–3) linked Fuc. Thus, some homology was observed between the oligosaccharides of the white nosed coati and the Ezo brown bear.

Studies on the milk sugars of another carnivorous mammal, the hooded seal (*Crystophora cristata*, Order Pinnipedia) [42] showed that the concentration of a trisasaccharide (Fuc(α1-2)Gal(β1-4)Glc), was again similar to that of lactose and higher oligosaccharides were also detected. These oligosaccharides contained lactose, lacto-N-neotetraose, lacto-N-neohexaose and para-lacto-N-neohexaose as core units and had the H antigen at their non-reducing ends. This oligosaccharide pattern resembled the patterns seen in the white-nosed coati and the Ezo brown bear insofar as the oligosaccharides contained lacto-N-neotetraose or lacto-N-neohexaose as core units and H antigen at their non-reducing ends. However, the concentration of lactose and oligosaccharides in the hooded seal milk was much lower than those in the bear and coati milk. The milk of pinnipeds generally contains a lot of fat with little or no carbohydrate, and their young metabolise fat in preference to carbohydrate as their main energy source [43].

Although the milk of phocids such as the hooded seal and of the crabeater seal contains both lactose and free oligosaccharides [42,44,45], that of a number of otariid species, including the California sea lion [46], the Northern fur seal [47], and the Australian fur seal [42] contains neither. The biosynthesis of lactose, and of saccharides containing lactose at their reducing ends, is dependent on the presence of α-lactalbumin within the mammary gland. This protein has been shown to be entirely absent from mammary glands of the California sea lion [48], consistent with the absence of lactose or oligosaccharides in the milk of this species. It is likely that α-lactalbumin is absent also from the mammary glands of the Australian and the Northern fur seal and perhaps all otariids, but not from those of phocids.

The above data show that there is some similarity between the three species of bears, the white nosed coati and the hooded seal in relation to the chemical structures of their milk oligosaccharides and the ratio of oligosaccharides to lactose. It can be hypothesized that the ratio of oligosaccharides to lactose and the variety of oligosaccharides in milk might have been relatively high in the common ancestor of Ursidae, Procyonidae, Phocidae and Otariidae and, during the course of evolution, increased in the Ursidae but decreased in Phocidae. Both lactose and other saccharides disappeared entirely from the milk of Otariidae due to loss of the synthesis of α-lactalbumin. Support for this hypothesis would require studies on milk samples from a greater number of Carnivora and other species.

Other eutherians

The milk of many other eutherian species contains a variety of oligosaccharides [2] but the chemical structures of these are mostly unknown [3]. Recent studies using high-pH anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) suggest, surprisingly, that elephant milk contains relatively high concentrations of oligosaccharides including at least seven neutral and four acidic oligosaccharides (Table 1) [49].

Rat milk contains sulphated oligosaccharides, determined as Neu5Ac(α2-3)Gal(β1-4)Glc-6′-SO_3 [31,50,51] and Gal(β1-4)Glc-6′-SO_3 [51], in addition to significant concentrations of the unsulphated saccharide [52]. A canine oligosaccharide was determined to be Gal(β1-4)Glc-3′-SO_3 [53]. It is interesting that the sulfated positions are different in the two species. An additional significant feature of rat milk is the presence of 6-O-β-D-galactopyranosyl-myoinositol [54].

Monotremes (Infraclass Prototheria)

Monotremes have been regarded as primitive mammals because they exhibit some reptile-like features, including the fact that their young hatch from eggs. The time at which the monotremes diverged from the lineage of the other mammals is still very uncertain, although most authorities agree that it occurred during the Jurassic period. Calculations based on comparisons of α-lactalbumin sequences have suggested a date of about 175 million year ago [55], consistent with the most recent paleontological evidence [56].

Because of the unique position of the monotremes among mammalian species their milk sugars have been of special interest. In 1973, Messer and Kerry reported that free lactose was no more than a minor component of the milk saccharides of two monotremes, the platypus and the echidna [57]. The major oligosaccharides of echidna milk were found to be sialyllactose and fucosyllactose while that of the platypus was a tetrasaccharide, difucosyllactose. Subsequent studies showed that platypus milk contains low concentrations of other oligosaccharides [58]. The chemical structures of the monotreme milk oligosaccharides, as determined by NMR and by sequential exoglycosidase digestion [59,60] are shown in Table 1. The echidna sialyllactose was characteristised by the unique presence of an O-acetyl group at C-4 of its Neu5Ac residue [61,62]. Almost all of the oligosaccharides of platypus milk were found to be based on lacto-N-neotetraose or lacto-N-neohexaose as core units and they all contained fucose [60]. In eutherian mammals, fucose is present in small amounts only as part of the oligosaccharide moieties of glycoproteins. However, the concentration of saccharide-bound fucose in monotreme milk, about 0.2 ∼ 0.9%, is so large that it far exceeds the requirement for glycoproteins. One is therefore forced to conclude either that suckling monotremes, unlike other mammals, utilize fucose as an energy source, or that fucose has an unknown specific biological significance for these animals.

Marsupials (Inflaclass Metatheria)

The major differences between marsupials and placental mammals lies in their reproductive strategies. In marsupials the period of gestation is very short and, as a result, marsupials at birth are very altricial, i.e. small and undeveloped. In contrast, the period of lactation is long, much longer than in placental mammals of similar size.

The first analyses of the milk of marsupials were done by Bolliger and colleagues during the 1950's. The most surprising finding was the virtual absence of free lactose from the milk of the wallaroo (a macropod marsupial) and the brushtail possum and the presence of large amounts of other oligosaccharides of unknown structure [63,64]. Some 20 years later, Messer and his colleagues separated the milk oligosaccharides of two macropods, the grey kangaroo and the tammar wallaby, by gel chromatography on Sephadex G-25 [65,66] and subsequently determined their structures, mainly by ^{13}C-NMR spectroscopy (Table 1). The major neutral oligosaccharides of tammar wallaby milk included a trisaccharide, β1-3 galactosyllactose (Gal(β1-3)Gal(β1-4)Glc) [67], and a series of homologues comprising tetra- to hepta-saccharides [68]. These appear to be produced through the action of a β1-3 galactosyltransferase catalysing the addition of successive galactosyl residues to the non reducing end of the growing chain, beginning with lactose [69]. Tammar wallaby milk also contains a branched series of neutral oligosaccharides which contain GlcNAc [9,70], whose biosynthesis within the lactating tammar mammary gland involves a specific β6-N-acetylglucosamyltransferase [71]. In addition there is a third group of acidic oligosaccharides which contain sialic acid and a β(1–3) galactosyl sequence and two units of N-acetyllactosaminyl branches [72].

The concentration of oligosaccharides in the milk of these macropods is very high compared with that of other mammals (up to 13% in the tammar wallaby), and to show very considerable variations (1 ∼ 13%) with the stage of lactation [65,66].

Studies using mainly thin layer chromatography have shown that oligosaccharides very similar to or identical with those found in tammar wallaby milk are present in the milk of all other marsupials so far studied, including the red-necked wallaby [73] and several non-macropod marsupials such as the eastern quoll [74], brushtail and ringtail possums [75,76], the koala [77], as well as a South American species, *Monodelphis domestica* [78]. The available evidence thus strongly suggests that the presence of high concentrations of a variety of oligosaccharides in their milk, and of low concentrations of free lactose, is characteristic of marsupials.

Comparison of structures of milk oligosaccharides

Although there are many differences between placental mammals, monotremes and marsupials, the three Infraclasses have in common the fact that their young are suckled on milk which is produced from mammary glands. Further, the milk of almost all mammals contains lactose, either in the free form or at the reducing ends of oligosaccharides. Consistent with this is the fact that, with the exception of some pinnipeds [42,46,47], the milk of all species including the platupus [79] and the echidna [80], contain α-lactalbumin.

There are, however, major differences between placental mammals, monotremes and marsupials in the nature of their milk saccharides. In most placental mammals—with some important exceptions—free lactose is the predominant saccharide. By contrast, the milk of monotremes and almost all marsupials contains very little free lactose. Instead, monotreme milk contains lactose mainly in the form of tri- and tetrasaccharides and is rich in fucose while the sugars of marsupial milk consist largely of a series of galactosyl saccharides and their derivatives which range in size from tri- to at least octasaccharides. This type of oligosaccharide is quite unique because, with the exception of the trisaccharide, they have never been detected in the milk or colostrum of eutherians or monotremes. It is noteworthy that, in marked contrast to monotreme and eutherian milk oligosaccharides, none of the marsupial milk oligosaccharides contain fucose, suggesting that fucosyltransferase are absent from the mammary glands of lactating marsupials. These enzymes may have been lost in ancestral marsupials subsequent to their evolutionary divergence from eutherian mammals. It should also be noted that most of the monotreme milk oligosaccharides are found also in the milk of eutherians but not in that of marsupials. In this respect, therefore, eutherian mammals are more closely related to monotremes than to marsupials.

Gastrointestinal digestion and absorption of milk oligosaccharides

It is well known that in suckling eutherian mammals, the lactose in milk is normally digested to glucose and galactose by a β-glycosidase (lactase-phlorizin hydrolase, lactase), which is a transmembrane protein located in the microvilli of the brush border of the small intestinal mucosa. The products (glucose and galactose) are actively transported into the enterocyte and enter the blood circulation. One may ask whether milk saccharides other than lactose are digested and absorbed in a similar manner, but the answer is almost certainly in the negative. Even simple oligosaccharides such as fucosyllactose or sialyllactose cannot be digested by lactase; enzymes which would be required for their digestion, in this case α-fucosidase and neuraminidase, respectively, are not found in the brush border of the small intestine. Recent data showing that human milk oligosaccharides are resistant to enzymatic hydrolysis in the upper small intestine support this conclusion [81].

It is likely that a small proportion of milk or colostrum oligosaccharides is metabolically modified within the infant small intestine because the faeces of newborn babies have been found to contain significant quantities of A-active oligosaccharides including A-penta-, A-hexa and A-hepta-saccharides; these are probably synthesized from smaller oligosaccharides, such as A-tetrasaccharide (GalNAc(α1-3)[Fuc(α1-2)]Gal(β1-4)Glc) and lacto-N-fucopentaose I (Fuc (α1-2)Gal(β1-3)GlcNAc(β1-3)Gal(β1-4)Glc), which are found in breast milk and colostrum [82]. In addition, some of these oligosaccharides appear to be absorbed intact, perhaps via receptor-mediated endocytosis [83], and are then excreted in the urine. The major part, however, survives passage through the small intestine and enters the colon, where a proportion is fermented by the colonic microbial flora [84], the remainder being excreted *via* the faeces [85].

Little is as yet known about the fate of milk oligosaccharides in other placental mammals. There is evidence that in some species, milk oligosaccharides may be transported into the enterocytes of the small intestine by pinocytosis and hydrolysed to monosaccharides by lysosomal enzymes such as acid β-galactosidase, α-galactosidase, α-fucosidase, neuraminidase and β-N-acetylglucosaminidase. This seems to be the case in the suckling rat, whose lower small intestine absorbs milk immunoglobulins and other macromolecules by pinocytosis and contains a very active, presumably lysosomal, neuraminidase. It has been proposed [86] that the sialyllactose present in rat milk is passively transported into the enterocytes along with macromolecules and is then digested by the lysosomal neuraminidase. The free lactose produced thereby would then be susceptible to the lysosomal acid β-galactosidase.

A similar mechanism has been suggested for the digestion of milk oligosaccharides in marsupials. Histochemical and biochemical evidence has shown that lactase activity is completely absent from the brush border of the small intestine of suckling tammar wallabies and other marsupials. A very active acid β-galactosidase, however, was detected in the lysosomes and the supranuclear vacuoles of the enterocytes [87,88]; this β-galactosidase, unlike the eutherian brush border lactase, was able to digest β-(1–3) galactosides such as those found in tammar wallaby milk. These results led to the hypothesis that in suckling tammars the milk oligosaccharides are transported intact into the absorptive cells of the small intestine, presumably by pinocytosis or endocytosis, and are then hydrolysed to monosaccharides by the lysosomal acid β-galactosidase and other lysosomal enzymes including neuraminidase and N-acetylglucosaminidase [89]. In this way suckling tammars would be able to utilize the milk oligosaccharides as an energy source. With respect to monotremes, Stewart et al. [90] showed, biochemically, that a neutral lactase is absent from the small intestine of suckling echidnas but that several lysosomal glycosidases are present, including an acid β-galactosidase, neuraminidase and α-fucosidase. It was concluded that in suckling echidnas, as in marsupials, milk oligosaccharides are digested intracellularly by these lysosomal enzymes.

It may be that similar intracellular digestion occurs in the young of eutherian species, such as bears, in which the milk contains a greater concentration of oligosaccharides than lactose. This possibility obviously requires further investigation.

Biological functions of milk oligosaccharides

At one time it was thought that human milk oligosaccharides may have no specific function and that they arise neither by specific synthesis nor by degradation of glycolipids. Instead, they could be the incidental result of the high concentration of lactose found in milk, the disaccharide serving as a glycosyl acceptor for the various soluble glycosyltransferases found in milk [91]. This view is no longer held by leading authorities in this field (see below). We have seen, moreover, that in several species of mammals lactose is only a minor component and other oligosaccharides are dominant. In such species, milk oligosaccharides are likely to have specific biological functions but there is still no definite or single answer to the question of what these functions might be.

In those species, such as monotremes, marsupials, and bears in which milk oligosaccharides constitute a significant proportion of the total milk solids [38–40,57,65], they would presumably be important as energy sources. In view of the very high concentrations of oligosaccharides in the milk of marsupials compared with that of eutherians, it is difficult to envisage any other hypothesis for the main functions of marsupial milk oligosaccharides. It is noteworthy that these species are very altricial at hatching (monotreme) or birth (e.g. marsupials, bears) and it is possible that altricial neonates have a special need for milk oligosaccharides [92]. This need may not be restricted to species whose young are very small at birth relative to the size of the mother but may be related more specifically to neurological immaturity. For example, it has been recently suggested that in species such as humans and elephants, whose central nervous system is not fully developed at birth, the galactose and sialic acid present in milk oligosaccharides may be required for optimal development of the infant brain [49].

Altriciality or neurological immaturity may, however, be only one of a number of factors to be considered in relation to the possible functions of milk oligosaccharides. It is worth noting that the osmotic pressure exerted by a given mass of oligosaccharides dissolved in milk is lower than that exerted by an equal mass of lactose. This could be significant as it permits milk to contain a greater concentration of saccharides and electrolytes without it becoming hyperosmotic. One can speculate, therefore, that altricial newborns may have a greater requirement for saccharides and/or electrolytes such as sodium and potassium, than precocial newborns [66,74].

Some milk oligosaccharides may have specific functions. For example, the sulphated oligosaccharides present in rat milk may be of nutritional significance. Although sulfate is not an essential nutrient in mature mammals, the activity of the rate-limiting enzyme, cystein dioxygenase, develops only slowly postnatally in rats, suggesting that sulfate may be an essential nutrient in the neonate. In an experiment using [^{35}S], N-acetylneuraminyllactose sulfate was found to be hydrolysed in the gut of rat neonates, and the sulphur absorbed as inorganic sulfate [31]. The presence of this compound may permit the simultaneous delivery of two essential nutrients, sulfate and calcium, in early life, avoiding the precipitation of insoluble calcium sulfate in the milk [31]. The phosphorylated oligosaccharides found in bovine and equine colostrum [25,33–35], may similarly permit the simultaneous delivery of phosphate and calcium, avoiding the precipitation of calcium phosphate.

By way of contrast, it has been suggested that, in human infants, the function of milk oligosaccharides is primarily protective rather than nutritional [85]. Oligosaccharides may inhibit the adhesion of pathogenic microorganisms to the intestinal and urinary tract by acting as receptor analogues, thus preventing gastric and urinary infections. In addition milk oligosaccharides may function as prebiotics, promoting the growth of benign microorganisms, such as *Bifidobacterium bifidus*, within the lower gastrointestinal tract, and inhibiting the proliferation of pathogenic organisms. One can speculate that these oligosaccharides could have similar functions in non-human species. For example, the Gal(α1–3)Gal(β1-4)GlcNAc sequence in rabbit glycolipids was identified as a receptor for the binding of *Clostridium difficile* toxin A [93]. It is possible that the trisaccharide Gal(α1-3)Gal(β1-4)Glc, which is found in bovine [7], ovine [19], caprine [21] colostrum and bear [38–40], coati [41] and elephant [49] milk, is an inhibitor of the binding of this toxin to the intestinal mucosa in the suckling young of these species. Glycolipids containing N-glycolylneuraminic acid in the form of the trisaccharide sequence, Neu5Gc(α2-3)Gal(β1-4)Glc, were recognised to be receptors for the binding of *Escherichia coli* K99, an organism which can cause life-threatening diarrhoea in piglets [94]. The free trisaccharide, which is found in bovine [14,16,23] and ovine [20] colostrum, may therefore be an inhibitor of the binding of pathogenic organisms to the intestinal mucosa of newborn calves and lambs.

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