Sialic Acid Is an Essential Nutrient for Brain Development and Cognition

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Key Words

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Abstract

The rapid growth of infant brains places an exceptionally high demand on the supply of nutrients from the diet, particularly for preterm infants. Sialic acid (Sia) is an essential component of brain gangliosides and the polysialic acid (polySia) chains that modify neural cell adhesion molecules (NCAM). Sia levels are high in human breast milk, predominately as *N*-acetylneuraminic acid (Neu5Ac). In contrast, infant formulas contain a low level of Sia consisting of both Neu5Ac and *N*-glycolylneuraminic acid (Neu5Gc). Neu5Gc is implicated in some human inflammatory diseases. Brain gangliosides and polysialylated NCAM play crucial roles in cell-to-cell interactions, neuronal outgrowth, modifying synaptic connectivity, and memory formation. In piglets, a diet rich in Sia increases the level of brain Sia and the expression of two learning-related genes and enhances learning and memory. The purpose of this review is to summarize the evidence showing the importance of dietary Sia as an essential nutrient for brain development and cognition.

Contents

INTRODUCTION

Newborn infants undergo rapid growth and development, particularly with respect to their nervous system. The rate of initial brain growth exceeds that of any other organ or body tissue, and by 2 years of age, the brain is about 80% of the adult weight. Infants born preterm or small for their gestational age are particularly vulnerable in early life. Advances in reproductive and neonatal technologies have increased the proportion of infants born early or small for gestational age (20); however, long-term neurodevelopmental outcomes of preterm infants remain poor and are characterized by lower academic performance, attention deficit, hyperactivity disorder, anxiety disorders, and learning difficulties (8, 16). These cognitive deficits are likely to cause an overriding central nervous system (CNS) impairment with underlying brain structural changes (16, 17).

Rapid brain growth places exceptionally high demands on the supply of precursors and nutrients. These nutrients can serve as building blocks or as preformed units for newly synthesized tissues and cell growth. Failure to meet overall nutrient needs during this crucial period of brain growth has significant consequences for cognitive development (91). However, the importance of nutrition for brain development has remained an understudied area. Understanding the molecular basis of early nutrition on neurodevelopment is a major challenge facing investigators because of the complexity of the unanswered questions that are of increasing clinical and public health importance (187). As a consequence, many key questions of seminal importance remain unanswered (91). For example, long-chain polyunsaturated fatty acids, particularly docosahexaenoic acid (DHA) from human milk, have been routinely added to preterm infant formula as a constituent thought to enhance brain development, although the molecular details underlying this supposition remain obscure.

Other essential nutrients, however, have now been shown to enhance brain development, cognition, and memory in animals (99, 203). One such nutrient is sialic acid (Sia), a family of 9-carbon sugar acids (**Figure 1**), which is a key monomeric building block of brain gangliosides, and glycoproteins including polysialic acid (polySia). Sia is also an important constituent of human milk and many conventional foods. This review focuses on the current state of knowledge of the functional role and underlying molecular and biochemical mechanism of dietary Sia as an essential nutrient for cognitive development, learning, and memory in humans.

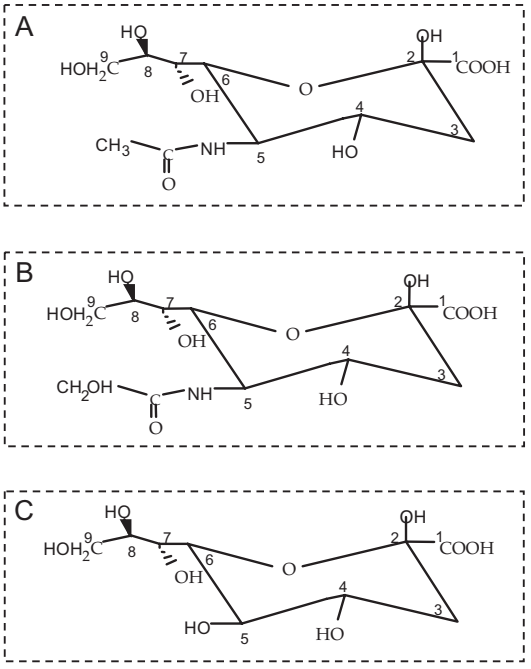

Figure 1

Members of the sialic acid (Sia) family. (*a*) *N*-acetylneuraminic acid (Neu5Ac) (present in neuroinvasive bacteria, human tissues, and foods). (*b*) *N*-glycolylneuraminic acid (Neu5Gc) (present in trout eggs, some foods, and human tumors). (*c*) Ketodeoxynonulosonic acid (KDN) (present in fish eggs and ovarian fluid, human fetal red blood cells, and ovarian cancers).

OCCURRENCE AND STRUCTURAL FEATURES OF SIA AND POLYSIA IN MAMMALS

General Properties and Structure of Sia in Mammalian Tissue

Sia is the generic term for the family of *N*- and *O*-substituted derivatives of neuraminic acid,

Sia: sialic acid

Neu5Ac: *N*-acetylneuraminic acid

Neu5Gc: *N*-glycolylneuraminic acid

KDN: ketodeoxynonulosonic acid

a 9-carbon acidic sugar molecule. More than 50 naturally occurring derivatives of Sia have been described (199). *N*-acetylneuraminic acid (2-keto-5-acetamido-3,5dideoxy-D-glycero-D-galactononulosonic acid; Neu5Ac) is the most common form of Sia in human glycoconjugates, including glycoproteins, glycosaminoglycans, gangliosides, and mucins (136, 149), and is expressed ubiquitously throughout the human body (**Figure 1**) (136). These negatively charged sugars are found in all vertebrate cell types and within essentially all tissues and body fluids, but are found less commonly in prokaryotes, plants, or invertebrates (151, 194). Many sialylated structures account for the remarkable structural diversity of glycoconjugates that function in neural development, synaptic transmission, cognition, and memory formation (11, 130) and in immune function (41, 76) and that serve as decoys for invading pathogens (129).

Hydroxylation, one of the protons on the *N*-acetyl group at C5 of Neu5Ac, gives rise to another type of Sia referred to as *N*-glycolylneuraminic acid (Neu5Gc) (**Figure 1**). Unlike Neu5Ac, Neu5Gc is expressed in all nonhuman primates, including our closest relatives, the great apes (102). Although Neu5Gc was once thought to be an oncofetal antigen expressed only on some human cancers, it is now known that humans express Neu5Gc on glycoconjugates at relatively low levels. This Neu5Gc is likely derived from eating a diet rich in red meat and milk products (9). Importantly, all humans have anti-Neu5Gc antibodies (113), which has potentially important implications for the biopharmaceutical industry, particularly for the development of human therapeutics and neutraceuticals, including postnatal dietary Sia supplementation for enhancing brain development and cognition.

In 1988, Inoue et al. reported another form of Sia present in sperm and fish eggs in which the acyl group on the C5 carbon atom was deaminated, giving rise to 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) (73) (**Figure 1**). Subsequent studies showed that human ovarian cancer cells and ascites cells obtained from these ovarian cancer patients contained elevated levels of free KDN compared with normal controls (74). No Neu5Gc was detected in these ovarian tumors, and comparatively little KDN was found conjugated or in CMP-KDN. High levels of free KDN were also found in fetal newborn cord red blood cells (RBCs). Interestingly, the amount of free KDN in fetal cord RBCs was ~2.4-fold higher than in RBCs from the mothers or from healthy nonpregnant woman (74). Lower levels of KDN were found in mononuclear cells. Free KDN was also identified in normal human ovaries. Although the significance of these findings is not clear, they suggest two possibilities. First, the enhanced expression of KDN in ovarian adenocarcinomas may be an "oncofetal" antigen, analogous to the oncodevelopmental expression of polySia in a number of human cancers. Thus, KDN could be a potential early warning signal for onset of disease or could serve as a marker for recurrence of the cancer. Second, the elevated level of KDN expression in fetal cord compared with maternal RBCs suggests that it may be developmentally related to RBC formation during embryogenesis. The potential role of KDN in normal development and malignancy is an important area for future exploration in this relatively new area of "sialobiology," or more specifically, "KDNology" (74).

General Properties and Structure of PolySia on NCAM in Mammals

PolySia is an unusual biological form of Sia that is a linear homopolymer composed most commonly of negatively charged α2-8-linked Neu5Ac residues. The polySia glycotope covalently modifies surface glycoconjugates on cells as evolutionarily diverse as microbes and man (136, 193). In mammalian cells, the Sia monomers (Neu5Ac, Neu5Gc, and KDN) can be dimerized (diSia), oligomerized (oligoSia), or polymerized (polySia) into linear, anionic chains consisting of repeating α2,8-linked sialyl residues (146, 194). Although many oligoSia/polySia chains in neural tissue consist of chain lengths with a degree of polymerization

(DP) of ~50 to 60 Sia residues, a subpopulation of chains with DPs ~150 to ~180 and extending to DP ~400 have been described on NCAM in polysialyltransferase transfected neuroblastoma cells, embryonic chick brain (106), and human natural killer cells (41).

Surprisingly, the pre-existence of α2,8-linked diSia and oligoSia structures with chain lengths up to 7 Sia residues was shown by Sato et al. to occur on a large number of embryonic and adult pig brain glycoproteins, including NCAM (145). Unexpectedly, these sialylated glycoproteins spanned the Mr range from >200 kDa to <45 kDa, thus expanding even further the diverse repertoire of surface glycoproteins expressing di-, oligoSia residues. These structurally distinctive polySia chains in mammalian neural cells are of critical importance in neurobiology, as they posttranslationally modify and dynamically regulate the function of NCAM during CNS development (106). Recent studies have shown that human natural killer cells isolated from peripheral blood leukocytes also contain polySia chains attached to the oligosaccharide chains on NCAM and that expression of this oncofetal glycotope modulates immune response (41). Chains with DP ~400 have been found on subpopulations of these immune cells. Interestingly, IL-2 activation of these cells produced more polySia chains than resting cells (41). The functional significance of this structural diversity remains to be determined.

NCAM is a member of the immunoglobulin superfamily of adhesion molecules and is widely expressed on the surface of cells in the CNS. It is the major carrier protein of polySia in vertebrate cells and functions to regulate cell migration, neurite outgrowth, axon elongation, and synaptic formation and plasticity (80, 141). During ontogeny, expression of polySia is abundant in nervous tissue, decreases shortly after birth but is expressed in selected regions of the adult brain undergoing neural plasticity (120, 127, 163) and in the regenerating nervous system (122). Recent studies demonstrate regeneration-promoting effects of polySia-NCAM in the motor system (2, 59). In vitro culturing of glial and neuronal cells on polySia showed that this glycotope promoted survival of the motor neurons and dorsal root ganglion neurons and viability of ventral mesencephalic progenitor cells in vitro (65). PolySia also provides a favorable substrate, particularly for culturing of Schwann cells, and showed no toxic effects for peripheral nerves, including sensory neurons and motorneurons (65). Schwann cells, the myelin-forming cells of the peripheral nerve, play a crucial role during regeneration. It is thus postulated that polySia may be a novel polymer for bioimplants to be used in tissue-engineered approaches for peripheral nerve reconstruction and cell transplantation strategies to restore damaged areas of the CNS (65).

DP: degree of polymerization

PolySia-NCAM: polysialic acid (polySia) on neural cell adhesion molecules (NCAMs)

Occurrence and Structural Features of Gangliosides in Humans

Gangliosides are complex glycosphingolipids that all contain one or more Sia residues, often in a terminal position. They are ubiquitously distributed in the cell membranes of all vertebrate cells. They constitute ~6% to 10% of the total lipid mass of the human brain, where they represent a quarter of the total conjugated saccharides and 70% to 80% of the conjugated Sia (188). The ganglioside family derives its name from the ganglion cells from which they were first isolated, so it is not surprising that the greatest number of ganglioside species is found in neural tissue and that their biological roles in this tissue are best understood.

Structurally, all gangliosides are amphipathic molecules that contain the hydrophobic ceramide moiety, derived from the amino alcohol, sphingosine, and a single fatty acid chain. Both the ceramide and fatty acid moieties show variations in their structures. The fatty acyl chain is attached to the ceramide moiety via an amide bond (87, 137, 179). The hydrophilic moiety is composed of terminal sialylated oligosaccharide chains that commonly contain the neutral sugars, glucose (Glc), galactose (Gal), N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc), fucose (Fuc), and the acidic Sia (Neu5Ac, Neu5Gc, and

GNE: UDP-N-acetylglucosamine-2-epimerase (UDP-GlcNAc-2-epimerase, EC 5.1.3.14)/*N*-acetylmannosamine kinase (ManNAc Kinase, EC 2.7.1.60)

CMP-Sia: cytidine monophosphate-sialic acid

CTP: cytidine triphosphate

KDN) residues. There is remarkable structural diversity in the oligosaccharide chains, which are linked to the ceramide moiety via a glycosidic bond. The number of Sia residues in the oligosaccharide chain are also used to classify the different ganglioside species, which are designated M (monosialo), D (disialo), etc. (175). More than 200 different ganglioside structures have been reported, but in higher vertebrates, 80% to 90% are composed of GM1, GD1a, GD1b, and GT1b (136) (**Figure 2**). The composition of the saccharide moiety is cell-type specific, depends on the developmental stage of the organism, and can change with the oncogenic state of a cell. The biological properties of gangliosides appear to be more influenced by the structure of their oligosaccharide portion than the membrane-bound ceramide (215). The ceramide moiety is of particular importance, however, in mediating a number of transmembrane-signaling pathways (63). They reside in the plasma membranes of vertebrate cells, presenting their oligosaccharide chains on the cell surface (87).

METABOLISM AND BIOSYNTHESIS OF SIA AND POLYSIA IN HUMANS

Biosynthesis of Sia in the Newborn: Importance of the Bifunctional Enzyme UDP GlcNAc-2-epimerase/ ManNAc Kinase (Gne)

In addition to dietary sources, humans and all other mammals have the capacity to synthesize Sia endogenously in all tissues starting with glucose or other product of glycolysis. However, the bifunctional enzyme UDP-N-acetylglucosamine-2-epimerase (UDP-GlcNAc-2-epimerase, EC 5.1.3.14)/N-acetylmannosamine kinase (ManNAc Kinase, EC 2.7.1.60) (Gne) is a key enzyme for initiating conversion of UDP-GlcNAc into ManNAc and regulating Sia biosynthesis (171, 185). ManNAc is an early substrate in the Sia biosynthetic pathway and is well upstream of the feedback inhibition site for this pathway [cytidine monophosphate-Sia (CMP-Sia) synthase]. The activated form of Sia, CMP-Sia, is synthesized in the nucleus (79), and its synthesis is regulated by feedback inhibition. Although GNE has been localized in the cytosol, it is also found in the nucleus (84), where Sia is activated. In this pathway, ManNAc-6-P condenses with phosphoenol pyruvate via an aldol condensation reaction to form the first Sia derivative, Neu5Ac-9-phosphate. A specific phosphatase dephosphorylates Neu5Ac-9-P to form Neu5Ac, which is then activated by cytidine triphosphate (CTP) to form its nucleotide donor, CMP-Neu5Ac.

An outline of the reactions involving the biosynthesis of Sia is shown in **Figure 3**. In rat liver, the activity of the rate-limiting moiety of UDP-GlcNAc-2-epimerase, is initially low, and rises to a maximum 15 days after birth (60). In the guinea pig, the activity is also low at birth and rises to a maximum level as in rats (60), interestingly, however, the activity of the epimerase in guinea pig liver is 2–5-fold lower than in rats (60). On this basis, we have postulated that newborn human infants might have a lower capacity for synthesizing Sia to meet the high demand for its use as a building block for polySia-NCAM and gangliosides, particularly in preterm infants. Inactivation of the epimerase by gene targeting causes embryonic lethality in mice (155).

Recently we carried out a molecular characterization of the Gne gene and examined its expression profile in 37-day-old piglets with and without dietary Sia supplementation using quantitative real-time PCR (207). We found that the Gne gene of piglets is highly expressed in the hippocampus, liver, and thymus, followed by the pancreas, frontal cortex, lungs, kidneys, heart, and spleen (207). The lowest level of expression was in skeletal muscle. Dietary Sia supplementation in developing piglets with active learning significantly increased the Gne mRNA expression levels two- to threefold in brain hippocampus and liver. These findings suggest that the enzyme activity is modified not only by dietary Sia, but also by other environment

stimulations. Therefore, up-regulation of the Gne gene and its cognate GNE enzyme provides the elevated levels of intracellular Sia required to meet the demands for the increased level of sialylated glycoconjugates, including polySia and gangliosides, during neural development (14, 207). Thus, Sia is an essential nutrient during periods of rapid neural growth and brain development in the newborn. Newborn human infants may not have adequate levels of Sia to meet the high demand for brain sialylglycoconjugate synthesis (204, 211). An exogenous source of dietary Sia is thus required for optimal and rapid brain growth during early neural development.

Importance of the Sialyltransferase Family of Enzymes in Synthesis of Sialylated Glycoconjugates

The biosynthesis of sialylated glycoproteins and glycolipids is catalyzed by a family of enzymes designated the sialyltransferases, which catalyze the transfer of a Sia from its activated sugar nucleotide precursor, CMP-Sia (CMP-Neu5Ac, CMP-Neu5Gc, or CMP-KDN) to the glycan moiety of an endogenous acceptor. More than 20 different human sialyltransferase cDNAs have been cloned and characterized and are classified into four linkage-specific families based on their acceptor substrates and types of glycosidic linkages. The *ST3Gal family* transfers Neu5Ac residues in α2,3-linkage to terminal Gal residues in glycoprotein and glycolipids. The *ST6Gal family* has two subfamilies, ST6Gal I and II, which both use Galβ1-4GlcNAc-R as the acceptor substrate. The *ST6GalNAc family* transfers Neu5Ac residues in α2-6 linkage to the GalNAc residues found in *O*-glycosylated proteins (ST6GalNAc I, II, and IV) and in glycolipids (ST6GalNAc III, V, and VI).

ST8Sia Family. The ST8Sia family catalyzes the transfer of Neu5Ac residues in α2,8-linkage to other Neu5Ac residues found in glycoproteins and glycolipids. Human sialyltransferases are type II transmembrane glycoproteins that reside predominantly in the lumen of the trans-Golgi compartment (66). They all contain a short *N*-terminal cytoplasmic tail, a unique transmembrane domain, and a stem region of variable length from 20 to 200 amino acids, followed by a large *C*-terminal catalytic domain (66). They catalyze the formation of different glycosidic linkages (α-2,3-, α-2,6-, α-2,8-, α-2,9-) and all differ in their acceptor specificities. Although most of these sialyltransferases catalyze the transfer of a single Sia residue to the nonreducing terminus of the glycan acceptor, an exception is the polysialyltransferases family described below.

There is limited information on the molecular details of the active sites of these enzymes, their mechanism of catalysis, or on the cellular mechanisms regulating their transcriptional expression (66). How the developmental changes of enzyme activities and glycoprotein glycosylation are controlled during the postnatal period is still not fully understood. In the small intestine of the rodent, the activity level of α-2,3- and α-2,6-sialyltransferases for both *N*- and *O*-glycans is high during the first week after birth, then declines until weaning is complete, and remains low thereafter (12, 27, 36). Concomitantly, the activity of the intestinal neuraminidases, the enzymes that cleave Sia from sialylglycoconjugates, is high in the mucosa of rat pups after birth then falls steadily until the end of lactation. This corresponds to the developmental profile of Sia levels in rat milk (38, 85). Dickson et al. suggested that the neuraminidases are digestive enzymes for the release of Sia from the various sialyloligosaccharides in milk (38). Thus, Sia released from dietary sources may likely serve as a substrate for synthesis of sialoglycoproteins and gangliosides by the sialyltransferases in young mammals. Interestingly, early-weaning rat pups had a slightly higher level of sialyltransferase activity in the small intestine than did late-weaning pups (64), yet synthesis of the donor substrate, CMP-Neu5Ac, catalyzed by CMP-Neu5Ac synthase, was low in suckling rats and rose to the maximal level between 30 and 60 days of age (133).

Biosynthesis of PolySia in Humans and Vertebrates

ST8Sia II and ST8Sia IV: α-2,8-sialyltransferase II and IV/ polysialyltransferases II & IV

Two Golgi-associated polysialyltransferases (polySTs), designated ST8Sia II and ST8Sia IV, are responsible for synthesis of the α2,8-linked polySia chains in vertebrate cells (44, 109, 152). The first report of polyST activity in brain tissue was by Troy and colleagues in 1985 (93). It was not until a decade after the discovery of brain polyST activity that both enzymes were cloned and expressed in mammalian cells (44, 109, 152, 195). Both polySTs catalyze the transfer of Sia from its activated sugar nucleotide precursor, CMP-Sia, to its major acceptor substrate, NCAM (28, 93). The two enzymes are highly conserved, but their expression is independently regulated in a time-dependent and tissue-specific manner. ST8Sia II is the dominant enzyme in the embryonic and early postnatal mouse, whereas ST8Sia IV prevails in the adult (70).

Based on transfection experiments, it was reported that ST8Sia II and ST8Sia IV can function cooperatively in polySia synthesis, giving rise to presumably longer polySia chain on NCAM than when the enzymes were used individually (7, 108). This observation was not confirmed, however, in reconstituted in vitro studies using purified polySTs and FcNCAM as an exogenous substrate. The DP was found to be essentially identical (106). Expression of the ST8Sia II and ST8Sia IV is independently regulated in time and is tissue specific. ST8Sia IV prefers the *N*-glycan on the sixth glycosylation site of the *N*-glycan on NCAM, but ST8Sia II does not show this preference (7). Although it has been shown that both ST8Sia II and ST8Sia IV can be autopolysialylated in vitro and in transfected cells (28, 29), there is no evidence that ST8Sia II is polysialylated in vivo (134).

ST8SIA II and ST8Sia IV show limited structural homology and are structurally and functionally distinct from the larger family of monosialyltransferases (192). Most notably, the polySTs all contain a unique basic amino acid domain of 32 residues immediately upstream and contiguous with the sialylmotif S domain (107). This cationic domain is essential for polysialylation, and now provides a selective target for therapeutics designed to inhibit polyST synthesis (107). PolySia expression is required for the metastatic spread of a number of human cancers (192). How dietary Sia can modulate expression of ST8Sia II and/or ST8Sia IV genes in the brain hippocampus and frontal cortex remains a challenging problem for future studies. Equally challenging will be studies to elucidate why di-, tri-, and oligosialylation is such a prevalent posttranslational modification on so many neural membrane glycoproteins.

Biosynthesis and Catabolism of Gangliosides

Ganglioside biosynthesis is carried out by a family of membrane-bound glycosyltransferases localized on the luminal face of the endoplasmic reticulum or Golgi membranes. These highly specific transferases catalyze the sequential transfer of each sugar from their activated sugar nucleotide precursors, e.g., CMP-Sia, UDP-GlcNAc, UDP-GalNAc, UDP-Gal, UDP-Glc, or GDP-Fuc, to the nonreducing terminus of the oligosaccharide chain on the endogenous acceptor. The addition of Sia residues to the terminal position of the ganglioside oligosaccharide moiety is catalyzed by different members of the sialyltransferase family, as described above (128). The contribution of glial cells to ganglioside synthesis is difficult to distinguish from that of neurons. Using cultured, radiolabeled hippocampal neurons, the major changes in ganglioside synthesis were shown to be restricted to the early stages of neuronal development, namely axonogenesis and rapid axon elongation (71). The significant increase in the synthesis of complex gangliosides (GM1, GD1a, GD1b, and GT1b) correlated with a reduction in the synthesis of glucosylceramide and ganglioside GD3 during axonogenesis (71). The ratio of A- to B-series gangliosides increased significantly during the stage of rapid axon growth (71).

Following ganglioside synthesis, the glycolipids are transported in membrane vesicles through the Golgi apparatus. This mechanism of intracellular trafficking is analogous to how all membrane-associated, and many secretory components, are translocated. This process initiates in the endoplasmic reticulum or *cis*-Golgi region, traverses to the medial-Golgi, and then to the trans-Golgi compartment before being transported and intercalated into the plasma membranes by vesicular translocation processes (212).

Catabolism of gangliosides occurs by the sequential action of specific lysosomal exoglycosidases that catalyze the sequential release of sugars from the nonreducing terminus of the oligosaccharide chain. A member of the family of sialidases usually initiates sugar chain degradation of the higher gangliosides (180), which are ultimately hydrolyzed in lysosomes to sphingosine and monosaccharides (191). Cytoplasmic and plasma membrane–associated sialidases have also been reported and may facilitate the recycling or reutilization of free Sia. Genetic defects in specific glycosphingolipid catabolic enzymes lead to a number of severe, often fatal, glycolipid-storage diseases, including Tay-Sachs, Gaucher, Krabbe, Fabry, Sandhoff, and Sialidosis, among others.

DISTRIBUTION OF SIA IN MAMMALS

Expression of Sia in the Central Nervous System

Sia is widely distributed throughout human tissues, the abundant source being found in the CNS. Neural cell membranes contain ~20-fold higher levels of Sia than other cellular membranes, suggesting Sia plays a unique structural and functional role in neural tissue (150, 203, 209). The predominant form of Sia in the mammalian brain is Neu5Ac, but in all nonneuronal organs and body fluids, except in humans, Neu5Gc is common (197). The majority of Sia in the human CNS is present in gangliosides (65%) and glycoproteins (32%), with free Sia accounting for the remaining 3% (21). Indeed, brain is the only organ in mammals that contains a higher level of Sia in gangliosides than in glycoproteins. Gangliosides are not uniformly distributed even within the human brain; the highest concentration is found in neuronal membranes, localized particularly at synapses where they exist in clusters surrounding membrane-bound proteins (3, 176). Human cerebral gray matter contains gangliosides at a level of 890 μg/g wet tissue, which is ~3-fold higher than the corresponding white matter, 15-fold greater than many large visceral organs including liver, lung, and spleen (181), and 500-fold higher than the intestinal mucosa (87, 177, 203). Human cerebella gray matter contains gangliosides at ~275 μg/g wet tissue, and the expression level follows the same trend as in cerebral gray matter, except that the level is about 70% lower than that of cerebral gray mater (181). The Sia concentration in the left lobe of the brain cortex is ~22% higher than that of the right lobe (209), suggesting that Sia levels may correlate with different biological functions associated with the left and right hemispheres (203). The highly concentrated amount of Sia in the adult brain gray matter underscores the essential role that Sia plays in neural structures and function (203, 209).

Human brain contains at least 12 structurally different types of gangliosides (**Table 1**), with GM1, GD1a, GD1b, and GT1b being the major species. The concentration and distribution of gangliosides change significantly during development. The relative increase of gangliosides during this period is more rapid than that of phospholipids. In humans, the level of brain gangliosides increases ~3-fold from the tenth gestational week to about 5 years of age. Gangliosides GM1 and GD1a increase ~12-fold to 15-fold during this same period. The most rapid increase in GM1 and GD1a occurs in the first two years of life, during the period of dendrite arborization, and the forebrain is characterized by neuronal differentiation, outgrowth of axons, and synaptogenesis (177). The profile of GD1b and GT1b expression is interesting because these are the predominant gangliosides during and shortly after the third trimester.

Table 1 The percent distribution of ganglioside Sia in normal human white matter and gray matter[a]

Type of ganglioside	Gray matter	White matter
GM1	14.9	21.6
GD1a	21.7	8.8
GD1b	18.2	16.9
GT1b	16.3	11.1
GM4	1.5	8.6
GM3	2.7	4.8
GM2	4.1	2.5
GD3	5.4	8.8
GT1a	1.8	2.2
GD2	8.0	3.1
GQ1b	5.0	2.7
GD1a- GalNAc	0.4	1.1

[a]Values represent means using high-performance thin-layer chromatography to quantitatively determine the amount of gangliosides Sia (4). Normal human white matter contains ganglioside-bound Sia 275 μg/g wet tissue, and gray matter contains 875 μg/g wet tissue.

However, their concentration declines rapidly, reaching its minimum around birth. Thereafter, GD1b and GT1b increase steadily to at least 50 years of age (173, 174). Developmental expression of total brain ganglioside levels in the human during the period from tenth fetal week to 80 years of age is summarized in **Figure 4** (177). The distribution of gangliosides in the gray matter of human adult brain is relatively enriched in the di- and polysialogangliosides, the rank order from high to lower expression being GD1a, GD1b, GTlb, and GM1. In white matter, the order is GM1, GD1b, GD1a, and GT1b (4). This implies that the activity of key biosynthetic glycosyltransferases required for ganglioside synthesis is not the same in various tissues (196). Thus, brain ganglioside synthesis is both tissue specific and developmentally regulated, underlying the fundamental role they play in tissue development and organization (197).

Our earlier studies demonstrated that the total Sia concentration in the human brain was 2-fold to 4-fold higher than in eight other mammalian species (209). These comparative differences have implications for evolutionary development and intellectual capacity. The negative charges on Sia residues on the cell membrane gangliosides facilitate the binding of positively charged neurotransmitters (149). Furthermore, changes in polysialylation occur during neurite cell migration, axonal growth, and synaptogenesis (108, 183).

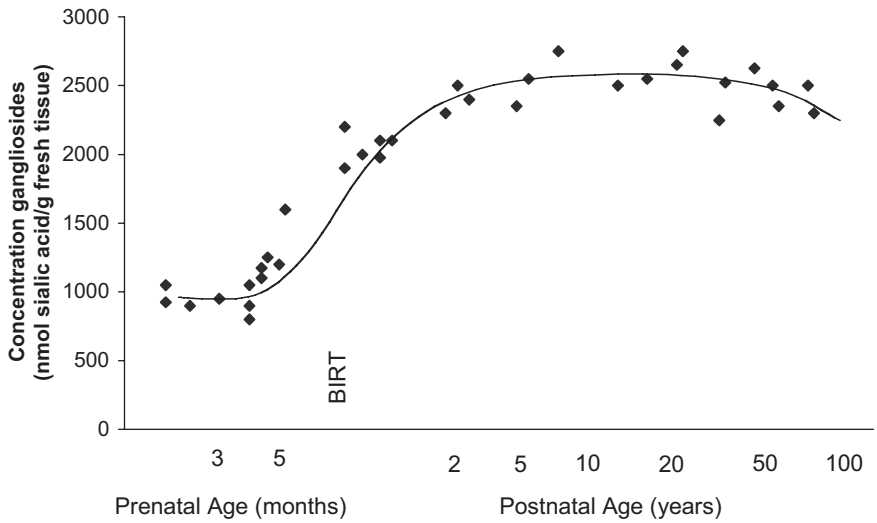

Figure 4

The total ganglioside concentration in human frontal cortex from early fetal stage to advanced age. Figure adapted from Svennerholm et al. (177).

Svennerholm et al. (179) reported that the concentration of major glycolipids in the frontal and temporal cortices and white matter of the human brain diminished continually from 20 years of age, with a 15% to 20% loss by 100 years of age. Gangliosides were an exception to the rule, with a decline of only 6% to 8%, most of which occurred after 70 years of age (178, 179). This provides further evidence in support of the hypothesis that gangliosides are essential throughout life and perhaps have a higher resistance to degradation than do other major brain lipids.

Expression and Distribution of PolySia in the Central Nervous System

In mammals, polySia-NCAM is both quantitatively and qualitatively distributed differently in the developing and adult nervous system (13). Although polySia is present in the cell-surface capsular polysaccharides of several neuroinvasive bacteria, including *E. coli* K1 and *Neisseria meningitidis* groups B and C (194), only six molecules in mammals are known to express polySia. These include two transmembrane glycoproteins, NCAM (53, 135, 189) and the α-subunit of the voltage sensitive sodium channel (220), a secreted glycoprotein present in human milk, CD36 (217), and neurophilin-2 in human lymphocytes (35). As NCAM is the major carrier of polySia, this moiety is expressed at high levels during early embryonic development and is associated with both neural and glial precursors during this period. The cellular distribution of polySia-NCAM can vary depending on both the developmental stage and specific region of the brain. Polysialylated NCAM is abundant in the developing nervous system; however, the majority of NCAM in adult tissues lacks this unique glycan. PolySia expression persists, however, during neuronal regeneration and in regions of the brain showing plasticity, including the hippocampus, dentate gyrus, amygdala, and olfactory bulb, as noted above. PolySia-NCAM is not detected in the neuroepithelium, ventricular zone, and spinal cord in the early phases of neurogenesis (114, 157, 159, 160). High expression levels of polySia-NCAM are found, however, during embryonic and early postnatal development in retinal ganglion cell axons, all glial cells, the optic nerve, neuroblasts, postmitotic neurons, Müller cells, and astrocytes (10). This suggests that some histogenetically plastic functions for these cells in the early and adult visual system (10). Highly polysialylated cells are unable to adhere to each other due to the steric hindrance and negative charge repulsion between cells. The repulsion of adjacent polySia chains and the imbibing of water cause an expansion of these space-filling molecules that occupy greater steric space (218). This prevents the binding of opposing NCAM molecules and other cell surface molecules. The intracellular concentration of Sia in the cell can regulate the polysialylation state of NCAM (15). On the basis of these facts, we inferred that increased amounts of Sia in dietary supplemented animals might increase the extent of polysialylation during development. Thus polySia, as an antiadhesive glycotope, plays critical roles in neural development by modulating the adhesive property of NCAM. The expression of polySia in the hippocampus throughout life facilitates the remodeling of synaptic connections, aids in neurogenesis, and facilitates neuronal migration (156). The hippocampus and surrounding regions of the medial temporal lobe are largely responsible for short-term memory formation and memory consolidation. Accordingly, polySia-NCAM involves a wide range of morphogenic events, including cell migration, neurite outgrowth, path finding, sprouting, regeneration, and synaptic plasticity (108, 132, 211, 214).

Expression of Sia in Extraneuronal Organ Systems

Sia is widely distributed throughout human tissues and body fluids, including saliva, gastric juice, serum, urine, tears, cerebrospinal fluid, and human milk (203). The concentrations vary physiologically with age, ethnicity, and normal/abnormal Sia metabolic status, as well as

dietary Sia intake. The normal range of total Sia level in serum/plasma of healthy individuals is 1.58 to 2.22 mmol L^{-1}, mostly bound to protein (~2 mmol L^{-1}), gangliosides (10 to 50 μmol L^{-1}), and then free Sia (~0.5 to 3 μmol L^{-1}) (165). Interestingly, Japanese have relativity lower serum Sia levels than do Americans (1.64 ± 0.15 versus 1.80 ± 0.18 mmol L^{-1}) (89) and Europeans (1.83 to 1.98 mmol L^{-1}) (165). This may reflect differences in the genetic, metabolic status and dietary intake of Sia between the different ethnic groups. Increases in the concentration of serum Sia levels have also been observed during pregnancy (33), inflammatory disease, atherosclerotic processes, diabetes, and some diseases related to cell injury. There is no significant difference in serum Sia levels in pre, peri-, and postmenopausal women (34). In various types of human cancers, increased levels of total serum glycoproteins and/or gangliosides have been reported, and in some cases, these levels have correlated with the degree of metastasis (165). Low levels of Neu5Gc have been observed in some human cancers, but this is of no diagnostic value as Neu5Gc is now known to be present in normal humans who have consumed a diet rich in red meat and milk products (184). No Neu5Gc was found in RBC membranes from healthy humans or in plasma glycoproteins or gangliosides from type A, B, AB, and O donors (23). Total plasma or urine levels of Sia are nonspecific for a given disease, but the presence of polySia is an important biomarker for a number of human cancers (192–194).

The level of Sia in 24-hour urine samples from healthy human donors is age related and shows an increase from early life to adulthood (51). These values range from 67.6 to 444.0 mumol/day in total Sia levels, 27.5 to 217.1 mumol/day in free Sia, and 40.1 to 226.9 mumol/day in protein/ganglioside-bound forms (51). In sialuria, a rare defect with excessive synthesis of Sia, free Sia levels can be elevated 70- to 200-fold (83). In these patients, there was no correlation between serum and urine Sia concentrations (83). Wang et al. showed that breast-fed, preterm infants had nearly twice the level of bound Sia in saliva as bottle-fed infants during the first three months of life (210). This finding confirmed an earlier study on full-term infants by Tram et al. (190). Consequently, the concentration of Sia in body fluids may play a structural and functional role in protecting mucosal surfaces (204). A summary of the concentration of Sia in human body fluids has been published (203).

SIA LEVELS IN HUMAN MILK AND CONVENTIONAL FOOD PRODUCTS

Human Breast Milk is a Rich Source of Sialyloligosaccharides

Human breast milk is unique among milk of eutherian mammals, as it is a rich source of more than 200 human milk oligosaccharides (HMOs) (186), and about 100 different complex structures have been elucidated (37, 203). These HMOs comprise the third-largest class of breast-milk solids after lactose and fat (75, 86). The level of HMOs ranges between ~21 to 24 g/L in colostrum and ~12 to 14g/L in mature milk (31). In contrast, the level of milk oligosaccharides in cow's colostrum is ~20- to 30-fold lower (0.7 to 1.2g/L) than in human milk (200). Neutral oligosaccharides, many of which are fucosylated, and acidic oligosaccharides, nearly all of which are sialylated, constitute the respective core molecules of HMO. The different structural and conformational types of these HMOs account for their different biological functions. Key biological functions of the HMOs, aside from providing a source of Sia and a number of growth factors and hormones, are thought to be their action as carbohydrate antagonists of intestinal bacterial infection in infants and their ability to stimulate growth of favorable intestinal microorganisms. Some of these natural oligosaccharides have been used as nutraceutical products in milk to enhance the presumed usefulness of HMOs, although there remains a paucity of information on the ideal carbohydrate structure to use and the efficacy of such supplementation.

The core sialyloligosaccharides in HMO comprise the trisaccharide sialyllactose, which consists of lactose at the reducing terminus and a Sia residue at the nonreducing end (112). Sialyllactose is one of the major components of HMO. More than 40 different sialyloligosaccharide structures, comprising ~50% of the identified structural HMO, have been described. These are classified under the following eight groups: Lactose, Lacto-N-tetraose, Lacto-N-neotetraose, Lacto-N-hexaose, Lacto-N-neohexaose, Lacto-N-octaose, iso-N-octaose, and "Deviant" (203). Sialyllacto-N-tetraose c, 6′-sialyllactose, and disialyllacto-N-tetraose are the most representative constituents in the family of sialyloligosaccharides (31, 172).

Sia is present in human milk sialyloligosaccharides as Neu5Ac (75, 203), attached to a penultimate galactose or GlcNAc residue via an α2-3 or α2-6 glycosidic linkage. These sialyl residues are synthesized by one of the specific monosialyltransferases, as described above (67, 75, 203). However, α2-6-linked sialylated oligosaccharides are present in significantly greater proportion than the α2-3-linked structures during early lactation (172, 203). This implies that α2-6 sialyltransferase activities are higher during early lactation in humans.

In contrast to the remarkable structural diversity in the family of HMOs, nearly 40 oligosaccharides are detected in bovine colostrum using high-performance mass spectrometry and separation method (186). Bovine milk oligosaccharides have a shorter oligomeric chain than those HMOs, with nearly 70% containing at least one Sia and at least seven of them containing Neu5Gc (186). Thus, the structure of milk sialyloligosaccharide is species specific. The structure of sialyloligosaccharides in human breast milk was summarized in a recent publication (186).

Comparative Features of Sia in Human and Bovine Milk and Infant Formula

Human milk contains relatively large amounts of sialylated oligosaccharides that are not found in significant quantities in bovine milk or infant formulas. The concentration of Sia in human breast milk varies with genetic, geographic, and dietary intake of the birth mothers. Furthermore, the variation in the reported concentration and distribution of these oligosaccharides in human milk is due in part to differences in methodology that result from the lack of standardized methods of analysis. However, all human breast milk studies have shown that the highest concentration of Sia is found in colostrum, with a gradual decrease as lactation progresses in women throughout different countries. We determined the range of Sia concentrations in Australian human breast milk and commercial infant formulas. The concentration of total Sia was the highest in colostrum (3.72 ± 0.15 mmol^{-1}) at full term and declined to 2.64 ± 0.14 mmol^{-1} seven to ten days after birth. This level declined further to 1.48 ± 0.07 mmol^{-1} after one month and to 0.73 ± 0.05 mmol^{-1} three months after birth. Pre-term human milk contained 13% to 23% more Sia than full-term milk throughout the first month of lactation (204). Coppa et al. reported that the concentration of the three major sialyloligosaccharides, sialyllacto-N-tetraose c, 6′-sialyllactose, and disialyllacto-N-tetraose, in human breast milk from women in Italy constituted ~60% to 70% of the total amount of all sialyloligosaccharides (w/w) (31). The sialyloligosaccharide levels showed a gradual decrement as lactation progressed, the concentration at day 90 being ~30% to 50% of that present in colostrum (31). However, the content of 3'-sialyllactose in human breast milk remained relatively stable at 0.32 to 0.55 mmol^{-1} throughout lactation, while that of 6′-sialyllactose showed a gradual decline during lactation (30).

The levels of Sia in glycoproteins and gangliosides reached maximum values in colostrum and gradually decreased as lactation progressed. Wang et al. showed that the total amount of glycoprotein-bound Sia in human breast milk declined during lactation from 1.18 ± 0.09 mmol^{-1} in colostrum to 0.29 ± 0.02 mmol^{-1} at 92 to ~121 days (204).

Human κ-casein, the major glycoprotein in breast milk, contains more carbohydrate by weight (40% to 60%) than bovine κ-casein (10%) (138). The oligosaccharide moiety of human κ-casein is rich in Sia compared with that of bovine κ-casein (90). κ-casein is a major source of glycoprotein-bound Sia in mature human breast milk (138). The human milk whey proteins, e.g., secretory IgA, contain ~12% carbohydrate by weight, whereas lactoferrin is composed of only ~6% carbohydrate by weight (138). Lactoferrin consists of an *N*-acetyllactosamine glycan with Sia at the nonreducing terminus (170). In an intriguing recent finding, Yabe et al. reported that α-2,8-linked polySia chains were expressed on the glycoprotein, CD36, in human breast milk. CD36 is a member of the B class of the scavenger receptor superfamily (217). PolySia was not detected in CD 36 from other species' milk. The concentration of polySia on the CD36 glycoprotein peaked one month after parturition and gradually decreased as lactation progressed (217). The function of polySia expression on such an important receptor molecule in human breast milk remains unknown. These findings thus underscore again the significant differences between human breast milk and bovine milk.

The Sia in gangliosides accounts for only ~1% of the total Sia in human milk and is found in the milk-fat globule membranes. GM3 is the major ganglioside in mature human milk (123, 139), and GD3 is the predominate ganglioside present in human colostrum and in mature bovine milk (123, 139). Other gangliosides species contribute <20% of the total amount of milk gangliosides. The total amount of ganglioside-bound Sia in human colostrum and mature human milk is about 9.51 ± 1.16 mg/L and 9.07 ± 1.15 mg/L, respectively (123). In contrast, the total amount of ganglioside-bound Sia in mature bovine milk is significantly lower (3.98 ± 0.25 mg/L) (123). Thus, both glycoprotein and ganglioside fractions in human breast milk contain lower levels of Sia than that of the free oligosaccharide fraction. Sia is one of the most variable fractions in human milk. The changes of concentration and distribution of Sia during lactation can be interpreted as a consequence of the aging process of cell secretion (201) and in accord with the physiological demands of the human infant.

Because sialylated oligosaccharides represent one of the predominate branches of the HMO blood group classifications, it is not surprising that the concentration is different among countries. Interestingly, there was a threefold difference in Sia levels among mothers at the same stage of lactation, even though the concentration varied within the source (94, 204). Viverge et al. reported that variation in the sialylated oligosaccharides is linked to the ABO blood group status of the mother (201). The high levels of Sia and low levels of galactose distinguished ABH secretors from nonsecretors. In the ABH secretor groups, A and H secretors had higher *N*-acetylglucosamine levels than did B and AB secretors, and lower galactose levels. The Lewis secretor groups were distinguished by having significantly higher fucose levels. Variations in the composition of sialyloligosaccharides during lactation between individuals may reflect changes in the complement of biosynthetic enzymes required for their synthesis. Such differences may also reflect a programmed adaptation of the milk composition to fit the need of the infant (94). Finally, genetic differences resulting from changes within the synthetic capacity or in environmental exposure to invasive microorganisms may be an additional factor. Currently, environmental factors, e.g., diet, have not been shown to influence oligosaccharide expression in human milk, but oligosaccharide expression itself is heterogeneous because of genetic variation among individual mothers. Further clinical studies are clearly needed in this area to better define/demonstrate the importance of these different factors. It will be of particular importance to determine how maternal dietary intake of a Sia-rich diet may influence the concentration of sialylated glycoconjugates in human breast milk and other secretions and tissues.

Despite advances in the design and formulation of infant formulas to better mimic human breast milk, the concentration of Sia

in bovine-milk-based infant formula is significantly lower than that in human breast milk. As noted above, both bovine milk and bovine-milk-based infant formulas contain sialyl- and neutral-oligosaccharide structures that are very different from those of humans. We studied 21 different infant formulas and found they contained less than one-quarter of the total amount of Sia found in one-month mature human milk. There was also a tenfold difference in Sia concentration among the different types of formula (204). The majority of Sia (70%) in infant formulas was bound to glycoproteins, whereas in human breast milk, most of the Sia (69% to 76%), whether from the preterm or full-term groups and irrespective of the stage of lactation, was present as free sialyloligosaccharides. In human breast milk, a smaller fraction of the Sia (21% to 28%) was bound to glycoproteins, while 3% was present as free Sia (204). This is in contrast to infant formulas, where only ~0.3 to 1.5% was present as free Sia.

Another striking difference between human breast milk and bovine milk or bovine-based infant formula is the presence of significant levels of Neu5Gc in all bovine-based milk products, and only trace levels in human milk. As described above, humans lack nearly completely the "animal" Sia, Neu5Gc, in all tissues, including human breast milk (184). In contrast, bovine milk contains about 3% of its total Sia as Neu5Gc (184), which therefore is a natural contributor to all bovine-milk-based infant formulas. Neu5Gc is found in human fetal tissues, likely derived from mothers' diets that may be rich in red meat or animal milk products. It is also present in some human carcinomas (92, 198) and at lower levels in normal human tissues. The enrichment of Neu5Gc in carcinomas and fetuses may be due to higher uptake by these rapidly growing tissues, likely by an increased macropinocytosis induced by growth factors (69). The exogenous Neu5Gc can be metabolically incorporated into cultured human cells (9). In rodent studies, pregnant mice were fed 1mg/ml Neu5Gc, and all newborn pups developed strong anti-Neu5Gc antibody reactivity (69). Thus, mice fetuses are capable of incorporating Neu5Gc from maternal sources (69). Consequently, bovine-derived Neu5Gc may more readily enter human infant cells via macropinocytosis to reach the endosomal/lysosomal pathway (9) and then be transported into the cytosolic/Golgi/nuclear compartment(s), where it is activated to form CMP-Neu5Gc. In the Golgi apparatus, the sialyltransferases can catalyze transfer of Neu5Gc to newly synthesized glycoconjugates (69). At present, we do not know the potential impact or long-term effects of an enhanced uptake of Neu5Gc for neonate or infant development, or for potential adult diseases, in particular a risk factor for cancer and heart disease. Because humans contain anti-Neu5Gc antibodies, this suggests that some caution should be exercised in the use of infant formula containing Neu5Gc as a neutraceutical until more adequate, well-controlled studies are carried out.

It is thus clear from the foregoing that the new generation of infant formula design must consider not only the concentration of Sia in human milk, but also the importance of the structure of the sialyl and neutral oligosaccharides and their forms (i.e., Neu5Ac/Neu5Gc). Human milk Sia is unique, species-specific, and a natural source of biologically complex nutrients designed for the human infant. Today, human milk sialyloligosaccharides cannot be duplicated by formula manufacturers. The challenge for nutritionists, food scientists, and glycobiologists is to develop an infant formula that closely imitates the nutritional complexity of human breast milk. The design for a human breast milk substitute has a long way to go.

Sia Concentration in Conventional Foods of Australia and the United States

The distribution of Sia is widely expressed on all mammalian tissues as either Neu5Ac, Neu5Gc, and more rarely as KDN. Animal foods, including red meat, fish, and poultry, contain high-biological-value protein and important micronutrients that are required to sustain adequate human health throughout life.

These animal foods also remain a core food in the diet of many countries today. Importantly, as described above, humans lack the ability to synthesize Neu5Gc because of an exon deletion/frameshift mutation in the human CMAH gene 2–3 million years ago (69). This mutation resulted in the complete loss of Neu5Gc expression in all human tissues (69). However, existing biochemical pathways allow exogenous Neu5Gc to be metabolically incorporated into cultured human cells (9). Consequently, Neu5Gc can be found at low levels in human carcinomas, fetal tissue, and normal tissue types, e.g., endothelium and epithelium of human surgical specimens at autopsy (184). This finding implies that Neu5Gc originates from exogenous dietary sources (184), generally red meat and milk products (69).

We recently analyzed the concentration of Sia in uncooked and cooked red meat, seafood, and poultry using an established high-performance liquid chromatography method (219). The concentration of total Sia in Australian raw meat was highest in beef followed by lamb, pork, and chicken. The percentages of Neu5Gc in each ranged from 0% to 48% (**Table 2**). Cooked ham contained the highest percentage of Neu5Gc in the tested foods. Cooked corn, surprisingly, contained relatively high Sia levels, 22% of which was

Table 2 Comparison of Sia concentrations in conventional foods in Australia[a] and the United States[b]

	Australia Sia concentration μg/g tissue				USA Sia concentration μg/g tissue			
Food	Neu5Ac	Neu5Gc	Total	Neu5Gc,% of total	Neu5Ac	Neu5Gc	Total	Neu5Gc,% of total
Beef	91.65	26.1	118	22.2	39.90	30.1	70	43
Beef, lean portion					39.70	22.3	62	36
Beef fat					50.80	31.2	82	38
Pork	50	9.85	60	16.4	108.50	25.5	134	19
Lamb	50.6	17.7	68	26.0	82.80	18.2	101	18
Ham (cooked)	21.5	20	42	48.25				
Chicken	52.6	–	53		75.92	0.076	76	0.1
Duck					19.98	0.02	20	0.1
Turkey	17.3	–	17*		45.95	0.046	46	0.1
Egg white	175	0.65	176	0.37				
Egg yolk	458.1	–	458					
Prawn	7.9	1.1	9	12.2				
Salmon	19.6	0.7	20.3	3.42	47.53	1.47	49	3
Cod					39.96	0.04	40	0.1
Tuna					31.97	0.032	32	0.1
Milk (cow 2%)					250.26	7.74	258	3
Milk (cow raw)					254.14	7.86	262	3
Butter					38.80	1.2	40	3
Cheese (cow)					153.60	6.4	160	4
Cheese (goat)					55.10	39.9	95	42
Corn (cooked)	39.1	21.7	61	35.6				

*Cooked turkey.

[a]The concentrations of Neu5Ac, Neu5Gc, and KDN were determined using Dionex high-performance anion exchange chromatography, pulsed amperometric detection with an ion exchange column. Range of values is shown for food items that were studied more than once. In these analyses, food samples were either raw or cooked and then directly subjected to acid hydrolysis (219).

[b]These analyses were carried out on raw food samples. Neu5Gc content was quantitatively determined after acid hydrolysis by the 1,2-diamino-4,5-methylenedioxy-benzene(DMB)–high-performance liquid chromatography analysis (184).

Neu5Gc. Interestingly, both chicken egg yolk and white contained high Sia levels, predominantly as Neu5Ac. Koketsu et al. reported the distribution of Sia in the eggs of original Silky fowl and showed that the Sia content in the yolk, albumin, and chalaza of a single egg were 205.2, 11.96, and 0.83 mg, respectively (82). The Silky yolk was entirely Neu5Ac; no Neu5Gc or O-acetyl Sia was detected. The structure of the major sialylglycan in Silky egg yolk was a disialyl-biantennary chain in which the Neu5Ac residues were α2-6 linked to glucose. No α2-3 linkages were observed. Thus, both chicken and Silky fowl eggs provide a good source of NeuAc and sialylglycan (82) for people without allergy to egg protein. Our own findings confirmed previous reports that Neu5Gc is rare in poultry and fish, but common in milk products, and enriched in red meats (184). Our results are similar to those reported by Tangvoranuntakul et al. (184), who analyzed 16 conventional foods in the United States.

Potential Impact of Neu5Gc Consumption on Human Health and Disease

In 2003, Tangvoranuntakul et al. reported the results of ingesting 150 mg purified Sia consisting of 95% Neu5Gc on normal human adults (184). They found that small amounts of Neu5Gc were incorporated into newly synthesized glycoproteins in saliva and facial hair clippings, but most Neu5Gc was excreted in the urine. Hence, this finding provides direct evidence that dietary Neu5Gc can be incorporated into glycoconjugates in the human adult. The incorporation of free Sia likely is even higher in newborns, since they have rapidly growing tissues and limited UDP-GlcNAc-2-epimerase activity. We do not know if human consumption of lamb, pork, beef, and milk products poses a potential risk because of their high levels of Neu5Gc (184). Normal humans have variable levels of circulating IgA, IgM, and IgG antibodies against Neu5Gc, with the highest levels comparable to those of the previously known anti-α-galactose xenoreactive antibodies. This suggests the possibility that these anti-Neu5Gc antibodies may cause some immune disorders. What is potentially more troubling, however, is the exposure of newborn infants to Neu5Gc. As noted above, human breast milk contains a considerable amount of Sia exclusively as Neu5Ac. In contrast, many infant formulas are based on bovine milk, and therefore an amount of the Sia is present as Neu5Gc. Hence, a formula-fed infant would be exposed to higher levels of Neu5Gc compared to infants who are exclusively breastfed. The potential long-term impact of the uptake of Neu5Gc in neonates on later health and disease remains to be determined. Pregnant mice that were orally fed 1 mg/ml free Neu5Gc in their drinking water from gestation day 13 through 18 failed to show that the fetuses incorporated Neu5Gc into their adult or fetal tissues, but Neu5Gc was incorporated into tumors (69). Thus, the expression of Neu5Gc in human cancers and fetal tissues is not likely to be due to an alternate "oncofetal" metabolic pathway. From an immunological perspective, the incorporation of Neu5Gc into glycoconjugates could potentially put these individuals at some risk because Neu5Gc can be antigenic when linked to glycoconjugates. Accordingly, knowledge of the Neu5Ac and Neu5Gc levels in conventional foods may help us better understand possible medical disorders involving the uptake of the "nonhuman" Neu5Gc from our diet.

METABOLIC FATE OF DIETARY SIA

Digestion and Absorption of Dietary Sia in Animals

During the past several decades, most research to study the absorption, transport, and function of Sia has been carried out using rats, mice, or piglets (115–117, 205) (**Table 3**). In spite of these efforts, the precise mechanisms and extent of digestion and absorption of dietary Sia-containing supplements are not fully understood, particularly developmental changes in the small intestine mucosa and how these

Table 3 Metabolic fate of radiolabeled Sia administration in animal models

Subjects	Dose and route	Compound	Amount radioactivity	Results and conclusions	Reference
8-, 12-, 16-, 20-, 24-, and 30-d well-fed and malnourished Sprague-Dawley rats	1 mg/50 g (0.5 mg/Kg) I.P.	[^{14}C]Neu5Ac	2.5 μCi [^{14}C]Neu5Ac/Kg body weight	^{14}C]Neu5Ac was more readily incorporated into the brain of well-fed pups than into undernourished rats. The maximum incorporation of [^{14}C]Neu5Ac was in gangliosides and glycoproteins of the cerebrum and cerebella in 12-d-old well-fed rats, but was at 16 d in undernourished pups. **Conclusion:** The rate of incorporation of Sia into gangliosides was higher than into glycoproteins in all groups.	(99)
12- and 16-d protein-restricted and well-fed Sprague-Dawley rat pups	I.P.	[^{14}C]Neu5Ac	2.5 μCi [^{14}C]Neu5Ac/Kg body weight	80% of the [^{14}C]Neu5Ac incorporated into the brains was found in the synaptosomal fraction, and the remainder was distributed among other subcellular fractions in proportion to the total Neu5Ac content in both well-fed and protein-restricted rats. Sixteen-d-old pup incorporated more Neu5Ac into the brain than did 12-d-old rats. **Conclusion**: These results suggested that Neu5Ac exerts its effects on behavior via the synaptic membrane.	(100)
20-d fasted mice for oral administration Wister rat 3-mo, 300 g bw for I.V. administration	Oral I.V.	N-acetyl-D-[2-[^{14}C],9–^{3}H]Neu5Ac mixture (double-labeled free Sia)	1.96 mg Neu5Ac containing 1.56 × 10^6 dpm each of 2-[^{14}C] and 9-[^{3}H] Neu5Ac (ratio 1:1) in 100 μl 0.2 M phosphate buffer pH at 7.4 100 μl/mice or rat	**Oral administration:** ^{14}C-radioactivity disappeared faster from intestine than ^{3}H. After 15 min, 95% of the radioactivity had passed from the stomach into the intestine. After 4 h, 90% of the radioactivity had been absorbed from the intestine. 10% of the ^{3}H and 1.3% of the^{14}C was retained in blood, liver, spleen, kidney, and brain. After 6 h, 60%–90% excreted in urine without chemical alteration. After 24 h, the level of Sia in blood, liver, spleen, kidney, and brain had decreased to 0.5% of the ^{3}H and 0.01% of the ^{14}C. ^{14}C was accounted for by the exhaled CO_2. **IV administration:** After 10 min, 90% of the radioactivity was excreted in the urine. After 4 h, 5% ^{3}H and 1.2% ^{14}C remained in blood, liver, spleen, kidney, and brain. **Conclusion**: Neither oral nor intravenous Neu5Ac can cross the cell membrane to any appreciable extent, except in the intestine. The small amount of Neu5Ac retained in tissues was largely cleaved by lyase, followed by metabolism of the reaction products.	(116)

20-d fasted C-57 mice	Oral	[^{14}C,^{3}H]Neu5Gc or [^{3}H]Neu5Ac mixture	1.96 mg Neu5Gc containing 1.42 × 10^6 dmp each of 2-^{14}C and 9-^{3}H Neu5Gc (ratio 1:1) in 100 μl 0.2 M phosphate buffer pH 7.4 87 μg Neu5[^{3}H]Ac2en containing ^{3}H at 1.07 × 10^6 dmp were dissolved in 47 μl 0.2 M phosphate buffer PH at 7.4	**Oral administration**: After 6 h, 90% of the total radioactivity was reabsorbed from the intestine and appeared in the urine. At this time, a total of 0.7% of the ^{3}H and 0.5% of the ^{14}C was recovered in blood, liver, spleen, kidney, and brain. **IV administration:** 90% of the radioactivity was excreted in the urine within 10 min. After 6 h, 1.3% of the ^{3}H and 1% of the^{14}C remained in the blood, liver, spleen, kidney, and brain. **Conclusions:** After oral or IV ingestion, low levels of Neu5Gc are retained in organs and body fluids.	(115)
20-d fasted C-57 mice 3-mo-old Wistar rat	Oral (mice) and I.V. (rat)	Mixture of [^{14}C]sialyl-lactose)/sialyl-[^{3}H]lactitol) Porcine submandibular gland mucin labeled with *N*-acetyl- and *N*-glycoloyl-[9–^{3}H]neuraminic acid Sialyl-[^{3}H]lactitol (IV) (double-labeled bound Sia)	87,000 dpm each of ^{3}H and ^{14}C radioactivity in 100 μl of 20 mM phosphate buffer pH 7.4 500 mg mucin containing 2 mg total Sia and 590,000 dpm-^{3}H radioactivity 2 mg Sia-[^{3}H]lactitol in 100 μl 20 mM phosphate buffer pH 7.4 representing 810,000 dpm	**Oral administration:** After 3 h administration, 50% of the oligosaccharide-bound, [^{14}C]sialyl-lactose/sialyl-[^{3}H]lactitol was excreted intact in the urine. Another 50% was hydrolyzed by sialidases, followed by excretion of 30% [^{14}C]–radioactivity as free Sia and 60% as nonanionic compounds expired as CO_2 within 24 h. [^{14}C]-radioactivity was less than 1% in the body of fasted mice after 24 h. Submandibular gland mucin: 40% of the ^{3}H–radioactivity (as free Neu5Ac and Neu5Gc; ratio 3:2) was excreted into the urine within 48 h; 30% of this radioactivity represented Sia while 70% was in other anionic and nonanionic metabolic products. Sixty percent of the nonanionic compounds was expired as CO_2 within 24 h. **IV administration:** 95% of administered sialyl-[^{3}H]lactitol was excreted intact in the urine in the first 30 min. Sialidase activity of whole mouse intestine was 20 μU/mg protein. **Conclusions**: (*a*) A portion of Sia bound to oligosaccharides and glycoproteins can be hydrolyzed in the intestine by sialidases and be reabsorbed at equal rates from the intestine. (*b*) Suckling mice metabolize a larger portion of sialyllactose compared to adult mice. (*c*) The rate of excretion/metabolism of Sia strongly depends on the form of Sia and the retention time of the molecules in the bowel.	(117)

(*Continued*)

Table 1 (*Continued*)

Subjects	Dose and route	Compound	Amount radioactivity	Results and conclusions	Reference
3-d-old Sprague-Dawley rats	Oral	0.1 ml of 1.5% [^{14}C]-Neu5Ac-lactose or 0.1 ml of 0.7% [^{14}C]-Neu5Ac	Specific activity: 1.3 × 10^5 dpm	[^{14}C]-Neu5Ac-lactose activity first appeared in the liver after 1 h, followed by its appearance in all other organs after 1.5 h. Maximum values were attained after 3 and 6 h. Thirty percent of the radioactivity was retained in the body and 70% was excreted by lung, kidney, and intestine after 6 h. The highest uptake occurred in liver, spleen, and brain. ^{14}C-Neu5Ac appeared in kidney and carcass at 30 min and in other organs after 1.5 h. It reached its maximal level between 1.5 and 2 h. **Conclusions**: (*a*) Absorption of the trisaccharide, [^{14}C]NeuNAc-lactose, was delayed by 30 min compared with free [^{14}C]Neu5Ac, and was retained to a greater extent in brain, liver and spleen. (*b*) [^{14}C]Neu5Ac-lactose was better utilized and absorbed by other organs.	(216)
Adult, *M/F* bw: 350 ~ 450 g Wister rats	I.V.	100 μg gangliosides containing [^{3}H]-GM1 or [^{14}C]-GM3	Specific activity: 27 μCi/mg for [^{3}H]-GMl; 9 μCi/mg for [^{14}C]-GM3	**Conclusions:** After 3 h, 75% of the labeled GM1 and 38% of the labeled GM3 were localized in the liver. After 3 h, the total radioactivity in kidney, lung, and brain was 10.6%, 5.2%, and 3.2% for GM1 and 3.87%, 3.9%, and 1.6% for GM3, respectively.	(42)
3-d-old piglet (*Sus scrofa*)	I.V.	5 μCi [6-[^{14}C]-Neu5Ac	Specific activity: 55 mCi/mmol	Two min after injection, 80% of the radioactivity was removed from the blood and by 120 min the remaining activity in blood was ~8%. After 120 min, the brain contained significantly more radioactivity (cpm/g tissue) than the liver, pancreas, heart, and spleen, but less than the kidneys. 0.23% of the total [^{14}C]-Neu5Ac was localized in the brain. **Conclusions:** (*a*) An exogenous source of Sia is capable of crossing the blood-brain barrier and being taken up by various tissues. (*b*) These findings suggest that dietary sources of Sia may contribute to early brain development in newborn mammals. (*c*) Piglets are a better animal model for the study of the metabolic fate of Sia in humans than any rodent model.	(205)

Abbreviations: d, day; wk, weeks; mo, months; bw, body weight; i.p., intraperitoneally; i.v., intravenous.

changes may affect the morphological and functional characteristics of the digestive enzymes. In rodents, oral administration of a mixture of [^{14}C]-sialyllactose/[^{3}H]-sialyllactitol to suckling mice showed that 50% of the administered dose was excreted unchanged in the urine after three hours. Another 50% of oligosaccharide-bound Sia could be reabsorbed from the intestine as free Sia, after its cleavage by sialidases, a finding that was confirmed by quantitatively measuring the levels of ^{14}C and ^{3}H in free Sia in the urine (117). In three-day-old mice, uptake of ^{14}C-labeled free Sia from the gastrointestinal tract into the blood and organs and its excretion by the kidneys occurred 1.5 hours faster than that of [^{14}C]-sialyllactose. Furthermore, sialylated glycoproteins were metabolized similarly, based on the finding of [^{3}H]-Neu5Ac in the urine of mice after oral feeding of porcine submandibular gland mucin labeled with [9-^{3}H]-Neu5Ac and Neu5Gc. The ratio of excretion/metabolic rate is strongly dependent on the retention time and nature of the sialylated molecules in the intestine. For example, 40% of the radioactivity in protein-bound Sia (mucin) was excreted into the urine as free Neu5Ac and Neu5Gc (ratio 3:2) within 48 hours. And about 80% of radiolabeled sialyllactose/sialyllactitol given orally was excreted as both free and bound Sia in the urine within 24 hours after oral administration (117). These findings indicate that sialylated glycoproteins have relatively longer retention times and slower digestion rates in the gut than does sialyllactose.

The high proportion of sialyloligosaccharide in human milk appears to provide optimal conditions for human infant absorption and utilization of milk Sia to meet developmental requirements. Furthermore, suckling mice metabolize a larger portion of sialyllactose than do adult mice (117). In adult rats, intravenous injection of doubly labeled [^{14}C, ^{3}H]-Neu5Ac resulted in less than 0.05% of the ^{14}C-label, and 0.16% of the ^{3}H-label, in the brain after two hours (116). In contrast, in 20-day-old rat pups, 0.4% of the ^{14}C-label (eightfold greater), and ~0.5% of the ^{3}H-label (tenfold greater) Sia was recovered in the brain two hours after an oral administration (116). The level of Sia incorporated into the brain of newborn piglets, who were given an intravenous injection of [^{14}C]-Neu5Ac, is therefore higher than in adult rats (0.23% versus 0.05%) but lower than that of three-day-old rats (0.23% versus 0.4%) (205). The latter difference might be explained by the two modes of administration (intravenous versus oral). It is possible that oral dosing is associated with slower absorption and therefore less rapid removal from the bloodstream than occurs with bolus intravenous administration. Thus, the metabolism of Sia may depend not only on the species and age but also on the method of administration and the form in which the Sia is presented, i.e., whether free or bound. The activity of the small intestinal sialidases is highest during suckling and decreases with age (38). In rats, this expression pattern correlates with changes in Sia content in milk during lactation (38). This suggests that the intestinal sialidases of suckling mammals function primarily to remove Sia from various sialylated milk glycoconjugates. Thus, free Sia released from dietary sialylglycoconjugate supplementation likely functions for synthesis of sialoglycoproteins and gangliosides by the young (38).

Digestion and Metabolism of Sialylglycoconjugates in Humans

In humans, HMOs appear to escape digestion and absorption in the small intestine of healthy infants when evaluated by the lactulose hydrogen breath test (18). These intact oligosaccharides are readily fermented, however, in the colon by the colonic bacterial microflora (18, 47). Colonic bacteria express many enzymes, including sialidases, that release free Sia from the sialylated oligosaccharide that then serve as substrates for bacterial energy metabolism (111).

In vitro digestion studies using enzymes prepared from human and porcine pancreas and intestinal brush border membranes (BBMs) also confirmed that neutral and acidic HMOs were resistant to hydrolysis by pancreatic and BBM hydrolases (47, 62). Sia was released from these

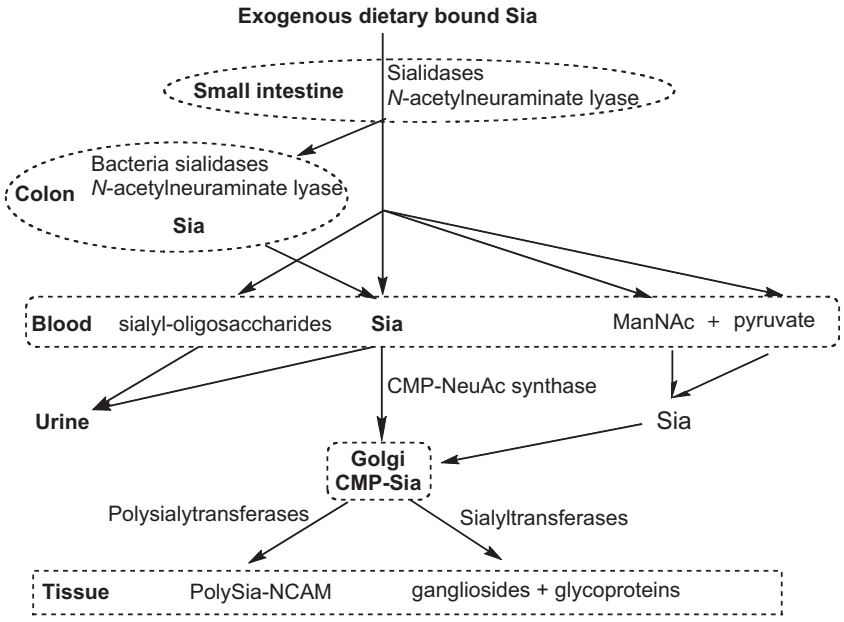

Figure 5

Metabolic fate of exogenous-bound Sia in mammals.

HMOs, however, by a pancreatic homogenate containing intracellular, lysosomal, and secretory enzymes. Intact HMOs may be absorbed across the intestinal mucosa and then taken into the bloodstream. This is based on the oligosaccharide profiles found in the urine and feces of breast-fed infants, which resemble the oligosaccharide profiles found in the milk consumed (111). HMOs can thus pass through the alimentary canal and enter the body. The conclusion that some dietary sources of Sia may be absorbed and metabolized by human infants is based principally on studies using suckling rodents (117, 216). Importantly, different types of HMOs likely have different rates of digestion, absorption, and excretion in human infants. The ingested HMOs function as prebiotics or as factors influencing the immune system of the intestine in breast-fed infants (111). Although it is not known which forms of sialyloligosaccharides are more readily absorbed and metabolized in humans, the Sia released from HMOs, glycoproteins, and glycolipids can be recycled or utilized by the sialyltransferases for Golgi-directed synthesis of new sialylglycoconjugates. Further study of the metabolic fate of dietary Sia is required. A summary of our current understanding of the metabolism of exogenous-bound Sia is shown in **Figure 5**.

Cellular-Based Evidence for Metabolism of Dietary Sia

A variety of human cells possess an efficient mechanism for the uptake of free Sia from surrounding media. These include B cell lymphoma lines, myeloid leukemia variants, adherent cell lines, and more recently, RBCs (22, 119). Bardor et al. showed the uptake of Neu5Gc and Neu5Ac by epithelial, skin fibroblasts, and human neuroblastoma cells (9). All three cells could import Neu5Gc, although fibroblast cells did so less efficiently. Free Neu5Ac in the culture medium can be readily incorporated and compensate for a Sia deficiency in endogenously hyposialylated cells, as observed by an increase in cell-surface sialylation (119). Competition experiments carried out using human cell lines showed that Neu5Ac and Neu5Gc both appear to use the

same pathways for entry and metabolism (9). Use of various specific inhibitors showed that Sia uptake into human cells occurred principally by pinocytosis (9, 184). For sialylglycoconjugates, the Sia that is released by lysosomal sialidases can be reutilized after activation by CTP to form CMP-Sia and can be incorporated into sialylglycoconjugates (9, 119). It is thus likely that human neural cells may also be capable of Sia uptake. In contrast to the uptake of Sia by pinocytosis, free Neu5Ac uptake in various human cells is nonsaturable, consistent with an endocytotic uptake mechanism. However, a number of endocytotic inhibitors did not significantly alter radiolabeled Neu5Ac uptake (119). Incubation of human RBCs with radiolabeled [4-9-^{14}C]-Neu5Ac for 30 minutes at concentrations ranging from 0.032 to 1.617 mmol/L also showed that free Neu5Ac was taken up and found intact in the cytosol (22). Moreover, Neu5Ac induced a more rapid sialylation of CD75 compared to cells exposed to ManNAc (119), which must first be converted to Sia before sialylation can occur. Thus, a cellular requirement for increased and rapid sialylation would benefit from Neu5Ac uptake rather than ManNAc. In contrast to ManNAc uptake, Neu5Ac uptake can effectively enter the Sia biosynthetic pathway downstream from ManNAc. This could be a distinct advantage to infants with limited capacity to synthesize endogenous Sia.

SIA AND PREGNANT WOMEN: EVIDENCE THAT SIA CAN CROSS THE PLACENTA FROM MOTHER TO THE FETUS IN HUMANS

There are no published studies providing direct evidence that Sia can cross the placenta from mother to the fetus in humans. This is due in part to ethical and technological limitations and difficulties imposed on carrying out stable isotope studies on mothers and their fetus. Multiple factors affect the sialylation and desialylation of sialylglycoconjugates, and these may change the level of Sia in different body fluids during pregnancy. A number of studies have shown that Sia concentration in both serum and erythrocyte membranes of healthy pregnant women increases significantly with advancing gestation (1, 33, 95) and persists for 12 weeks postpartum (33). Such elevation may be dependent on the concomitant increase in serum total Sia levels, and it has been hypothesized that these elevations during pregnancy are related to the sialyltransferase activity found in human placenta (110) and the increased demand for Sia during fetal development. A decrease or lack of Sia in placental tissue may contribute to a reduction in placental function, affecting the development of placental vessels and thus placental efficiency in the maternal-fetal exchanges of gases and metabolites. This may lead to a restriction in fetal growth and development (164). In early pregnancy, increased expression of α2–3-linked Sia residues in the deciduas surrounding the implantation site has been shown to be associated with the implantation of the embryo in the uterus of pregnant rats and mice (77). However, in the third trimester, Sia synthesized by the mother is thought to cross the placenta and contribute to fetal growth (19). Moreover, the total Sia concentration, in particular 2–6-linked Sia in amniotic fluid of healthy pregnant women, is significantly increased during pregnancy (19). The sialylglycoconjugates in the amniotic fluid likely play a critical role in the growth and tissue remodeling of the fetus as well as reflecting maturation of the fetus. A rigorous analysis of the sialylated glycotope expression profile in amniotic fluid may ultimately be useful in prenatal diagnosis as another predictive factor for the well being of mother and child.

Saliva is often regarded as a mirror of the body in reflecting tissue fluid levels of various metabolites. Pregnancy is associated with an increased concentration of Sia in maternal saliva. For example, mean salivary Sia levels were increased during gestation from ~0.175 mmol/L at 10 weeks gestation to ~0.485 and ~0.420 mmol/L at 21 and 40 weeks gestation, respectively. This period correlates with the period of rapid Sia accumulation in fetal brain (143). The smooth and viscous nature of saliva is

a direct result of the negative charge exerted by the Sia moiety of mucin glycoproteins. Salivary sialylated glycoproteins also interact with bacterial cell walls through different mechanisms to facilitate a protective and lubricative effect.

The mean Sia content in urine is also elevated during pregnancy from ~32 mmol/mol at 0–12 weeks pregnancy and increases with advancing gestation to ~54 mmol/mol at 26–40 weeks pregnancy. This level remains constant until at least two weeks postpartum (131). The increase in the levels of Sia in circulation during pregnancy is correlated with and reflects the high Sia secretion found in the urine.

Briese et al. (19) measured the Sia concentration in maternal, retroplacental, and cord blood samples of 126 pregnant women between 28 and 42 weeks of gestation. They showed significant correlations ($p < 0.01$) between maternal and retroplacental blood on the one side and between maternal and the cord blood on the other side. This suggests that the mother synthesizes most of the Sia, which crosses the placenta to contribute to the fetal growth in the third trimester. The high concentration of sialylated components in early human milk, but not infant formula, may thus provide for this increased and continuing need for Sia to ensure normal development.

In conclusion, an increase in the level of Sia in plasma, RBC membrane, saliva, amniotic fluid, and placenta during healthy women's pregnancies suggests the mother will maintain a sufficient supply of Sia to the uterus to maintain it in a relaxed state throughout gestation. The Sia concentration in healthy women during pregnancy is summarized in **Table 4**.

EVIDENCE THAT DIETARY SIA CAN BE ABSORBED AND CROSS THE BLOOD-BRAIN BARRIER

An exogenous source of Sia capable of crossing the blood-brain barrier (BBB) and of being incorporated into sialylglycoconjugates in different regions of brain tissue was observed

Table 4 Sia concentration in plasma, erythrocyte membranes, amniotic fluid, and urine during pregnancy in healthy women

Source/fluids	Findings	Reference
Serum	The mean serum Sia concentration during pregnancy increased from 1.63 mmol/L during weeks 5–8 of pregnancy (n = 7) to 2.06 mmol/L (n = 37) at 37 weeks of pregnancy.	(1)
Plasma	The mean plasma Sia concentration increased from 22.3 mmol/L $\times 10^{-2}$ from 0~12 wk pregnancy (n = 11) to 25.8 mmol/L $\times 10^{-2}$ after 26–40 wk pregnancy (n = 28) and to 34.6 mmol/L $\times 10^{-2}$ at 1–14 day postpartum (n = 10).	(131)
Erythrocyte membranes	The Sia concentration in RBC membranes was 96.30 ± 34.20 μg/mg protein in healthy pregnant women (HPW) (n = 25), gestational age 28 to 32 weeks. This compared to 42.33 ± 15.85 μg/mg protein for healthy nonpregnant women (HNPW) (n = 10). There was no change in the erythrocyte membrane Na^+/K^+-ATPase activity between HNPW and HPW.	(95)
Saliva	The mean salivary Sia concentration increased during gestation from 53.8 ± 7.6 mg/L at 10 wk to 149.6 ± 62.5 and 129.3 ± 16.5 mg/L at 21 and 40 weeks gestation, respectively.	(143)
Amniotic fluid	The relative amount of α2,6-linked Sia residues increased from 965 ± 299 (mean ± SD; arbitrary units) during the second trimester to 1458 ± 328 in the third trimester and increased further to 1806 ± 292 in the perinatal period. The relative level of α2,3-linked Sia residues remained the same from the beginning of second trimester (694 ± 238 arbitrary units) to the 37th week of pregnancy (639 ± 346), then increased to 964 ± 251 during the perinatal period (n = 6). The level was 947 ± 190 (n = 6) at 38–40 wk of pregnancy and 1203 ± 284 (n = 22) at 39–41 wk of pregnancy.	(121)
Urine	The mean Sia level in urine was elevated during pregnancy from 31.8 mmol/mol during 0–12 wk pregnancy (n = 11) and increased with advancing gestation to 54.0 mmol/mol at 26–40 wk pregnancy (n = 27). There was no decrease in the urinary Sia levels before delivery or during the 1–14 days postpartum (55.4 mmol/mol, n = 7).	(131)

for both oral administration or intravenous injection of radiolabeled free or bound Sia in rodents and newborn pigs (116, 117, 205, 216). In the newborn piglet, the brain contained significantly more radioactivity [0.23% of the labeled dose, (cpm/g tissue)] than did liver, pancreas, heart, and spleen, which contained less radioactivity than kidneys at 120 minutes after intravenous administration of [^{14}C]-Neu5Ac (205). Within the brain, the mean percentage of total injected radioactivity was highest in the cerebrum (~0.175), followed by the cerebellum (~0.0295), and then thalamus (~0.029) (205). These differences were highly significant ($P < 0.001$). In 3-day-old rats, oral administration of [^{14}C]-Neu5Ac or [^{14}C]-sialyllactose showed that 2.8% and 3% to 4% of the dose appeared in the brain after 1.5–2 hours and 6 hours, respectively (216). Oral administration of [^{14}C,^{3}H]-labeled sialyllactose and [^{3}H]sialyl-mucin to 20-day-old mice showed that both bound forms of Sia could be absorbed and incorporated into neural tissues (117). The uptake of isotope accelerated rapidly and reached its maximum level at 6 hours for sialyllactose and 10 hours for the protein-bound form of sialyl-mucin, respectively (117). The study also showed that gangliosides can cross the BBB by binding to bovine serum albumin (153). Thus, an exogenous source of Sia in the bloodstream (115–117) can cross the BBB and be used for synthesis of new sialoglycoconjugates. However, many factors can influence the efficacy of absorption, digestion, and incorporation of dietary Sia into brain tissue, including age, species, amount and form of Sia, and the route of administration. Human milk is a rich dietary source of Sia (26, 219), which is a major factor contributing to the higher levels of brain Sia in infants fed human milk (204, 208) but not infants fed formula milk.

The precise molecular mechanism(s) of how exogenous Sia can cross the BBB is not fully understood. From isotope studies, it has been inferred that dietary sources of Sia are first taken into the bloodstream and cross the BBB by either diffusion or a carrier-mediated process. It is known, for example, that disruption of the BBB by injury allows blood gangliosides to penetrate the BBB (153). Mice with brain lesions induced by treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) showed a greater accumulation of [^{3}H]-GM1 in the ventricular spaces two to four hours after an intraperitoneal injection (147). When the BBB is not disrupted, gangliosides may cross membranes of vascular endothelial cells that line the capillaries by lateral movement to reach the contra-luminal side, where they are released, or they may cross the BBB using a mechanism analogous to that proposed for proteins, including a receptor-mediated endocytotic process (153). The receptor-mediated endocytotic mechanism suggests that serum albumin may function in the transport of ganglioside across the BBB (153), which could be facilitated by specific albumin receptors on the surface of vascular endothelial cells (154). Future study is clearly needed to investigate the molecular mechanism of how different forms of Sia can cross the BBB and be incorporated into neural tissue to enhance cognition and memory.

EVIDENCE THAT EXOGENOUS ADMINISTRATION OF SIA CAN ENHANCE LEARNING AND MEMORY: PIGLET AND RODENT STUDIES

Advances in understanding the importance of prenatal nutrition on brain development require the use of animal models. During the past several decades, most research on the effects of Sia as a dietary supplement to enhance learning and memory has been carried out in rodents (25, 99). We have chosen instead newborn piglets as our preferred animal model because the structure and function of their brains more closely resemble that of human infants, in particular preterm infants (101, 124). Similar to humans, the newborn piglet is less developmentally mature, and its body weight is relatively small in relation to its mature weight. For this reason, both newborn piglets and low-birth-weight infants are vulnerable to developmental deficits.

Moreover, unlike rats, the pig's digestive system shares similar physiology and anatomical structures with human infants and has comparable nutrient requirements. In both humans and piglets, birth occurs in the midst of a developmental spurt in brain-mass accretion (21). These considerations thus make the piglet more ideally suited for an in-depth analysis of learning and memory at various levels not possible with humans, especially neural, biochemical, and molecular genetic analyses.

Effect of Dietary Sia Supplementation in Piglets: Results of Molecular and Cognitive Studies

In our original piglet model studies, we supplemented a commercial source of Sia derived from cheese whey known as caseinglycomacropeptide (CGMP), containing 60 mg/g Sia, to three-day-old piglets for five weeks. The dose levels of total Sia intake were 40 mg/kg/day (the control group; CGMP-free diet), 85 mg/kg/day (low dose level); 180 mg/kg/day (middle dose level); and 240 mg/kg/day (high dose level) (211). Learning performance and memory were assessed using an easy and difficult visual cue in an eight-arm radial maze (211). Brain ganglioside, sialylglycoprotein concentrations, and mRNA expression levels were determined quantitatively for two learning-associated genes, Gne and ST8SiaIV. In a dose-dependent manner, dietary Sia supplementation was associated with faster learning in piglets fed the higher doses of Sia, and they made fewer mistakes in the maze than did those fed lower levels. Concomitantly, there was a corresponding dose-related increase in the amount of Sia in the frontal cortex, particularly in sialylated glycoproteins, and a two- to threefold increase in mRNA levels in the hippocampus for both Gne and ST8SiaIV (206, 207, 211). A number of studies have demonstrated that learning leads to an increase in the incorporation of Sia into the brain. There is also compelling evidence from Reutter and colleagues that the intracellular concentration of Sia regulates the polysialylation of NCAM (15). Increasing Sia availability by overexpressing GNE using GNE-sialuria-transfected cells, or the addition of ManNAc (the product of the GNE reaction and physiological precursor of Sia), dramatically increased the amount of polySia on NCAM (15). Our findings suggest that increased levels of ST8Sia IV may also up-regulate Sia synthesis in the developing brain. This may be a molecular mechanism to explain how increased dietary Sia level correlates with increased learning performance and also with an increase in Gne expression. These results indicate that both Gne and ST8SiaIV may function cooperatively to increase the synthesis of polySia on NCAM during brain development. Because neonates have a lower capacity for the de novo synthesis of Sia (60), it is possible that a diet deficient in Sia may reduce early learning capacity, in turn lowering the demand for endogenous synthesis of polySia-NCAM, and resulting in a greater need for Sia. **Figure 6** summarizes our proposed pathway and mechanism by which exogenous and endogenous sources of Sia may influence Sia metabolism in the brain (207).

We have now demonstrated that Sia concentration in brain cortical tissues correlates with evolutionary development in higher animals (209). Humans were clearly at the top of the developmental list, with twice as much brain Sia as the chimpanzee, our closest relative (209). Importantly, we have also found that Sia concentration in the frontal cortex of breast-fed infants is higher than the levels of formula-fed infants (208). In breast-fed infants, but not in formula-fed infants, the level of ganglioside Sia correlated significantly with ganglioside ceramide DHA and total n-3 fatty acids (208). This suggests that Sia and DHA are structurally and functionally linked in the infant brain and may act together to benefit early brain development and cognition (203, 208). Taken together, these findings suggest that the increase in sialylglycoconjugates resulting from a dietary supplement of Sia plays a crucial role in consolidating learning behavior. Because there is

only limited capacity for endogenous synthesis of Sia during early life when the demand for Sia is high, the exogenous dietary supplementation of Sia found in human milk may be more critical to neural development and cognition than heretofore recognized. Because infant formulas are deficient in Sia nutrients (24, 203, 204), premature infants who receive artificial feeding are likely to be at greater risk of a neural deficit.

Dietary Supplementation Studies of Sia in Rodents

The effect of repeated administration of Sia on learning and memory has been extensively studied in rodents (97, 99, 125, 126). The main obstacle and limitation to these studies is the process of artificially feeding very tiny rat pups of <14 days old, which influences the normal body weight gain. The artificial, forced feeding does not mimic the real-life situation. For example, there are adverse metabolic effects resulting from stress associated with artificial, forced feeding. As a consequence, most studies on rats are done after they are weaned from their mother, but by this time it is too late in the present context because brain growth is nearly complete. Because of these serious limitations, it is clear that the rat pup is far from an ideal animal model system for nutritional neurodevelopment studies.

Morgan & Winick demonstrated that behavioral changes after Sia administration in rat pups correlated with an increased level of Sia in the brain (98). These effects were shown to persist into adulthood using a Y maze, where treated animals showed greater learning performance (98). They also found that most exogenous Sia localizes to the synapse (100), and they suggested that Sia may influence the movement of positively charged neurotransmitters and transmitter release to alter existing synaptic morphology (98–100). The results suggested that brain Sia content played an important role in influencing behavior. When Neu5Ac was injected after 30 days of age, by which time rat brain growth was complete, neither behavior nor brain biochemical parameters were affected (96). This implies that for exogenous Sia to be incorporated into the brain, it must be given much earlier during the period of rapid brain growth. Overall, these observations suggest that dietary Sia plays a fundamental role in facilitating learning and in determining the final levels of Sia in the brain. The dietary effect of free and bound forms of Sia supplementation on learning behavior in mammalians are summarized in **Table 5**.

LTP: long-term potentiation

LTD: long-term depression

MOLECULAR MECHANISMS UNDERLYING THE ROLE OF SIA ON BRAIN DEVELOPMENT AND COGNITION

Possible Functions of PolySia-NCAM in the Central Nerve System: Role of PolySia on Brain Development and Cognition

A number of diverse functions have been postulated for the role that polySia-NCAM may play in the CNS (192, 193):

- Implicated in embryonic neural development and neuronal plasticity;
- Involved in the long-term potentiation (LTP) and depression (LTD) in the hippocampal CA1 region and spatial learning;
- Mediates cell adhesive interactions including neurite fasciculation, neuromuscular interactions, and cell migration;
- Acts as an antiadhesive glycotope by decreasing NCAM-mediated cell adhesion;
- The amount of polySia on NCAM is critical for normal morphogenesis and neural development;
- Influences cell-cell apposition and regulates contact-dependent cell interactions by increasing the intercellular space between cells;
- Participates in the establishment of neuronal connections and modulates neurite outgrowth;
- Can activate second messenger signaling pathways in primary neurons;
- Can influence the formation of new neural circuits in the dentate gyrus and in

Table 5 Evidence that exogenous administration of free Sia, sialyloligosaccharides, sialylated glycoproteins, or gangliosides can enhance learning behavior in mammals*

Animals	Treatment	Age or weight during treatment	Memory or behavior test used	Results and conclusions	Reference
Young animal					
Sprague-Dawley rats	Rat milk substitutes: (*a*) choline chloride (250 mg/l) and 43 mg/l gangliosides (*b*) choline chloride (250 mg/l) and 67 mg/l gangliosides (*c*) chloride (1250 mg/l) and 43 mg/l gangliosides	5-d-old male pups fed artificially via gastric cannulae for a period of 20 min every 2 h, 5- to 6-d-old fed 29% bw, 7-d-old fed 33% bw, increased by 2% per d to 43% when 18 d old P19 to P49 fed laboratory chow	A variation in the delayed matching to place task in the Morris water maze	**Conclusions:** (*a*) All rats were equally proficient at using spatial short-term memory, regardless of the ganglioside or choline content of the preweaning diet. (*b*) There was no significant difference in the Sia content per mg brain weight in all groups.	(202)
Sprague-Dawley rat pups from well-fed or undernourished pregnant rats	1 mg Neu5Ac/50 g bw, administered I.P.	14-d-old rat pups repeatedly injected until 21 d old, then tested on day 21. Rats remained on casein diet until 6 mo	Novel object exposure, electrified Y maze at 6 mo	**Conclusions:** (*a*) Administration of Neu5Ac was associated with increased cerebral and cerebellar ganglioside and glycoprotein Sia concentrations in all groups. Both well-fed and undernourished treated rats spent more time with novel object. Treated animals learned Y maze significantly faster at 6 mo of age. (*b*) Increased levels of sialylated brain glycoconjugates are associated with increased learning.	(99)
Wistar 2BAW rats	10 mg/kg GM1, administered subcutaneously	Rats treated daily from 3 to 15 d of age and tested at 4 and 24 mo. by the passive avoidance test	Elevated plus maze with visual cues, electrified step-down task	**Conclusions**: (*a*) GM1-treated animals avoided aversive arms and had increased latency to step-down task. (*b*) Administration of GM1 facilitated maturation of the CNS that persisted into adulthood and with aging.	(167)
Sprague-Dawley rats	I.P. and feeding by catheter	14-d-old rats fed Neu5Ac (20 mg/kg) for 8 consecutive days	N/A	**Conclusions:** (*a*) Administration of Sia by either IP or catheter resulted in significantly elevated levels of gangliosides and sialylglycoproteins in the cerebrum and cerebellum compared to the control group. (*b*) Catheter feeding and IP routes were similarly effective.	(25)

C57BL/6 rats	40 mg/kg GM1, given I.P.	30-d-old rats given daily injections of GM1 for 3 wk then left for 1 wk before testing when rats were 2 mo old	Novel object exposure, radial maze without extramaze cues	**Conclusions:** (*a*) Treated animals froze less but reared more, suggesting that GM1 reduced fear. (*b*) GM1-treated group improved performance in maze at beginning of training, but not at the end.	(49)
Piglet (*Sus scrofa*)	Oral Sia dose level at 40, 85, 180, and 240 mg/kg/d	3-d-old milk supplemented protein-bound Sia for 35 days	Easy and difficult discrimination and memory tests using 8-arm radial maze with early and difficult visual cues	**Conclusions**: (*a*) In both memory tests, the Sia supplementation groups learned significantly faster than the control group, with a dose-response relationship in the difficult but not the easy task. (*b*) In the hippocampus, there was a significant dose-response relationship between level of Sia supplementation and the mRNA levels for both ST8Sia IV and GNE. This corresponded with a proportional increase in sialylglycoprotein levels in the frontal cortex.	(211)
Adult and aging animals					
Wistar rats	0.8 μmoles Neu5Ac /10 μl, given I.V.	Treated 30 min before training (bw 200 g)	Electrified Y maze brightness discrimination	**Conclusion**: I.V. administration of Neu5Ac improved retention performance.	(125)
Wistar rats	Separate 200 μg/10 μl doses of GM1, GD1a, GD1b or GT1b and 0.8 μmol/ 10 μl Neu5Ac, given I.V.	Rats treated 1 or 4 h before training (Males; bw 200 g)	Electrified Y maze brightness discrimination	**Conclusions:** (*a*) Neu5Ac improved retention when applied 1 h before training. A greater improvement in acquisition and retention observed when GM1 was injected 1 h and 4 h before training. (*b*) GT1b improved retention performance when injected 1 h before training. GD1a and GD1b injections had no effect on learning.	(126)
Wistar rats	GQ1b, GT1b, FucGD1b, GD1a, GD3, and GM1 containing 20 or 40 mg/kg dose injected I.P.	7-wk-old rats injected twice, immediately after training session and then before the first retraining session	Electrified two-way shuttle box	**Conclusions:** (*a*) Ganglioside-treated groups took longer to reach test criteria for both dosage levels when provided immediately after training. (*b*) Injection of dose 2 h after training improved performance in retraining session. Thus, immediate injection treatment impaired memory formation.	(68)

(*Continued*)

Table 5 (*Continued*)

Animals	Treatment	Age or weight during treatment	Memory or behavior test used	Results and conclusions	Reference
Sprague-Dawley rats	Chow containing 1% Neu5Ac or 1% sialyllactose (SL) or 1% glactosylated Neu5Ac (GN). P.O.	8-wk-old male, bw 300 g Feeding 2 wks	T maze learning test (twice/d) Morris swimming maze test (twice/d)	**Conclusions:** (*a*) Brain ganglioside content was significantly higher in the GN and SL groups, but not in the Neu5Ac group, compared to the control groups. (*b*) SL and GN group learned faster at day 1 trial of T maze learning test and day 1 and day 2 trials of Morris swimming maze test compared to the control group. However, the difference between the groups was not statistically significant.	(142)
C57BL/6 rats	I.P. injections of GM1 for either 1, 3, or 7 d; doses were 80–200, 20–120, 5–80 mg/kg, respectively	Aged 4, 5, and 6 wks. Treated daily for 1, 3, or 7 d at different doses, then tested 1 wk later	Electrified step-through inhibitory avoidance task	**Conclusions**: (*a*) Treatment group increased latency. All age groups improved when treated for 7 d. (*b*) The two eldest groups showed greater improvement than the younger rats. Thus, older rats were more sensitive to the improved effects of GM1 administration.	(50)
Male mice (EPM-M1)	GM1 injected I.P. at 50 mg/kg (10 ml/kg)	3-mo-old mice treated for 14 d, trained the next day, then tested on d 20, 25, 30	Electrified plus maze with extramaze visual cues	**Conclusion:** GM1-treated mice required less time in aversive arms under a number of different test conditions. Thus, the beneficial effects of GM1 on learning and memory can be observed in normal animals.	(166)
Human volunteers	Oral, 150 mg porcine sialomucin (5% Neu5Ac, 95% Neu5Gc)	Healthy human adults		**Conclusion:** Neu5Gc can be rapidly absorbed, incorporated into newly synthesized sialylglycoproteins, and subsequently excreted into the urine.	(184)
Deficient rodents					
Sprague-Dawley rats Age-related cognitive deficit in senescent	GM1 administered I.P. at 30 mg/kg	3-mo-old "young" and 20- to 22-mo-old "aged" rats treated for 30 d. Some rats received daily I.P. injections for 30 d, then saline for the next 15 d (discontinued treatment)	Morris water maze with extramaze cues	**Conclusions:** (*a*) GM1 enhanced memory in aged rats, but not in young or control rats. (*b*) Treated aged rats did not perform better than control young rats. (*c*) I.P. administration of ganglioside GM1 can attenuate memory deficits associated with aging.	(56)

C57BL/6 mice Genetic deficit in cholinergic neural systems	GM1 administered I.P. at 30 mg/kg	1-mo-old male rats were treated for 3 wks (chronic administration) before testing	Retention of passive avoidance of shock: animals must remember to remain stationary to avoid a mild electrical shock	**Conclusions**: (*a*) GM1 treatment improved retention performance in adult C57BL/6 intact male mice in a passive avoidance test, suggesting that GM1 can increase cholinergic neural parameters. (*b*). I.P. administration of GM1 can improve lesion-induced behavioral deficits and also age-dependent behavioral dysfunctions in intact animals.	(48)
Wistar EPM-1 rats	GM1 administered I.P. at 50 mg/kg (1 ml/kg)	5-mo-old adult and 20- to 27-mo-old rats; repeated injections for 7 d Tested hours after last dose	Electrified two-way shuttle box	**Conclusions:** (*a*) Old rats treated with GM1 increased passive avoidance responses compared to old control group. (*b*) Control adult rats had significantly greater responses compared to control old rats. (*c*) GM1 treatment of old treated rats showed no significant difference compared to control (no GM1) adult rats.	(169)
Sprague-Dawley rats, unilateral entorhinal cortex (EC) lesions	GM1 injected I.P. at 30 mg/kg body weight	Male 90-d-old injected daily with GM1 for 14 d	Hebb-Williams maze tasks with integration of spatial cues	**Conclusions:** GM1-treated rats showed improved maze performance, making significantly fewer total and repeat errors. This suggests GM1 may be involved in the neuronal sprouting observed after EC lesions.	(61)
Wistar EPM-1 rats and Swiss EPMM1 mice. Amnesia induced by scopolamine (cholinergic antagonist) in both rats and mice	GM1 administered I.P. at 50 mg/kg	Four-month-old male Wistar EPM-1 rats injected I.P. for 7 d. 3-mo-old EPMM1 mice injected I.P. for 14 d	Passive avoidance and maze tests	**Conclusions:** (*a*) GM1 improved memory in aged but not adult rats. (*b*) GM1 treatment can improve learning/memory deficits induced by scopolamine administration. (*c*) The beneficial effects of I.P. administered GM1 on learning and memory involves the cholinergic circuitry.	(168)
Ischemia-induced cognitive deficits in rats	L-PDMP (synthetic ceramide analog) stimulates ganglioside biosynthesis	Male 200–250 g I.P. twice/d for 6 d PDMP in 5% Tween 80 in saline at 20 mg/ml	8-arm radial maze test: Animals must remember which arms they have not yet visited	**Conclusion:** L-PDMP treatment increased p42 MAP kinase levels, which is involved in synaptic transmission.	(72)

*Studies typically used male animals. Saline or glucose used for the control groups. The majority of learning or memory tests described used an electrified apparatus with an aversive response. These tests generally determine how long it takes for an animal to associate an action (i.e., entry into a radial arm or stepping onto a floor) to an electric shock or aversive experience. Animals that learn this relationship quickly will take longer to perform the action associated with shock/aversion (increased latency). This is observed during the retraining period in the absence of the shock/aversion. Results are compared to the response of control animals. Abbreviations: d, day; wk, weeks; mo, months; bw, body weight; i.p., intraperitoneally; i.v., intraventricular.

reorganization of the piriform cortex in the adult rat;
- Can regulate intramuscular nerve branching during embryogenesis;
- Can influence the interaction of cells of the preimplantation mammalian embryo;
- May couple the morphogenic effects of adhesion and synaptic activity-dependent processes;
- May mediate the formation of fiber cell gap junctions and adherence junction during lens cell differentiation;
- May control the migration and maturation of dopaminergic cells of the developing mesencephalon;
- Functions in the internalization of the antennapedia homeobox peptide involved in late expression of some homeogenes in the CNS.

The most fundamental role of polySia, however, is to promote developmentally regulated and activity-dependent plasticity in cell-cell and cell-matrix interactions from embryos to adults, thereby facilitating changes in the structure and function of the nervous system (140). The covalent modification of NCAM with polySia confers unique properties on the adhesion molecule that influence both homophilic and heterophilic binding and thus cellular activity. For example, the embryonic, or highly polysialylated, cells are relatively nonadherent, in contrast to the adult, or poorly polysialylated, cells (45, 140). The reduced adhesiveness is postulated to be due to the steric hindrance and negative charge repulsion between cells that express these highly hydrated, space-filling molecules that occupy greater steric space (218). This activity is believed to result from the ability of the negatively charged polySia chains to occupy a large hydrated volume and thereby cause a direct physical hindrance to cell-cell contacts (46). When a presynaptic neuron that expresses NCAM is in the presence of postsynaptic neurons that either do or do not express NCAM, the presynaptic neuron will make more synaptic connections with the NCAM-expressing postsynaptic neuron (39). Accordingly, maximum stability or formation of synaptic connections will occur preferentially to a postsynaptic neuron expressing NCAM.

Polysialylation of NCAM is developmentally controlled, and the highly polysialylated form is prevalent principally during the early stages of embryonic brain development. In embryonic chick brain, the maximal period of polySia-NCAM expression occurs around E12 to E14 (141). Although polySia can be found in most regions of the brain during this time (139, 140), there is loss in polySia as development proceeds to prenatal and early postnatal stages. In contrast, adult NCAM, which contains about one-third the amount of polySia as the embryonic form, has reduced capacity for plasticity. However, certain regions of the brain, including the hippocampus, hypothalamic nuclei, olfactory system, subventricular zones, and the dentate gyrus where neurogenesis is ongoing, continuously express polySia-NCAM (5, 161). These regions exhibit life-long plasticity and thus are able to alter morphology and presumably functional parameters mediated by polySia in the adult. The axons of neurons often retain polySia to bundle, sprout, and branch appropriately during axon path finding (162, 182). The polysialylated axons are capable of synaptic remodeling (157, 158) and neuronal-glial plasticity during both embryonic and adult neurogenesis.

PolySia-NCAM has received the most attention of the sialoglycoproteins for its role in learning and memory. In the hippocampus, the plasticity of synapses is changed in response to learning. And the induction of LTP, an increase in synaptic efficacy, is believed to underlie learning and memory mechanisms. NCAM knockout mice have been shown to exhibit deficits in neuronal functions, including impaired hippocampal LTP in CA3, deficits in spatial learning and memory due to an abnormal formation of the hippocampus (32), and abnormality in circadian rhythm and motor coordination (26, 103). Both ST8Sia II and ST8Sia IV are expressed in different spatial and temporal patterns in neural tissue. Mice deficient in ST8Sia IV have a decrease in polySia in specific regions of the brain and impaired LTP, and

LTD in Schaffer collateral-CA1 synapses in the adult hippocampus (43). Mice lacking ST8Sia II, however, do not show an impairment in hippocampal synaptic plasticity, but rather show misguidance of infrapyramidal mossy fibers and the formation of ectopic synapses in the hippocampus (6). ST8Sia II-deficient mice exhibited higher exploratory drive and reduced behavioral responses to Pavlovian fear conditioning (6). Double knock-out mice of ST8Sia II and ST8Sia IV led to a severe phenotype with specific brain-wiring defects, progressive hydrocephalus, postnatal growth retardation, and precocious death (213). The specific defects in major brain fiber tracts are postulated to cause the lethal phenotype (213). Very recently, the phenotype of triple knock-out mice lacking NCAM, ST8Sia II, and ST8Sia IV has revealed a completely unexpected finding in which there was a complete reversal of the phenotype of the double knock-out of ST8Sia II and ST8Sia IV. This intriguing finding is the first example showing that polySia is essential in controlling NCAM functions during development of the CNS (213).

PolySia expression in the hippocampus facilitates the remodeling of synaptic connections, aids in neurogenesis, and facilitates neuronal migration (156). The hippocampus and surrounding regions of the medial temporal lobe are largely responsible for short-term memory formation and memory consolidation. Thus, polySia plays a crucial role in neural development by modulating the adhesive property of NCAM. As noted, it is involved in a wide range of morphogenic events, including cell migration, neurite outgrowth, path finding, sprouting, regeneration, and synaptic plasticity (108, 132, 214).

An important function for polySia-NCAM is to mediate many diverse neural processes through modulation of brain-derived neurotrophic factor (BDNF) signaling. BDNF plays an important role in many aspects of neural development and plasticity in the CNS and recently has been implicated in LTP and rapid modifications of synaptic function (52, 88). BDNF also has been implicated in fast modifications of synaptic transmission and is proposed to facilitate or even trigger synaptic potentiation. Accordingly, polySia-NCAM may function to sensitize pyramidal neurons to BDNF, thereby modulating activity-dependent synaptic plasticity (103).

A number of studies have shown that the level of Sia incorporated into neural tissues increases with learning. Specifically, ganglioside synthesis increases after long-term active-avoidance conditioning in rats (148). Since NCAM is the major carrier protein of polySia in the CNS, changes in the polysialylation state appear to facilitate memory formation (54, 58). Adult rats exhibit a transient increase in polysialylation within the hippocampus ~12 hours after a learning paradigm, including passive avoidance tasks (40, 55, 57, 58, 104, 105), spatial mazes (55, 104, 105, 118, 144), fear conditioning (144), and olfactory-learning tasks (54, 81). The degree of polysialylation returns to basal levels by ~14 hours post-training (104) and does not continue to increase with further trials of the respective learning task. Rather, the level reflects that seen at the 12-hour post-training period, irrespective of improved performance (104). Intracerebroventricular administration of anti-NCAM 6 hours post-training resulted in amnesia at the 48-hour recall, but not at 24 hours, when NCAM polysialylation was maximal (132). In addition to the cells within the hippocampal area, neurons in the entorhinal cortex, an important memory center in the brain and the septal nuclei, also exhibited significant increases in polysialylation post-training (55, 118). This cellular behavior is most likely in response to task-associated stimuli rather than retrieval from previously stored task-associated memory (104). This strongly suggests that the transient polysialylation is involved in processing information rather than information storage (57). Based on the extensive work that has examined this response, it is believed that the changes in polysialylation frequency of the neurons are a vital event leading to altered synaptic plasticity required for memory consolidation (54, 57). These observations suggest that the

polySia plays a fundamental role in facilitating learning.

Postulated Functional Roles of Gangliosides in Brain Development and Cognition

In the CNS, ganglioside expression is correlated with many neurophysiological functions including, neurogenesis, synaptogenesis, synaptic transmission, and cell proliferation. The precise mechanism(s) of how gangliosides mediate these diverse events is not fully understood. In previous reviews, the mechanisms for possible involvement of neuronal gangliosides in brain development and memory formation were discussed (78, 130, 203).

FUTURE PERSPECTIVES

Although much has been learned at the observational level about the role of Sia as a dietary supplement, many unsolved problems exist and much remains to be discovered. Every question answered appears to generate additional questions. In spite of the mostly phenomenological studies to date, there remains a dearth of information about the fundamental molecular mechanisms underlying how dietary Sia can have such a profound effect on the structure and function of the CNS and its impact on cognitive development and memory. It will be important in future studies to seek answers to the following questions:

1. Is the "memory" of an early dietary Sia supplementation experience retained throughout many cell generations, and to be expressed, or of value, in later life?
2. What is the long-term impact of the uptake of the nonhuman Sia, Neu5Gc, in neonates on later health and disease?
3. Which source of Sia from milk is most readily absorbed and can influence cognition in the human infant?
4. What is the most suitable animal model that most closely mimics the human infant in order to carry out preclinical studies to assess the impact of dietary Sia supplementation on neurodevelopment? To date, it appears that the piglet model best fulfils the requirements to evaluate the structure, function, and molecular and biochemical mechanisms of dietary Sia on brain development, cognition, and memory.

Current evidence strongly supports the concept that the Sia in human breast milk is an essential micronutrient for early brain development and cognition. Randomized human clinical trials are needed to determine if dietary Sia supplementation can enhance cognitive function in human infants.

SUMMARY POINTS

1. The rapid growth of infant brains places extraordinarily high demands on the supply of precursors and nutrients that serve as building blocks for newly synthesized tissues and cell growth. Suboptimal nutrition during the critical period of early brain development is recognized to have irreversible consequences for cognitive function into adulthood. Human breast milk is a species-specific food containing many unique nutrients required for optimal early brain growth and cognition. Many of these nutrients are not provided by either bovine milk or infant formula.
2. Sia, a family of 9-carbon acidic sugar molecules, are key monosaccharide units in brain gangliosides and glycoproteins, including the polysialic acid (polySia) glycotope on neural cell adhesion molecules (NCAM). Sia is also a major component of human milk oligosaccharides that are essential nutrients for brain development and cognition.

3. PolySia on NCAM plays an important role in cell migration, neurite outgrowth, branching, neuronal path finding, regeneration, and synaptic plasticity. Removal of polySia from NCAM results in developmental and behavioral defects in rodents. PolySia is essential in modulating NCAM functions during development of the CNS. Brain gangliosides also play a crucial role in mediating cell-cell interactions, cell connection, and the transmission and storage of information.
4. N-acetylneuraminic acid (Neu5Ac) is the most common form of Sia in humans and many foods. Humans lack the ability to synthesize N-glycolyneuraminic acid (Neu5Gc) because of an exon deletion/frameshift mutation in the human *CMAH* gene. This mutation results in the loss of Neu5Gc expression in human tissues. However, Neu5Gc can be metabolically incorporated into human cells and tissue after ingesting a diet rich in red meat and/or milk products. All humans have variable levels of circulating anti-Neu5Gc antibodies, thus raising potential concern about the consumption of a Neu5Gc-rich diet. Correlatives studies in humans and mice have shown that the dietary uptake of Neu5Gc from red meats and bovine milk may lead to some human inflammatory diseases.
5. Human breast milk contains a significantly high level of Sia, principally as Neu5Ac bound to milk oligosaccharides. Bovine milk and bovine-milk-based infant formulas contain a small amount Sia with Neu5Gc. The long-term impact of the uptake of Neu5Gc in neonates and on later health and disease remains to be determined.
6. Sia is ubiquitously distributed throughout human tissues, the most abundant source being in the CNS. Neural cell membranes contain ~20-fold higher levels of Sia than other cellular membranes. Human brain gangliosides constitute ~6% to 10% of the total lipid mass of the human brain, where they represent a quarter of the total conjugated saccharides and 70% to 80% of the conjugated Sia. These levels increase ~threefold from the tenth gestational week to ~5 years of age.
7. The activity of the rate-limiting bifunctional enzyme, UDP-GlcNAc-2-epimerase, which is responsible for synthesis of Sia, is initially lower in rat pup and guinea pig. This suggests that newborn human infants may also have a lower capacity for synthesizing Sia to meet the high demand for sialylglycoconjugate synthesis in synaptic membranes, in particularly preterm infants. Feeding a diet rich in Sia has been shown to increase the levels of Sia in neural tissues, leading to enhanced learning and memory in rat pup and piglets, and to up-regulate expression of two learning related genes, Gne and ST8SiaIV.
8. Normal brain development and active learning increase the requirement for sialylated structures, including brain gangliosides and polySia-NCAM. The higher level of ST8SiaIV activity may also up-regulate expression of Gne, thereby coupling endogenous Sia synthesis to polySia and ganglioside synthesis. Thus, during times of high Sia demand, the inhibitory feedback of CMP-Sia on GNE is minimized. An exogenous source of Neu5Ac is critical for the rapid brain growth in human infants. A clinical trial study is needed to determine the role of dietary Sia in infant nutrition and brain development.

FUTURE ISSUES

1. What is the precise metabolic fate of dietary Sia in humans, and how are the developmental changes of enzyme activities regulating Sia metabolism and glycoprotein glycosylation controlled during the postnatal period?
2. Which forms of Sia from milk are most readily absorbed and can influence cognition in the human infant?
3. What is the potential long-term impact of the uptake of Neu5Gc from red meat and bovine milk products in neonates on later health and disease?
4. A more suitable animal model that closely resembles the human infant, such as piglets, is required to examine the metabolic fate of human milk Sia and the mechanisms of how dietary Sia affects brain development and cognitive function.
5. A randomized human clinical trial is required to determine the safety and efficacy of the role of a dietary supplement of Sia in brain development, cognition, and memory.

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of this review.

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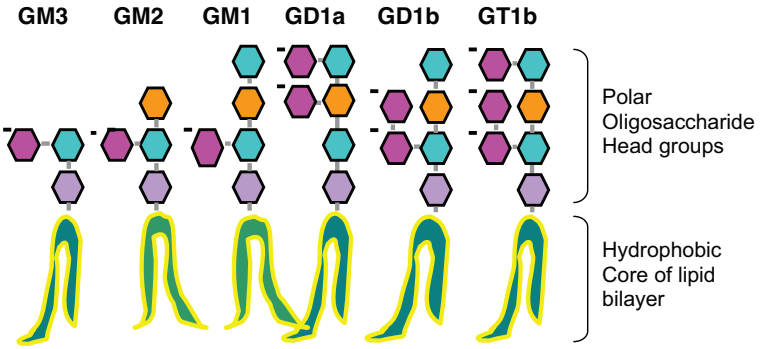

Figure 2

The chemical structures of some gangliosides showing the position of Sia. Figure adapted from Wang et al. (235).

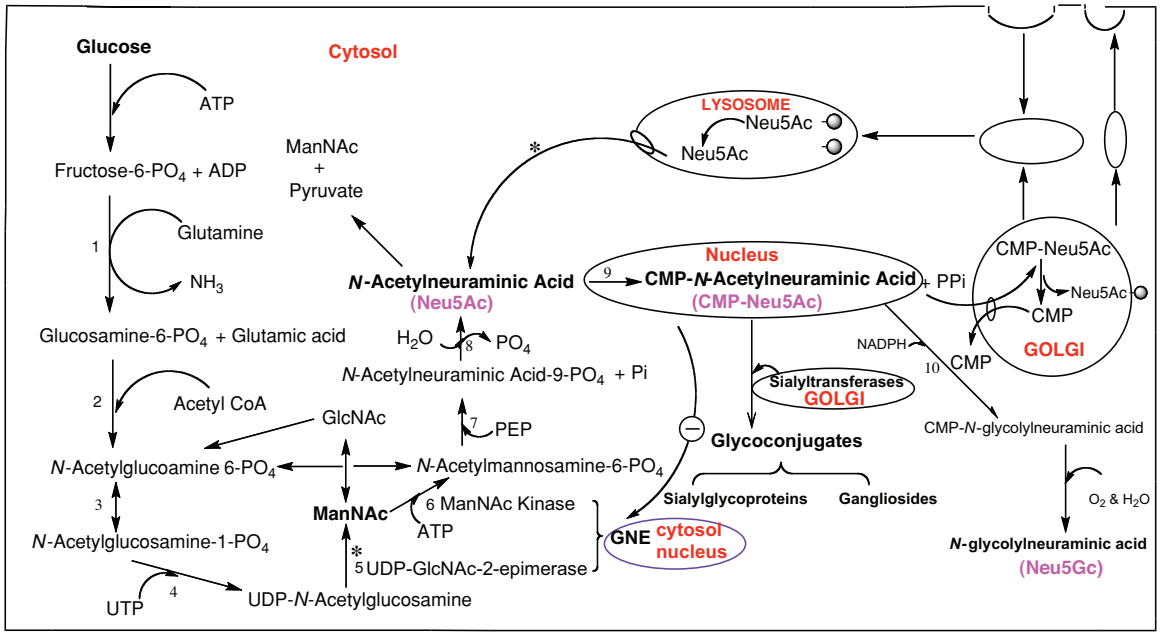

Figure 3

Summary of the metabolic pathway for synthesis of Sia. Enzymes: 1. Glucosamine-6-phosphate synthase; 2. glucosamine-6-phosphate *N*-acetyltransferase; 3. *N*-acetylglucosamine-6-phosphate mutase; 4. UDP-*N*-acetylglucosamine pyrophosphorylase; 5. (*key enzyme) UDP-*N*-acetylglucosamine-2-epimerase (activity is initially low in young rats); 6. *N*-acetylmannosamine kinase; 7. *N*-acetylneuraminate-9-phosphate synthase; 8. *N*-acetylneuraminate-9-phosphate phosphatase; 9. CMP-*N*-acetylneuraminate synthetase; 10. monooxygenase. Figure modified from Wang et al. (211).

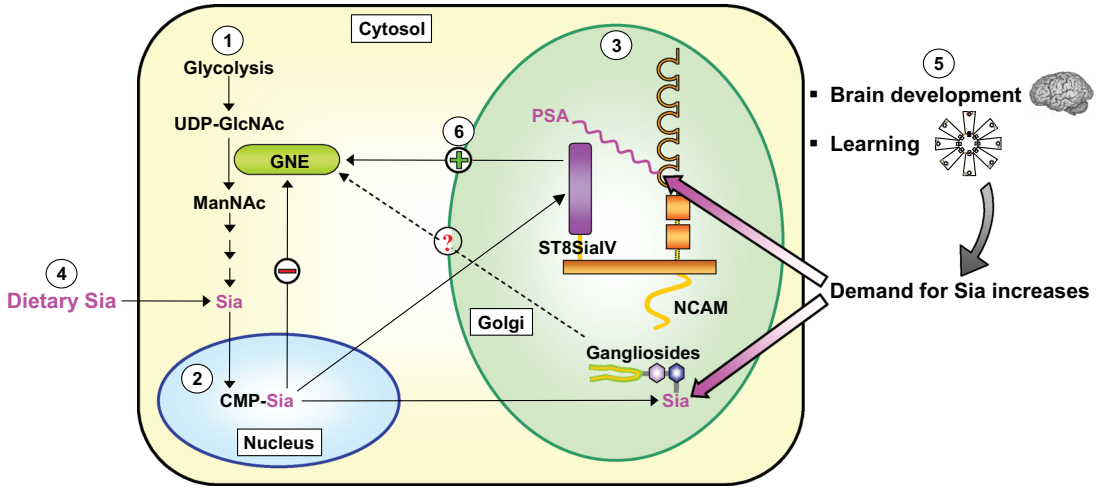

Figure 6

Proposed mechanism by which dietary sialic acid and learning influence cellular function during brain development. 1. Endogenous Sia is produced in the cytosol from glucose or the products of glycolysis. The epimerase moiety of the bifunctional enzyme of GNE is responsible for the rate-limiting step, converting UDP-GlcNAc to the Sia precursor, ManNAc. GNE activity is low in neonates (60). 2. In the nucleus, Sia is activated to CMP-Sia, the substrate for sialyltransferases such as the polysialyltransferase, ST8SiaIV. 3. Within the Golgi apparatus, Sia residues are transferred onto acceptor substrates including gangliosides and the NCAM. The intracellular concentration of Sia drives the synthesis of polySia on NCAM (15). CMP-Sia exerts negative feedback inhibition on GNE, limiting excess production of free Sia (15). 4. Supplementation of the diet with Sia bypasses GNE, increasing the amount of Sia available for metabolism and hence CMP-Sia for polySia synthesis. 5. Normal brain development and active learning increase the requirement for sialylated structures, including brain gangliosides and NCAM (177, 148). 6. We propose that increased learning and/or higher ST8SiaIV activity automatically increase the expression of GNE, thereby coupling endogenous Sia synthesis to polySia synthesis. Thus, during times of high Sia demand (learning and brain growth), the inhibitory feedback of CMP-Sia on GNE is minimized. 7. The mechanism whereby ST8SiaIV might up-regulate GNE expression is not clear. It is also unclear if gangliosides might up-regulate GNE expression during development. Figure modified from Wang et al. (211).

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Errata

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