

# Influence of dietary gangliosides on neonatal brain development

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*Gangliosides are sialic acid-containing glycosphingolipids. Gangliosides are found in human milk; understanding of the potential role of gangliosides in infant development is emerging, with suggested roles in the brain and gut. Ganglioside accretion in the developing brain is highest in utero and in early neonatal life, during the periods of dendritic branching and new synapse formation. Further, brain contains the highest relative ganglioside content in the body, particularly in neuronal cell membranes concentrated in the area of the synaptic membrane. Gangliosides are known to play a role in neuronal growth, migration and maturation, neuritogenesis, synaptogenesis, and myelination. In addition to their roles in development and structure of the brain, gangliosides also play a functional role in nerve cell communication. It is less well known whether dietary gangliosides can influence the development of cognitive function. This review summarizes current knowledge on the role gangliosides play in brain development.*

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## INTRODUCTION

Identified over a century ago, gangliosides are lipids named for brain ganglion cells from which these molecules were initially isolated.<sup>1</sup> Research indicates that gangliosides are components of plasma membranes in most vertebrate cells.<sup>2</sup> Gangliosides have been isolated from skeletal muscle, smooth muscle, liver, pancreas, spleen, placenta, thymocytes, lymphocytes, erythrocytes, plasma, amniotic fluid, and milk.<sup>3</sup> In addition to being present in milk, gangliosides are found in other foods of animal origin. A complex group of compounds, gangliosides are vital nutrients that exhibit a variety of important functions from cell differentiation and proliferation to cell death. This review focuses on dietary gangliosides and the role of gangliosides in influencing membrane structure and function which impact growth and development of various organs and modify some disease states.

## GANGLIOSIDE CLASSIFICATION

The new lipids metabolites and pathways strategy (LIPIDS MAPS) classification scheme<sup>4</sup> closely follows the IUPAC rules<sup>5</sup> and divides lipids into eight primary categories: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides. Gangliosides are members of the sphingolipid category and, more specifically, the acidic glycosphingolipid class. Gangliosides are the most complex glycolipids, with a number of different species identified,<sup>4</sup> and they form the basis of this review.

## NOMENCLATURE AND CHEMICAL STRUCTURE OF GANGLIOSIDES

There are two systems for naming a ganglioside structure. Due to simplicity, the nomenclature introduced by

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Svennerholm<sup>6</sup> is often chosen over the IUPAC system.<sup>7</sup> According to Svennerholm's method, all gangliosides start with "G", and the remainder of the nomenclature is based on the sugar portion of the molecule.

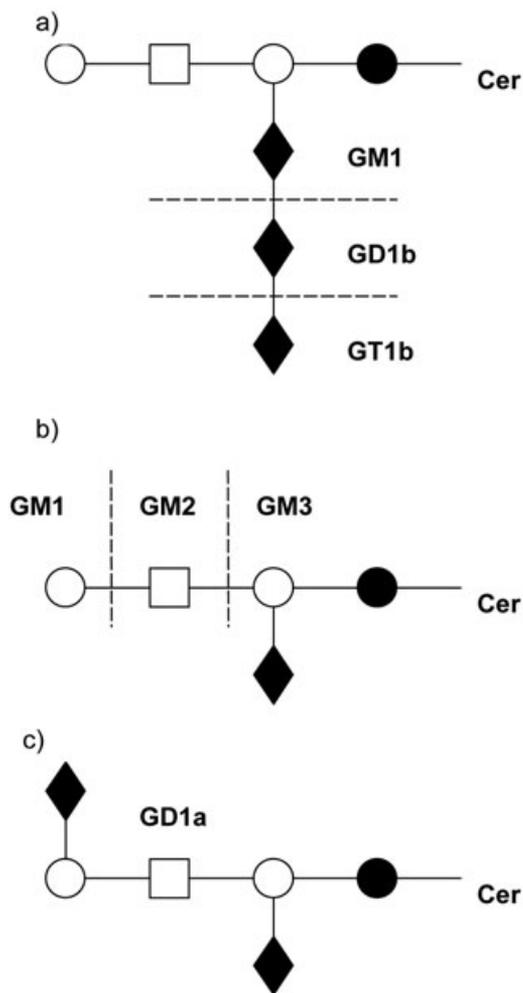
### Sugar moiety

A wide variety of ganglioside glycans (Figure 1) can be formed by a combination of glucose (Glc), galactose (Gal), N-acetyl galactosamine (GalNAc), and sialic acid. Following the letter "G" is a letter that indicates the number of sialic acid residues present in the molecule, e.g., "M" (mono), "D" (di), "T" (tri), "Q" (quad). The third

part of the nomenclature is a number that describes the carbohydrate sequence attached to the ceramide. In some cases there is a lowercase letter attached at the end to designate where the sialic acid residue is attached (Figure 1a,c). Further complexity of the glycan moiety is derived by the possible addition of fucose or from modification of the sialic acid hydroxyl groups with addition of acetyl or other groups. The addition of acetyl (4-, 7-, or 9-O-acetylation) has major effects on bioactivity or on recognition by other molecules. Although the most common forms of sialic acid are N-acetyl neuraminic acid and N-glycolyl neuraminic acid, more than 30 have been shown to exist in nature.<sup>8</sup>

### Ceramide moiety

Analysis of tissue gangliosides indicates a few selected fatty acids are linked to ceramide. Contrary to this, milk gangliosides are found to have a wide variety of ceramide-linked fatty acids. The fatty acid profile of milk gangliosides varies between species<sup>9</sup> and with the stage of lactation.<sup>10-12</sup> The importance of these compositional variations is starting to be explored. Potential implications of these fatty acid variations with respect to immunosuppression have been reported.<sup>13</sup> In brain tissue gangliosides (human and rat) the ceramide-linked fatty acid composition also changes with age (in utero and post birth) as does the sphingosine component, with increasing proportions of C-20 sphingosine and decreasing proportions of C-18 sphingosine.<sup>14</sup> Any possible link between the variation in the ceramides of milk over lactation and the variation in brain gangliosides ceramides within the corresponding time period is unexplored, but human milk gangliosides are dominated by C16:0, C18:0, and C18:1<sup>12</sup> early on in lactation, with variation increasing as lactation progresses; the human brain gangliosides are also dominated by those three fatty acids plus C20:0 early in life.<sup>14</sup>



**Figure 1 Chemical structures of some gangliosides.** (a) Chemical structure of a ganglioside showing how attachment of N-acetyl neuraminic acid (NANA) relates to the nomenclature of the molecule, i.e., with one NANA, the molecule is called monosialic. (b) Chemical structure of a ganglioside showing how numerical subscript refers to the number of hexoses in the molecule (which also affects the distance of migration on thin layer chromatography plates). (c) GD1a.

### GANGLIOSIDES IN FOOD

Dietary sphingolipids are ingested as part of meat, fish, eggs, milk, and some vegetable products such as soybeans.<sup>15</sup> Daily intake of sphingolipids has been calculated to be 0.3–0.4 g/day.<sup>15</sup> Gangliosides make up a small percentage of sphingolipid intake. Dietary gangliosides have been isolated from eggs.<sup>16</sup> Egg yolks have been reported to contain 2.5, 8.5, and 1.5 mg, respectively, of the gangliosides GM4, GM3, and GD3.<sup>16</sup> Gangliosides have also been isolated from chicken livers,<sup>17</sup> amounting to 330 nmol/g of liver, with GM4 and GM3 comprising the main gangliosides. Ohashi<sup>18</sup> looked at the ganglioside distribution in fat from various animals and found

remarkable interspecies variation, especially in the composition and relative concentration of complex gangliosides. A substantial amount of disialosylganglioside, however, was commonly found in all animal fat tissues tested.<sup>18</sup> Plants are not a source of gangliosides as they lack the pathway to synthesize sialic acid. Current literature suggests it is difficult to reliably assess and calculate the actual ganglioside content of a diet. This is, in part, dependent on the procedure used for extraction of crude gangliosides. A comparison of four methods for extraction of gangliosides from dairy products resulted in a wide and variable range of values.<sup>19</sup> Consequently, sphingolipid and ganglioside values are not contained in food composition tables.

### Milk and lactation

Ganglioside composition has been studied most frequently in milk and milk products. In milk, gangliosides are almost exclusively associated with the milk-fat-globule membrane.<sup>20</sup> During lactation, gangliosides are derived mainly from the apical plasma membrane of apocrine secretory cells in the mammary gland.<sup>20</sup> Changes in the ganglioside content of mammalian milk and its distribution during lactation imply that gangliosides play a role in newborn development.

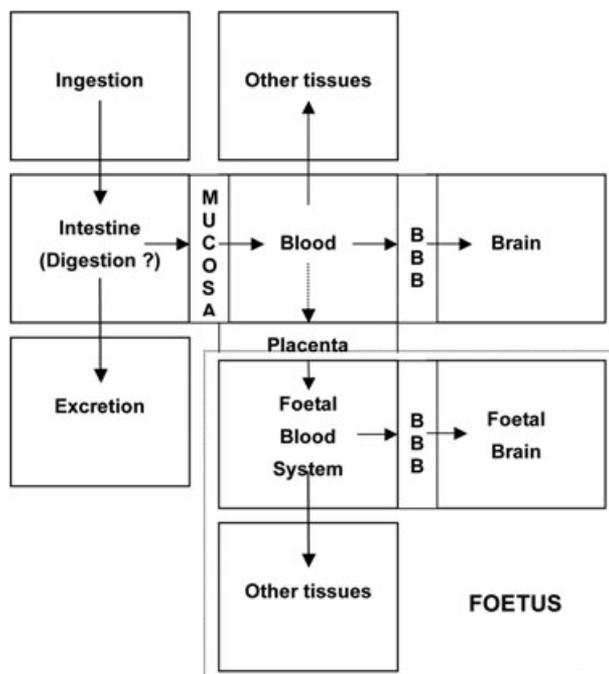
Comparative ganglioside analysis of milk indicates cow's milk has a much lower ganglioside concentration than human milk. Likewise, cow's-milk-based infant formula has a lower ganglioside concentration compared to human milk.<sup>21</sup> Human colostrum, or early milk, contains a higher total ganglioside concentration compared to mature milk.<sup>12,22</sup> This pattern is also noted in cow's milk.<sup>10,23,24</sup> In cow's milk, it was reported that another slight rise in ganglioside content was observed from milk samples analyzed in later lactation.<sup>10,25</sup> While total ganglioside concentration fluctuates, individual ganglioside concentrations also vary among species and stages of lactation.

In human milk, GD3 is the predominant ganglioside in early stages of lactation while GM3 becomes more predominant in later stages.<sup>12,21,26,27</sup> GD3 is also the predominant ganglioside in cow's milk, making up about 60–70%, with GM3 and GT3 comprising most of the remainder.<sup>10,26</sup> More recent analysis, however, suggests a ganglioside preparation from bovine milk contained more GD1a and GD1b than GM3 or GT3.<sup>28,29</sup> This discrepancy may be related to species variability, milk-sampling procedures that affect the amount of fore-milk and hind-milk collected, stage of lactation at which the milk was obtained, or extraction methodology.<sup>19,30</sup> At this point there is no evidence to suggest whether or not maternal diet influences the amount of gangliosides in milk.

### METABOLISM OF GANGLIOSIDES

Gangliosides added exogenously to culture media are taken up by a variety of cells, including astrocytes, glioma, pituitary tumor cells, neuroblastoma cells, fibroblasts, red and white human blood cells, leukemic and non-leukemic lymphocytes, and intestinal mucosa.<sup>29,31–41</sup>

To gain an understanding of ganglioside metabolism, studies have injected gangliosides into animals and monitored ganglioside fate. Before cell uptake, gangliosides occur in aqueous solutions in the form of micelles, monomers, and oligomers.<sup>42</sup> Based on this, it is speculated that gangliosides would be in one of these forms in blood. In mice, injected gangliosides (GM1) were taken up by the liver, internalized into cells, and metabolized.<sup>43</sup> Studies using sphingosine C-3 (<sup>3</sup>H)-labelled GM1 in adult C3H/He male mice demonstrated that ganglioside was mainly incorporated into the liver and rapidly metabolized.<sup>44</sup> Research in pregnant rats<sup>45</sup> showed that injected ganglioside GM1 was distributed into maternal organs and crossed the placenta as early as 30 min after injection. Ganglioside was then taken up by fetal tissue. A substantial amount of labeled GM1 was incorporated into maternal brain, suggesting GM1 crosses the blood-brain barrier<sup>45</sup> (Figure 2). This work indicates GM1 is utilized in various organs for synthesis of polysialogangliosides.<sup>45</sup> In a reperfusion study, milk-derived GM3 was shown to cross human placentae and milk-derived GD3 was taken



**Figure 2 Uptake of dietary gangliosides by the body.** Each tissue that receives the gangliosides modifies them to the specific ganglioside signature of that tissue at that stage of life.

up by the placenta.<sup>46</sup> Recent data suggest that supplementing the diet of rat dams during pregnancy can significantly increase the levels of brain gangliosides in the pups soon after birth.<sup>47</sup>

## DIGESTION AND ABSORPTION

GM3 and GD3 gangliosides in human milk reach the intestinal tract intact after passing through the acidic environment of the infant's stomach. Over 80% of the sialic acid in GM3 and GD3 structures remained intact under acidic conditions (pH 3–5) similar to the conditions found in an infant's stomach.<sup>48</sup> The pH in an infant's stomach is higher (pH 3–4) compared to the pH in an adult's stomach (pH 2).<sup>49</sup> Lactonization (gangliosides containing inner ester linkages) of sialic acid occurs at lower pH<sup>48</sup> levels and has been shown to protect the sialic acid monomer from destruction under acid conditions.<sup>50</sup> Dietary ganglioside can be absorbed in the small intestine and transported to different membrane sites in the body<sup>29</sup> (Figure 2).

### Incorporation into enterocytes

Once absorbed by the intestine, dietary ganglioside may be remodeled in the enterocyte, inducing changes in total membrane ganglioside content. GM3 is the major ganglioside in the enterocyte of humans and animals.<sup>51,52</sup> A decrease in GM3 level in rat intestine was reported following a 2-week feeding protocol with a GD3 ganglioside-enriched lipid diet.<sup>29</sup> The authors also observed an increase in the level of GD3 in the intestine compared with the control animals.<sup>29</sup> The alteration of ganglioside level in the intestine could potentially change developing enterocyte function.

### Localization in the brush-border membrane

GM3 and GD3 are localized, respectively, in the apical and basolateral membrane in the enterocyte.<sup>29</sup> This difference in localization implies different biological and/or physiological functions for gut development and protection.<sup>29</sup> Localization of GM3 at the apical membrane provides direct exposure to the lumen and supports a role for GM3 in the interception and inactivation of bacterial toxins. Concentration of GD3 in the basolateral membrane supports a role for GD3 in immune cell activation via access to local immune factors present in the lamina propria of the intestine. Tight junctions serve to partition the cytosolic membrane into apical and basolateral domains. Tight junctions of the gastrointestinal epithelial cells regulate intestinal permeability and barrier function. The expression of tight-junction proteins protects the

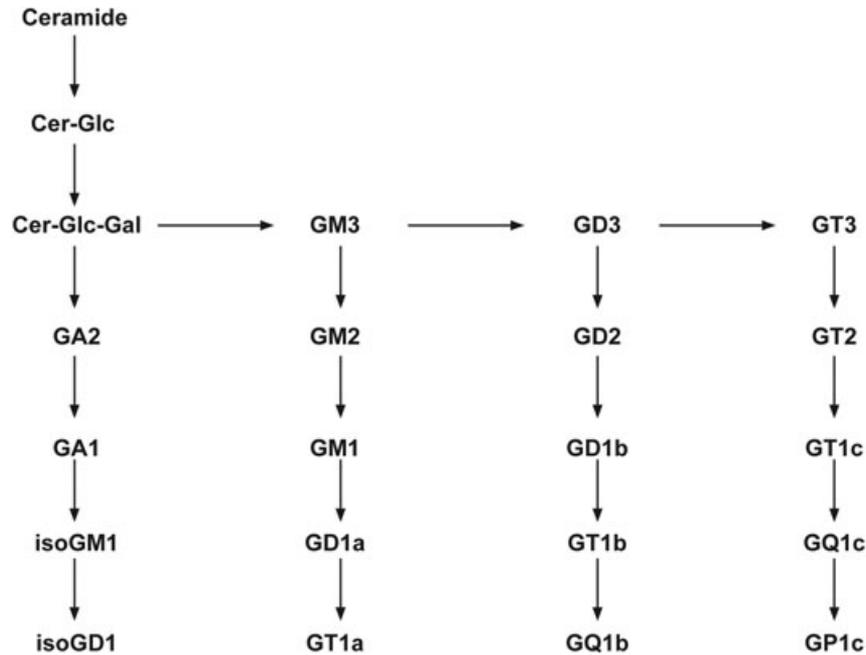
host from bacterial toxins, viruses, and dietary antigens, while maintaining distinct fluid-containing compartments.<sup>53</sup> Dietary ganglioside has been shown to inhibit degradation of tight-junction proteins in acute inflammation.<sup>54</sup>

### Interconversion and biosynthesis

Exogenous gangliosides are metabolized via two main routes. One route involves direct glycosylation in the Golgi apparatus (Figure 3), yielding higher homologous gangliosides<sup>55</sup> or modified gangliosides.<sup>56</sup> The other route involves catabolism in lysosomes<sup>57</sup> and results in concurrent formation of intermediates of both the saccharide and lipid components.

Ceramide is synthesized in membranes of the endoplasmic reticulum and functions as the lipid anchor of gangliosides. Upon transport to the Golgi apparatus, a glucose residue can be added to ceramide to produce glucosylceramide. Glucosylceramide can be further modified in the luminal leaflet of the Golgi by addition of galactose to form lactosylceramide. The precursor for most gangliosides is lactosylceramide. There is an ordered addition of nucleotide-activated sugars, which is assisted by specific glycosyltransferases.<sup>55</sup> Following synthesis in the Golgi, gangliosides enter the plasma membrane. Here, gangliosides are assembled into cholesterol- and sphingolipid-rich caveolae and rafts. These microdomains function as signaling platforms. Park et al.<sup>58</sup> indicated that dietary ganglioside is directly incorporated into the microdomain. Specific diet-induced change in the GM3 and GD3 content of microdomains may have implications for a variety of cellular functions.

Larson et al.<sup>59</sup> measured glycolipids in the feces of breastfed infants and found that lactosylceramide, the product of either acid or enzymatic desialylation of GD3 or GM3, was a minor component in stools. Further reports indicate that lactosylceramide did not accumulate in feces until after the introduction of formula or solid foods. This was irrespective of whether breast milk was consumed and parallel to a decrease in the amount of extended glycosphingolipid structures. GM3 and GD3 were identified in the feces of breastfed infants. Both gangliosides were present at 1 and 3 months in breastfed babies, but GM3 disappeared following the introduction of solids or infant formula. After 3 months, GD3 was gradually lost from the feces of breastfed babies. These data coincide with the dietary supply of gangliosides, with the drop in GD3 in human milk relative to GM3<sup>50</sup> being reflected in the feces' ganglioside content as well as the observation that GD3 is the dominant ganglioside in infant formula and in the feces of infant-formula-fed babies. A number of *Bifidobacterium* isolated from infant feces produce sialidases.<sup>60</sup> Sialidases desialylate ganglio-



**Figure 3 Pathways of ganglioside biosynthesis.**  
Abbreviations: Cer, ceramide; Glc, glucose; Gal, galactose.

side in the colon but data suggest that gangliosides may survive passage through the intestinal tract. The relative amount of gangliosides metabolized or absorbed to the amount of ganglioside excreted is not known. It is known that feeding GD3 increases GD3 levels in rat intestinal mucosa and plasma.<sup>28</sup>

### Plasma levels

Ganglioside GM3 is reported as the major ganglioside in human serum.<sup>61</sup> When the diet contained GD3 and GM3, GD1a was reported to be the major ganglioside in plasma from rats.<sup>29</sup> Total ganglioside levels in plasma increase following dietary ganglioside supplementation in rats, with no apparent change in composition of individual gangliosides.<sup>29</sup>

Clearly, dietary gangliosides produce increased ganglioside levels in the blood and other organs. What remains to be determined is the degree to which the rise in blood ganglioside is a result of the same gangliosides ingested or whether ganglioside ingested is remodeled in the enterocyte and released into the blood as newly formed ganglioside.

### BIOLOGICAL EFFECTS AND FUNCTIONS OF GANGLIOSIDES

Gangliosides are both structural and functional lipids. Structurally, gangliosides form part of the membrane lipid. Gangliosides are located at the surface of the cell

membrane with the hydrophilic oligosaccharide portion extending into the extracellular space. As the size of the glycan molecule increases, the hydrophilic portion of the molecule also increases. The ceramide portion of the ganglioside molecule is hydrophobic. The hydrophilic and hydrophobic portions together make the ganglioside molecule amphiphilic. Other physical properties of glycosphingolipids (such as high-phase-transition temperatures) affect the membrane and lipoprotein properties of the cell. Understanding the functional role of gangliosides as a bioactive lipid is developing. Since ganglioside profiles vary from one tissue to another,<sup>20</sup> there is speculation that individual gangliosides could have different functions. Among the known cellular functions of gangliosides are cell adhesion and recognition, intracellular trafficking of lipid raft components, cell differentiation and proliferation, cell death, modulation of ion channels, calcium influx and neurotransmitter release, and signal transduction in immune function. The basis for most of these functions revolves around signal transduction pathways. The predominant distribution of gangliosides in the plasma membrane enables gangliosides to modulate cellular response through signaling platforms. Signaling platforms are membrane microdomains that are also known as lipid rafts and caveolae. These domains are enriched in cholesterol and sphingolipids, among which gangliosides appear to be structurally important.<sup>62,63</sup> From the position in microdomains, gangliosides have been reported to modulate the function of various receptors.<sup>64,65</sup>

## GANGLIOSIDES AND BRAIN STRUCTURE

Lipids of complex structure and composition are abundant components of the human brain. Much of the complex structure is a result of the high concentration of gangliosides in brain. Compared to large visceral organs or the intestinal mucosa, respectively, brain contains 15 to 500 times more ganglioside.<sup>66</sup> Ganglioside is three times more concentrated in cerebral grey matter compared to white matter.<sup>66</sup> The ganglioside profile of neural tissue changes significantly during brain development. Between weeks 16 and 22 of human gestation, ganglioside concentration increased twofold in the frontal cortex and increased 30% in the hippocampus.<sup>67</sup> This high concentration persisted for up to 30 weeks in most cortical regions. In the cerebellar cortex, ganglioside concentration continued to increase for up to 4 months postnatal.<sup>67</sup>

## GANGLIOSIDES AND BRAIN FUNCTION

During growth and development, the composition and metabolism of lipids changes in the brain.<sup>68,69</sup> The complex and diverse brain lipid system plays a role in modifying the fluidity, structure, and function of brain membranes.<sup>70-74</sup> An analysis of different regions in infant brain tissue during the first 13 weeks of life determined the fatty acid accretion rates required for synthesis of structural lipids.<sup>75</sup> An increase in specific fatty acids in fetal brain tissue during the third trimester of pregnancy is particularly important in the normal rise in brain cellularity that occurs during this period of development.<sup>76</sup> Primary cultures of nerve tissue from prenatal rat brain<sup>77,78</sup> and postnatal chick brain<sup>79</sup> have shown the ability of nerve cells to synthesize polysialogangliosides,<sup>80</sup> and the expression of polysialogangliosides during fetal development can be transient.<sup>81</sup> Development of the visual system and visual acuity occur alongside development of other brain regions. Similar to development in other brain regions, the visual system reveals critical periods for development. Lipid composition in retina also changes with developmental stage.<sup>82-84</sup> Analysis of lipid profiles during development and aging in human and rat brain indicate regional differences in lipid composition, including gangliosides.<sup>80,85-87</sup>

The association between gangliosides and neurons was first established over 80 years ago, when gangliosides were observed in grey matter of normal and pathological brains.<sup>1</sup> Gangliosides are found in abundance in the plasma membrane and synaptic membranes of nerve cells.<sup>88-90</sup> The majority of gangliosides in most vertebrate neurons are derived from the gangliotetraose family, which includes gangliosides with four hexoses (i.e., GM1 and its sialylated derivatives).<sup>91</sup> Compared to other

gangliosides, GM1 has been associated most commonly with brain function and is the ganglioside used most commonly in brain-related research.

The physiological roles of gangliosides in brain are not fully understood. Studies confirm that neuronal and glial ganglioside expression is developmentally regulated.<sup>92-94</sup> Changes in ganglioside concentration and composition coincide with the progress of neuronal and glial differentiation and maturation.<sup>95</sup> Dramatic changes in the distribution and quantity of gangliosides that occur during cephalogenesis are evident.<sup>92</sup> The ganglioside 9-O-acetyl GD3 is a potent promoter of neurite outgrowth and is expressed in different regions of the nervous system of both developing and adult rats. Mendez-Otero and Santiago<sup>96</sup> demonstrated the functional role of 9-O-acetyl GD3 in neuronal motility. It is generally accepted that gangliosides are involved in synaptic transmission, neuronal metabolism, development, and maintenance of neural tissue.<sup>94,97-99</sup>

During development, the concentration and distribution of brain gangliosides change significantly.<sup>95</sup> It has been noted that changes in ganglioside composition may be a consistent feature of brain differentiation.<sup>100,101</sup> In early stages of brain development, Stojiljkovic et al.<sup>102</sup> verified the presence of GM1 and GM3 gangliosides in neuronal and glial cells. Glial-neuronal contacts made during migration of neuroblasts to the neurons final morphologic destination involved both GM1 and GM3.<sup>102</sup> The presence of GM3 in proliferative cells in the ventricular zone of the telencephalic wall was previously verified.<sup>102</sup> The human brain's center for memory and learning is the hippocampus and it shows a 30% increase in ganglioside concentration between 16 and 22 weeks of gestation, but after birth, the ganglioside concentration starts to decrease along with the proportions of GD1a.<sup>67</sup> The decreasing proportion of GD1a is observed until 20-30 years of age.<sup>67</sup> This suggests that the pattern of ganglioside concentration in adult human brain also changes continuously.<sup>86,87,103</sup> Gangliosides contain the majority of sialic acid within the central nervous system<sup>104</sup> and sialic acid is understood to be an important "brain nutrient." Segler-Stahl et al.<sup>103</sup> reported that the concentration of ganglioside-bound sialic acid decreased considerably with age. More specifically, ganglioside-bound sialic acid concentrations increase in frontal white matter, reaching a peak between 40 and 50 years of age.<sup>86</sup> Ganglioside-bound sialic acid decreases around the age of 60 years.<sup>86</sup> Thus, a plateau in the level of ganglioside-bound sialic acid was observed between the ages of 20 and 70 years, after which there was an accelerated decrease in ganglioside concentration.<sup>86</sup> These patterns of development are difficult to accurately stage as the aging rate in the different brains analyzed cannot be assessed or standardized.

The role of gangliosides in brain function can be assessed by studying how brain function is affected when a specific ganglioside is absent, or when a defect in ganglioside metabolism exists. A defect in ganglioside synthesis has been shown to underlie an inherited form of epilepsy,<sup>105</sup> suggesting a link between ganglioside and this neurological disorder. By disrupting a gene for a key enzyme in complex ganglioside biosynthesis (GM2/GD2), Sheikh et al.<sup>106</sup> produced mice that expressed only simple gangliosides (GM3/GD3). In these animals, axonal degeneration in both the central and peripheral nervous system, demyelination in peripheral nerves, and decreased myelination were observed in the central nerves. These results provide evidence that complex gangliosides function in central myelination and in maintaining the integrity of axons and myelin,<sup>106</sup> stabilizing brain function.<sup>105</sup>

### **Axonal sprouting/neuronal development**

Gangliosides are implicated in neural development. GM3 induces apparent cell differentiation of neural cells.<sup>107</sup> Roisen et al.<sup>92</sup> exposed primary cultures of sensory ganglia and an established neuroblastoma cell line to media containing mixtures of bovine brain gangliosides. These ganglioside mixtures enhanced neurite development and metabolic activity of both sensory ganglia and neuro-2a neuroblastoma cells. Exogenous ganglioside enhanced axonal sprouting in primary embryonic neurons and established neuronal cultures, suggesting gangliosides play a key role in regulating neuronal maturation.

Mechanisms underlying neuritogenic and/or neuroprotective effects associated with dietary gangliosides are thought to involve  $\text{Ca}^{2+}$  modulation<sup>108</sup> through various interactions including with protein kinases such as calmodulin-dependent protein kinase.  $\text{Ca}^{2+}$  plays a pivotal role in mediating a host of cellular processes, and gangliosides contribute in several ways to the complex  $\text{Ca}^{2+}$  system through modulation of ion channels, transport/exchange proteins, and  $\text{Ca}^{2+}$ -utilizing enzymes.<sup>108</sup>

### **Neural transmission**

Gangliosides are components of the plasma membrane, are enriched in the synaptic junction,<sup>109</sup> and are, thus, ideally localized for a role in neural transmission. Gangliosides modulate ion channel function<sup>108</sup> and receptor signaling, two functions that are essential for the successful transmission of nervous impulses.<sup>105</sup>

The role of gangliosides in brain function is, in part, attributed to the sialic acid portion of the molecule, with the main function involving transmission of neural

impulses across synapses in the brain. It is believed that the sialic acid on gangliosides around the synaptic cleft modulates calcium ion levels via  $\text{Ca}^{2+}$ -ganglioside interactions.<sup>110-118</sup> Calcium ions are critical mediators in neuronal responses. During periods when there is no action potential in the presynapse, the presynaptic membrane is closed. This is due to tight  $\text{Ca}^{2+}$ -ganglioside associations with the aid of negatively charged terminal sialic acid residues on the ganglioside molecule. An action potential (nervous impulse) travelling from the presynapse to the postsynapse changes the electric field strength and/or ion concentration of the presynaptic membrane containing gangliosides. As a result, the gangliosides are reoriented and calcium ions are released from binding sites on the ganglioside molecule. Membrane permeability is altered, leading to an influx of calcium ions through ion channels into the presynapse. The increase in calcium ion concentration in the presynapse initiates a number of intracellular responses and signal cascades, which leads to release of neurotransmitters into the synapse. Neurotransmitters cross the synapse to the postsynaptic membrane and then bind to specific receptors. This binding results in an influx of sodium ions into the postsynaptic cell and a local depolarization of the postsynapse. In the presynapse, resting potential is restored as the transmitter is degraded, calcium ions are returned to the extracellular space by ganglioside-modulated  $\text{Ca}^{2+}$ -ATPase, and the tight membrane formed by the interaction between calcium ions and gangliosides is reformed.<sup>119</sup>

### **Behavior and memory**

Rahmann<sup>119</sup> provides a review of brain gangliosides and memory formation. The structural basis for memory formation is supported by molecular facilitation of neuronal circuits. This occurs through stabilization of synaptic contacts. Neurological, ultrastructural, phylogenetical, ecophysiological, ontogenetical, biochemical, physicochemical, and bioelectrical evidence was provided by Rahmann to support the role of gangliosides in facilitating neural circuits in synapses, thus supporting a role for gangliosides in memory formation.<sup>119</sup>

A link between behavior and gangliosides has also been examined. Although not specific to dietary gangliosides, the effects of malnutrition on ganglioside metabolism in rat pups has been investigated.<sup>120-122</sup> Malnutrition reduces the ganglioside content of the brain<sup>121</sup> and produces rats with significantly different open-field behavior.<sup>120</sup> Post-weaning sialic acid supplementation increased ganglioside concentrations and reduced the expected behavioral abnormalities expected from malnutrition.<sup>122</sup> The supplemented animals also learned the Y-maze quicker.<sup>122</sup> Environmental stimulation was also

shown to prevent most abnormalities associated with early malnutrition but, interestingly, small changes in ganglioside metabolism in rat whole brain were also observed following short-term environmental stimulation.<sup>120</sup> In this model, improvement in behavior was associated with both increased brain-cell size and increases in brain gangliosides and glycoprotein sialic acid content.<sup>120</sup>

### Ganglioside supplementation studies

There have been a number of studies looking at supplementing the supply of gangliosides to variously aged animals and humans. Given the complexity of the ganglioside group of molecules, the range of subject ages, cognitive state and disease/disorder state, and the outcomes measured, the picture produced is far from clear. Inconsistent results have been obtained with respect to the role and efficacy of supplemented gangliosides in the learning performance of animals. Some have shown improvement<sup>123–126</sup> and others have not.<sup>127,128</sup> Inconsistent results may be due to variations in the composition of ganglioside tested.

Between 1980 and 2000, a mixture of four different cerebral gangliosides derived from bovine brain (Cronasial™) was tested for efficacy in the treatment of various neurological disorders.<sup>129–134</sup> The mixture contained 21% GM1, 40% GD1a, 16% GT1b, and 5% other gangliosides. Mixed results obtained from these studies may be attributed to the source or isolation methodology from which the ganglioside was obtained or to the treatment administered for different disorders.

Mei and Zheng<sup>125</sup> tested whether gangliosides would enhance the learning and memory processes of rats. Cognitive function and memory at various ages after receiving placebo or injected bovine brain gangliosides was tested. The results showed that gangliosides enhanced the learning ability of neonatal, adult, and aged rats ( $P < 0.001$ ). Likewise, the results of the memory tests showed that there was a significant difference in memory retention in ganglioside-treated rats compared with those not receiving gangliosides. For example, in one memory test, after 1 month, those that received gangliosides had a 97.5% retention compared with 91.1% retention for those receiving the placebo ( $P < 0.05$ ).

Park et al.<sup>29</sup> fed weanling rats for 2 weeks with bovine-milk-derived gangliosides at 0.02% of the diet and measured the subsequent ganglioside content in brain, plasma, and intestinal mucosa. Compared to animals fed the control diet, animals fed the ganglioside diet exhibited significantly higher total brain, plasma, and intestinal mucosa ganglioside content. Although there was a higher overall content of gangliosides in the brain tissue of the rats fed gangliosides, there was no difference in ganglioside species distribution.<sup>29</sup> Dietary ganglioside also

altered the total ganglioside level in the retina and GD3 content during retinal development in rats.<sup>28</sup> In rats fed a ganglioside-supplemented diet for 2 weeks from the time of weaning at a level of 0.02% of the diet, a 39% increase occurred in the ganglioside content in retina. Supplementation with ganglioside also produced concurrent changes in retinal phospholipid composition.<sup>28</sup> Changes in retinal phospholipid composition may affect light adaptation via alteration of electric potential in retinal rod outer segments. Thus, changes in ganglioside and phospholipid composition could potentially lead to enhanced development of retinal function in neonates. Behavior, memory, and learning were not investigated in either of these studies.

Rat pups orally supplemented from 10 days until 80 days of age with milk-derived lipid ingredient to provide gangliosides at 0.01% of the diet showed significant improvement over control but showed no significant differences in brain ganglioside concentration.<sup>135</sup>

When rat dams were supplemented during pregnancy and lactation with a milk-derived lipid ingredient to provide gangliosides at 0.01% of their diet, 2-day-old pups had significantly higher levels of gangliosides in their brains.<sup>47</sup> At weaning, the pups' brain ganglioside concentration was still higher but not significantly. With no further supplementation, novelty recognition and spatial memory was not different from the control group.<sup>47</sup>

To determine if dietary gangliosides alter the lipid profiles of synaptosomal membranes and myelin fractions of the developing rat brain, Watson and Clandinin<sup>136</sup> fed weanling rats either a diet supplemented with gangliosides or a control diet. Results indicate that dietary gangliosides did not alter lipid profiles, except GQ1b in myelin and GM4 in synaptosomal membrane.

The study of Wang et al.<sup>137</sup> is not strictly a supplementation study but it is interesting. Wang et al.<sup>137</sup> measured ganglioside and protein-bound sialic acid in the frontal cortex of infants that had died of sudden infant death syndrome. They report that the infants fed human milk have an average 32% higher brain ganglioside content ( $P = 0.013$ ). Protein-linked sialic acid concentrations were also higher ( $P = 0.01$ ) in infants fed with human milk than infants fed infant formula. Since animal studies<sup>122</sup> indicate decreased learning ability with lower brain ganglioside levels, and human milk has higher ganglioside levels<sup>21</sup> than infant formula, there may be a link between infant ganglioside consumption and cognitive health. Given the roles of gangliosides in brain development, the higher ganglioside levels in infants fed human milk suggest an increase in synaptogenesis and differences in neurodevelopment in infants fed human milk. These data provide the first evidence of differences in brain sialic acid concentrations between

breastfed and formula-fed infants, suggesting there may also be potential for differences in brain development and cognition.

Xu and Zhu<sup>138</sup> reported evidence of the effects of exogenous and orally administered gangliosides on the brain functions of 2230 children suffering from cerebral palsy. They report that the oral ganglioside treatment improved the neurological symptoms associated with cerebral palsy, in particular muscle tension, limb function, language ability, and intelligence. The study lacked detailed statistical analysis and a control group, but the authors state that treatment resulted in faster improvement with younger children (0–3 years).

## CONCLUSION

Initially isolated from neural tissue, gangliosides are now known to exist in all mammalian cells. Gangliosides are complex molecules associated with expansive roles in the body. Although research on gangliosides spans decades, only recently have the vast functions and diverse potential of these molecules begun to be appreciated.

The position of gangliosides in membrane microdomains renders them structurally important for modulating receptor function and inflammatory signaling. This could have implications for preventive strategies and therapies of various disease states.

Structural observations, including the presence of gangliosides in the central nervous system and in the plasma membrane of neurons, suggest that gangliosides are strongly associated with neural functioning. Gangliosides play both a structural role and a functional role in the brain, as membrane components as well as agents/modulators of neural transmission. Previous research provides some evidence to suggest that oral supplementation of gangliosides to humans improves mental function/behavior. However, given recent advances in isolation technology, purity, and source, and the fact that few supplementary studies have been undertaken, this may be an encouraging area for further research. In particular, it may be useful to investigate the benefit to premature infants and to those that have suffered neurological damage at birth, in addition to investigating maternal supplementation, and whether there are windows in an infant's brain development during which ganglioside supplementation may have greatest benefit.

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