Published in final edited form as:

Semin Cell Dev Biol. 2010 August ; 21(6): 655–662. doi:10.1016/j.semcdb.2010.03.011.

METABOLIC MANIPULATION OF GLYCOSYLATION DISORDERS IN HUMANS AND ANIMAL MODELS

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1. INTRODUCTION

The metabolic chart is not flat since all metabolites in various pathways are not equally represented in time and space within an organism. For the most part, we do not know the sources or flux rates of metabolites. Patients with metabolic defects are sometimes treated by dietary manipulations [1,2,3], because they have hypomorphic alleles, allowing for direct or indirect stimulation of an injured pathway. Here, we focus on a few Congenital Disorders of Glycosylation (CDG) and related diseases that impair sugar chain (glycan) synthesis with specific reference to the expectations, and surprises when monosaccharide supplements are given to patients or animals with inherited glycosylation deficiencies (table 1). These examples provide insights and cautions for studies covering the four-dimensional metabolic chart.

2. BIOSYNTHETIC PATHWAYS AND GLYCANS

2.1 Sources of Precursors

De novo and salvage metabolic pathways for monosaccharides are shown in Figure 1 [4]. Monosaccharides can be derived from the diet, or salvaged (reutilized) from degraded endogenous glycans.

Animal cell glycans made within the ER/Golgi contain nine precursor sugars: glucose, mannose, galactose, N-acetylglucosamine, N-acetylgalactosamine, sialic acid(s), fucose, xylose and glucuronic acid. All can be derived exclusively from glucose. Some occur in the diet or are indirectly salvaged from glycans degraded within lysosomes [4]. The relative contributions of de novo, salvage and dietary pathways are quite variable but mostly unknown [5,6]. How the preferences translate into physiology/pathology probably depends on the relative biosynthetic loads of different tissues. For instance, during embryonic development chondrocytes carry a heavy glycosylation burden [7], and in postnatal growth, hepatocytes make most plasma glycoproteins.

2.2 Glycosylation Pathways

The N-glycan precursor is assembled on a carrier lipid (dolichol-P-P) into a 1sugar unit containing 9 mannose, 3 glucose and 2 N-acetylglucosamine residues which is transferred to Asn within a Asn-X-Thr/Ser sequence of newly synthesized proteins in the ER. The protein-bound chains are trimmed, processed and extended within the Golgi. Membrane-associated

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proteins in the ER, Golgi, lysosome and on the cell surface contain N-glycans as do extracellular matrix and secreted proteins. O-GalNAc linked glycans are usually found in large clusters on secreted and cell surface mucins typically produced by epithelial cells. Some of the O-GalNAc containing proteins also function as ligands for lectins such as the selectins, which are important for trafficking of leukocytes [8]. Glycophospholipid anchors and glycosphingolipids, often occur in lipid rafts [9]. Proteoglycans are made on a limited number of core proteins. They begin with xylose linked to Ser and are extended with glycosaminoglycan chains, e.g., heparan sulfate, chondroitin- and dermatan-sulfate, which influence morphogen and growth factor distribution [10]. Quantitatively smaller, more recently appreciated important glycosylation pathways include O-Fucose (on Notch receptors and ligands) [11], O-glucose (Notch receptors/ligands) [12], and O-mannose (primarily on α -dystroglycan at neuromuscular junctions) [13,14]. The occurrence of monosaccharides in these pathways is shown in Table 2.

3. GLYCOSYLATION DISORDERS

An estimated 2–3% of the genome encodes proteins used for biosynthesis and/or recognition of glycans. In just over a decade, >40 different human genetic disorders were discovered that affect one or more glycosylation pathways [15]. Below we focus on several disorders where the patients' pathology results from a limited supply of biosynthetic precursors.

3.1 CDG-Ia

3.1.1 Metabolic Steps—CDG-Ia and Ib involve the earliest steps of mannose metabolism. Mannose-P can be made from fructose-6-P using phosphomannose isomerase (MPI) or from mannose imported directly from an extracellular source (diet or turnover) via a hexose transporter. It is then converted into Man-1-P using phosphomannomutase 2 (PMM2, Man-6-P $\rightarrow$ Man-1-P) which then leads to GDP-Man.

3.1.2 Patients—This disorder is caused by deficiency in PMM2 (<25% normal), which limits the amount of mannose flux into the glycosylation pathway resulting in sub-optimal occupancy of N-linked sites [16]. It is unknown whether other glycosylation pathways are also affected.

CDG-Ia patients are usually born at a normal weight, but are often symptomatic at birth and therefore show evidence of developmental abnormalities [17]. The babies often have an unusual distribution of fat above their buttocks along with inverted nipples. Symptoms which appear early after birth include stunted growth, crossed eyes, hypotonia (floppiness at the trunk) and highly variable developmental delay. They usually fail to develop a normal-sized, functional cerebellum. It is unknown whether this is due to disrupted migration of cells moving into the cerebellum, impaired growth, stress-related cell death or a combination of these. Children have abnormal skeletons, seizures, coagulopathy, and indications of liver damage [18,19,20,21]. The high demands placed on the chondrocytes that create the environment for proper bone ossification and on hepatocytes, which synthesize plasma proteins including clotting factors may help to explain some of these symptoms. Some children develop cardiomyopathy, pericardial effusions and ischemia. Mortality is about 20% in the first 5 years and is often due to severe infections.

3.1.3. Therapy—There is no biochemical or molecular therapy for this disorder. Impaired glycosylation seen in CDG-Ia patient cells improves when mannose is added to the culture medium. However, mannose supplements for patients are ineffective, probably because any additional Man-6-P formed by hexokinase would likely be catabolized by the fully active MPI. Mannose might be more effectively used if the degradation of pathway of Man-6-P via MPI were partially blocked [16]. Another idea is that the deficient substrate Man-1-P could

be supplied as a cell-permeable pro-drug that would subsequently be converted into the parent compound for incorporation into glycans [16]. To date neither proposal has produced positive results. Direct enzymatic assays show that some patients have up to 25% residual enzymatic activity. Perhaps stabilizing mutated protein or modestly increasing the flux through the pathway could improve some symptoms in patients, especially those with high residual activity.

3.1.4. Animal Models—CDG-Ia is the most common of these rare glycosylation disorders with over 800 documented cases [22]. Ablation of the *Pmm2* mouse gene is lethal within two days of fertilization [23]. Ablation of the first step in the N-glycosylation pathway also shows very early lethality [24]. True mouse models of CDG-Ia may require construction of hypomorphic alleles in mice based on homology with the patients' mutations. Such constructs and studies are ongoing [25].

Recent studies in *C.elegans* used a sub-phenotypic dose of the N-glycosylation inhibitor tunicamycin along with RNAi technology to identify genes that caused a synthetic or enhanced deleterious phenotype. Of the 500 genes identified were a monosaccharide transporter, a subunit of the oligosaccharyl transferase complex, and several sugar transferases including 4 used for synthesis of the lipid precursor glycan. Nearly 200 candidate genes had unknown functions. Heterozygous mutations in some of these may help explain the wide phenotypic variability seen in CDG patients. [26].

3.2 CDG-Ib

3.2.1 Metabolic Steps—See 3.1.1

3.2.2 Patients—CDG-Ib is caused by mutations in *MPI* encoding phosphomannose isomerase (MPI, Fruc-6-P↔Man-6-P) which leads to under-occupied glycosylation sites similar to CDG-Ia. However, there are no obvious developmental abnormalities *in utero*. Ductal plate malformation in the liver during development contributes to later complications such as liver fibrosis [27]. Patients are born normally, but may suffer from hypoglycemia, failure to thrive (growth), coagulopathy, and intestinal abnormalities including protein-losing enteropathy (loss of plasma proteins via paracellular leakage in the small intestine) especially when patients are exposed to infection or stress. They have no dysmorphic features and have normal intelligence, neurologic and psychomotor development. This is surprising, since this defect occurs in the metabolic step immediately prior to PMM2, where patients have a much more severe phenotype. A number of CDG-Ib patients died prior to a correct diagnosis of impaired glycosylation [28,29,30].

3.2.3 Therapy—Daily dietary supplements of mannose reversed nearly all of the symptoms of this disorder, except for the liver fibrosis or bile duct abnormality [31]. Within a few weeks, abnormal glycosylation, coagulopathy, and enteric protein loss resolved [32]. A two-year-old child with CDG-Ib and short stature [33] became normal within a few months of starting mannose therapy. Patients usually have 2–15% residual MPI activity. Normally, human plasma mannose concentration is 1% that of glucose or ~55uM. Drinking 0.15gm/kg mannose 4 times per day raises the concentration to about 150–200uM with a turnover T1/2 of about 4 hr [34]. Mannose normally provides only about 2–4% of the mannose found in N-glycans in most tissues and cell types. Fibroblasts are an exception because they derive much more from mannose [35]. By far the great majority (96–98%) of mannose is derived from glucose (Sharma and Freeze, unpublished studies). Surprisingly, very small increases in mannose concentration is sufficient to rescue impaired glycosylation in the patients. Under normal conditions, MPI also “burns” Man-6-P by dispatching it into glycolysis as Fructose-6-P. No studies have been done to determine exactly the contributions

of mannose and glucose to the glycosylation pathways in humans. It is likely to vary in different tissues depending on the ratio of PMM2: MPI since both compete for the same substrate, Man-6-P. Cell and animal models using stable isotopes and mass spectrometric analysis are likely to be informative [36].

3.2.4 Animal models—Ablation of the mouse *Mpi* causes embryonic lethality at ~ 11.5d. The embryos showed growth retardation and placental hyperplasia. More than 90% failed to form yolk sac vasculature and one-third failed chorioallantoic fusion. Moreover, the embryos showed abnormalities in heart formation [37]. Glycosylation of the embryo appears to be sufficient based on normal lectin (concanavalin A) staining. Mouse sera contains ~100uM mannose, and adding mannose to the drinking water increases plasma mannose levels by up to 10-fold [38]. Attempts to rescue the *Mpi* null embryos by providing the dams with mannose had a decidedly negative effect: *Mpi* null embryos died even earlier and were resorbed [37] due to the accumulation of very high (mM) concentrations of Man-6-P, since it cannot be catabolized. Increased Man-6-P inhibits several glycolytic enzymes and depletes ATP, presumably by engaging in a futile cycle of phosphorylation and dephosphorylation. Other metabolic consequences may also occur as the embryos attempt to cope with the toxic levels of Man-6-P. Since Man-6-P is produced only within the cell, Man-6-P toxicity is cell autonomous. Temporal or tissue specific *Mpi* knockout constructs are unlikely to be useful since they would probably cause cell death wherever excision occurs.

As with lethal *Pmm2* knockout, another option is to knock in a patient-like mutation to create a hypomorphic allele in the mouse. A recent abstract suggests that this approach may be successful since it yields viable embryos [39]. Whether they will model CDG-Ib is not yet clear, but this system should have the advantage of quantitatively measuring the contributions of glucose and mannose to various glycosylation pathways with and without mannose supplements.

No other animal models of CDG-Ib or MPI-deficiency have been reported. Zebrafish morphants should also be amenable to study [40].

3.3 CDG-IIc (Leukocyte Adhesion Deficiency, Type II)

3.3.1 Metabolic Steps—GDP-Fucose can be derived from two sources; 1.) GDP-Man via a two-step reaction in the cytoplasm or 2.) Fucose→Fucose-P→ GDP-Fucose, which must be transported into the ER or Golgi using GDP-Fucose transporters.

3.3.2 Patients—CDG IIc, aka, Leukocyte Adhesion Deficiency (LAD), type II is an autosomal recessive disorder first discovered in 1992 [41]. Patients had mental retardation, facial dysmorphism, recurrent infections and rare Bombay blood group, which is caused by the absence of the fucosylated H-antigen. The patients also had leukocytosis (elevated circulating leukocytes), immunodeficiency and lacked fucosylated glycans on the leukocyte cell surface due to a defective GDP-fucose transporter, *SLC35C1* [42,43]. Fucosylated selectin ligands are required for leukocyte rolling on endothelial cells via their binding to E-, P- and L-selectins prior to firm adhesion and diapedesis.

3.3.3 Therapy—One CDG-IIc patient homozygous for *SLC35C1* mutation received daily oral fucose supplements. Within two days, the leukocytosis resolved, recurrent infections ceased, and he gained significant weight. His psychomotor condition also improved, most likely as a result of a more stable medical status. Fucosylated selectin ligands were re-expressed within 40 days of starting therapy facilitating binding of neutrophils to endothelium [44]. Exogenous fucose also improved fucosylation of IgM, but surprisingly there was no expression of α -1,2-fucosylated H antigen even 10 years after beginning

therapy. This indicates that the utilization of exogenous fucose was cell type specific and restricted to certain glycans. Presumably fucose was taken up by the recipient cells, phosphorylated to fucose-1-P, which then increased the endogenous pool of GDP-Fucose. Other CDG-IIc patients homozygous for another mutation did not benefit from fucose therapy [45], showing that elevated GDP-fucose levels cannot correct all patients.

3.3.4. Mouse Model of CDG-IIc—*Slc35c1* knockout mice were generated [46] and about one-third mice died within first week. *Slc35c1* deficient mice had 5.7% normal GDP-fucose transport activity. They showed leukocytosis, granulocytes that failed to adhere to E- and P-selectins and impaired leukocyte rolling in endothelial venules similar to CDG IIc patients. Another study showed defective leukocyte rolling to cremaster venules and lymphocyte homing to lymph nodes, but normal lymphocyte homing to the spleen, which probably is able to provide some adaptive immunity in *Slc35c1*^{-/-} mice [47]. Fucose supplements partially normalized fucosylation in *Slc35c1*^{-/-} fibroblasts thus suggesting an alternative transport mechanism.

3.3.5 Related Mouse Models—Hypofucosylation in other genetically engineered mice helps us to understand the mechanism of fucose rescue. The de novo pathway of GDP-Fucose synthesis begins with GDP-Man and requires two enzymes: GDP-mannose-4,6 dehydratase and GDP-4-keto-6-deoxymannose-3,5-epimerase-4-reductase (FX). Breeding heterozygous *Fx*^{+/-} mice very rarely produced *Fx*^{-/-} progeny; most died in utero and before weaning [48]. Providing dietary fucose to the dams was unable to rescue these embryos to term, but maternally derived (salvaged) fucose did provide some fucosylated proteins. Two rare *Fx*^{-/-} male survivors were crossed with *Fx*^{+/-} females, which gave increased survival of *Fx*^{-/-} animals from *Fx*^{-/-} X *Fx*^{+/-} crosses and *Fx*^{-/-} intercrosses. A variable contribution of the mixed genetic background increased *Fx*^{-/-} survival potential. *Fx*^{-/-} mice were smaller, infertile and suffered from diarrhea and leukocytosis. However, fucose supplementation reversed these symptoms and restored fertility. It also led to a very rapid normal expression of P-selectin ligand, similar to CDG-IIc patients. How did such rapid improvement occur?

A likely explanation is that the loss of fucosylation not only prevents leukocyte diapedesis, but also induces myeloproliferation by signaling through the Notch pathway [49]. Multiple EGF modules on Notch receptors are modified by protein O-fucosyltransferase 1 (Pofut1). These are further elongated by the Fringe family of GlcNAc-glycosyltransferases [50]. Notch ligands normally suppress myeloid differentiation of progenitor cells and enhance expression of Notch target genes, but fucosylation-deficient myeloid progenitors are not sensitive to suppression by these ligands. In this mouse model there was also a secretory epithelial cell hyperplasia [51] and impaired lung fibrosis due to impaired myofibroblast differentiation during inflammation [52]. Providing fucose to these *Fx*^{-/-} mice reversed the abnormal phenotypes.

Another knockout mouse lacking a specific α 1,6-fucosyl transferase (*Fut8*) for N-glycans shows growth retardation and loss of progeny during postnatal development [53]. Lack of this specific fucosylation also led to down-regulation of the EGF receptor, TGF- β receptor and integrins, which regulate several important events in growth and development. Impaired receptor signaling in CDG-IIc patients might explain growth and developmental retardation.

3.3.6. Models in other organisms—*Gfr* is the *Drosophila* ortholog of human GDP-fucose transporter. *Gfr* null mutants showed a wing phenotype typical of reduced Notch signaling [54], which could be rescued by wild type human GDP-fucose transporter, but not by human gene with the R147C mutation. The animal models and CDG-IIc patients underscore the importance of fucosylation in growth and development. Correcting the

abnormal phenotypes with fucose in both patients and models helped us to understand (i) the underlying mechanisms leading to CDG-IIc pathology, (ii) the large influence of genetic background on survival (iii) distinction of contributions from salvage pathway *vs.* *de novo* pathways.

3.4 Hereditary Inclusion Body Myopathy (HIBM)/Distal Myopathy with Rimmed Vacuoles (DMRV)

3.4.1. Metabolic Steps—Sialic acids (parent precursor N-acetylneuraminic acid) is efficiently salvaged from degraded glycans in the lysosome [55,56]. A small portion of dietary sialic acid is also incorporated into glycans [57,58]. Sialic acid is synthesized *de novo* from UDP-GlcNAc using the bifunctional enzyme UDP-GlcNAc epimerase/kinase (GNE) converts the substrate to N-acetyl mannosamine-6-phosphate (ManNAc-6-P), which then condenses with pyruvate to form N-acetylneuraminic acid and activated to CMP-Sialic acid by CTP. GlcNAc released from salvaged glycans also contributes to sialic acids by direct conversion to GlcNAc-6-P and epimerization to GlcNAc-1-P. The relaxed substrate specificity allows this enzyme to use ManNAc as well.

3.4.2 Patients—Hereditary Inclusion Body Myopathy (HIBM)/Distal Myopathy with Rimmed Vacuoles (DMRV) is an autosomal recessive, adult onset myopathy that does not affect children [59,60]. It begins in the distal leg muscles and progresses to the proximal muscles while sparing the quadriceps, and usually leads to wheelchair-restricted mobility. It is caused by mutations in *GNE*, which encodes N-acetyl glucosamine epimerase-kinase [61] that are rather mild with only 30–60% reduction in enzymatic activity [62]. Mutations can occur in either of the two catalytic domains with mutations in one domain affecting the activity of the other [63]. Cells from patients have sialic acid contents that overlap with controls, and depending on the study, some patients may show hyposialylation of α -dystroglycan and neural cell adhesion molecule (NCAM) [64].

The onset and rate of progression varies widely, even in patients with the same mutation and very similar genetic background. The observed penetrance is 99%, so there may be compensating or protective mechanisms in place [62]. Although the geographic distribution is broad, the highest incidence is in the Persian Jewish population where the carrier frequency of a founder mutation (M712T) is approximately 1:20 leading to a prevalence of 1:1600 [62]. Japan has a significant population (perhaps 400) of DMRV patients. The disease is also called Nonaka myopathy [65,66].

Several studies suggest that the production of sialylated glycans in the muscle is reduced, although this is still unsettled question. The enzyme is localized in the Golgi and in nucleus where CMP-Sialic acid synthase is found, leading some to speculate that it may also have a regulatory function [67].

The identification of GNE mutations in this disorder was surprising since mutations in an allosteric site of the protein that binds CMP-Sia to down regulate its activity, also produce another rare disorder called sialuria [68]. Patients with this condition accumulate large amounts of sialic acid in their cells and urine because they cannot decrease the rate of endogenous synthesis when salvaged or dietary sialic acid is sufficient. This implies that sialic acid flux is variable and needs to be closely regulated. Unlike sialuria patients, HIBM2/DMRV patients do not accumulate sialic acid.

3.4.3 Therapy—There is no currently approved therapy for HIBM2/DMRV, although it is reasonable to suggest that sialic acid or ManNAc supplements might be beneficial. One pilot clinical study provided 4 patients with a one-month course of intravenous immune globulin that contains 8 μ moles of sialic acid/g. All patients showed a significant, measurable

increase in localized muscle strength, but no improvement occurred based on immunohistochemical staining or immunoblotting of muscle biopsies for α -dystroglycan [69]. Presumably the transient improvement involved the endocytic uptake and lysosomal degradation of the infused sialylated protein, release of sialic acid within the lysosome, and its transport into the cytoplasm to bypass the block in the impaired muscle. Alternatively, the effects may have been indirect through an unknown mechanism. Uptake of exogenous free sialic acid occurs via non-clatherin mediated endocytosis [70] while ManNAc likely enters cells by diffusion directly into the cytosol. No clinical trials on ManNAc efficacy have been done since, ManNAc does not naturally occur in the diet unlike fucose and mannose and would require additional animal safety and toxicity studies after clearing pending regulatory approval. Since the pathway for ManNAc entry into cell is different from the uptake of a sialylated glycoprotein this would be expected to affect the tissue distribution and the efficiency of utilization of these different substrates.

3.4.4 Mouse models—GNE activity is high in liver, salivary glands and intestine, but very low in other organs and muscle [71]. Knockout of *Gne* in mice is lethal at 8.5 d in embryogenesis [72]. Proliferation of embryonic stem cells from these mice is directly correlated with GNE-expression and the cellular sialic acid concentration [73], which may explain embryonic lethality.

Two research groups made knockin mutations in mice. The first was the common Persian Jewish mutation (M712T) in a C57BL/6J background [74]. Residual enzymatic activity in homozygous mice was about 20% of control. Homozygotes were born, but died of kidney failure within the first 3 days. They showed glomerular hematuria, proteinuria, and podocytopathy. This resulted from an abnormal glomerular basement membrane and podocyte foot processes, with reduced sialylation of the major podocyte sialoprotein, podocalyxin. When ManNAc (1g/kg/day) was provided to the dams at mating and onward, 43% of the mice survived and they showed reduced pathology with improved morphology and function of the foot processes and glomerular basement membrane. Sialylation of podocalyxin improved with ManNAc treatment. The surviving mice showed no signs of muscular pathology. Providing ManNAc increased the enzymatic activity of Gne in both wild-type and homozygous mutant mice, possibly due to substrate stabilization.

Why this mutant strain showed kidney-specific pathology is unknown. Clearly, rescue with ManNAc suggests correction of a sialic acid deficiency. In that case it must have required maternal delivery of ManNAc to the embryo and utilization of the precursor for sialic acid synthesis.

The second group introduced a different mutation (D176V), which is prevalent in the Japanese population [75,76]. They bred *Gne*^{-/+} mice with transgenic mice carrying multiple copies of mutated human gene to produce a *Gne* null C57Bl/6 mice that carried five copies of the mutated human gene in the a *Gne*-null background. The mice were born normally but grew poorly and had a significantly higher mortality T_{1/2}~50 weeks. Sialic acid content in proteins in plasma, liver and spleen were dramatically reduced by 60–90% and only by ~20–30% in muscle and heart. They had poor muscle performance by 30 weeks of age and β -amyloid deposits accumulated in the myofibrils by 32 weeks. By 42 weeks they developed a muscular pathology resembling the human patients with accumulation of rimmed vacuole inclusions.

Most intriguing was their therapeutic intervention. Muscle atrophy and weakness are completely prevented by providing sialic acid, ManNAc, or sialyllactose in the drinking water of 5–6 week old mice. Even the lowest dose (20mg/kg body weight) of any of these sialic acid metabolites was equally effective in preventing the onset of disease. Sugar

administration showed quantitative improvement in muscle performance, muscle sialic acid content, and reduced number of rimmed vacuoles and amyloid inclusions. It prevented atrophy of skeletal muscles and enhanced the proportion of muscle fibers with normal diameter and decreased the number of atrophic fibers. These results showed that sialic acid metabolism is indeed one of the key factors in the pathomechanism of the disorder. The doses are quite modest and most surprising for sialylactose, which is only 45% sialic acid. These preventive studies did not assess therapeutic reversal of established myopathy.

Previous studies on the metabolism of sialic acid and sialyl lactose in rats showed that both could be absorbed from the intestine, and 60–90% of the sialic acid is excreted into the urine within 6 hr [11]. Intravenous injection gave similar results. Only a small amount of radiolabeled material remained in the tissue. Most of it was degraded and not salvaged. These few animal studies suggest that the majority of these molecules go to “waste”.

Whether sialic acid, ManNAc or sialylactose will be effective therapies for patients is an open question. Expense is one consideration. Reagent grade sialic acid, ManNAc and sialyl lactose have not been tested for safety, which is required if they are intended to treat a specific disorder.

One of the most abundant sources of sialylactose is human milk [77], specifically the whey, but it is also abundant in milk from elephants and spiny anteaters [78,79]. The potential availability of these untested sugars for a population of patients aware of their 99% probable prognosis is a powerful motivation to look past issues of safety and dosage [62]. Again, this exposes our limited basic knowledge of the non-flat metabolic chart and the need to understand contributions of de novo, dietary, and salvage pathways.

4. PERSPECTIVES and CONCLUSIONS

These examples of human glycosylation disorders illustrate several points.

- Small changes in glycan precursor flux cause significant but unpredictable changes in glycosylation in selected cells and tissues.
- Hypomorphic alleles in different genetic backgrounds can influence the ability of sugars to rescue pathological phenotypes.
- The relative contributions of de novo, salvage and dietary sources of monosaccharides are mostly unknown beyond a few experiments in cultured cells.
- The fate of monosaccharides released from turnover of endogenous glycans and dietary sources needs to be investigated since it may have therapeutic impact for patients.
- Additional models of impaired glycosylation are needed to assess the precursor contributions to major and minor pathways. Tractable experimental systems such as zebrafish and state-of-the-art metabolomic approaches may be useful to track sugar precursor origin and utilization.
- Better fundamental understanding of the 4 dimensional metabolic chart may help create new therapeutic opportunities.
- The public's positive attitude toward nutritional/dietary alterations as potential “natural” treatments provide both reason and opportunity to investigate basic science underlying sugar metabolism.

Many human glycosylation disorders were discovered in the last decade. Most likely, new ones that affect glycosylation will be discovered the near future using state-of-the-art genetic

mapping and high throughput sequencing technologies. Both forward and reverse genetic approaches in model systems will certainly encounter glycosylation insufficiencies or stressful conditions that unmask them. In these conditions, it will be important to consider sugar dietary modifications to ameliorate the phenotype as shown in the examples here and in settings where providing GlcNAc in the drinking water modulates signaling pathways and branching of N-glycans [80]

Acknowledgments

This work was supported by R01 DK55615, The Rocket Fund, and by Challenge Grant 1RC1HD0641501. HHF is a Sanford Professor.

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Figure 1. Biosynthesis and interconversion of monosaccharides. The relative contributions of each pathway under physiological conditions are unknown. (*Rectangles*) Donors; (*ovals*) monosaccharides; (*asterisks*) control points; (6PG) phosphogluconate; (PEP) phosphoenolpyruvate; (KDN) keto- deoxy-D-glycero-D-galactonononic acid; (Dol) dolichol.

Glc		Man		Gal
GlcNAc		GalNAc		Sia
Fuc		Xyl		GlcA

Table 1

Disease names, genes, and enzymatic defects, and typical symptoms.

Disease Name	Gene	Enzymatic Defect	Symptoms
CDG-Ia (PMM2-CDG)	<i>PMM2</i>	Man-6-P→Man-I-P	psychomotor retardation, hypotonia, esotropia, lipodystrophy, cerebellar hypoplasia, storke-like episodes, seizures
CDG-Ib (MPI-CDG)	<i>MPI</i>	Man-6-P↔Fru-6-P	hepatic fibrosis, protein-losing enteropathy, coagulopathy, hypoglycemia
CDG-IIc (SLC35C – CDG)	<i>SLC35C1</i>	GDP-Fuc(c)→GDP-Fuc(g)	recurrent infections, persistent neutrophilia, psychomotor retardation, microcephaly, hypotonia
HIBM/DMRV	<i>GNE</i>	UDP-GlcNAc→ManNAc-6-P	adult onset with progressive distal and proximal muscle weakness; spares quadriceps

Table 2

Monosaccharides and Glycosylation Pathways in Animals

Monosaccharides in Animal Glycans.

	Glc	Man	Gal	GlcNAc	GalNAc	Sia	Fuc	Xyl	GlcA
<u>Major Pathways</u>									
N-GlcNAc	+	++	++	++	+	++	++	+	+
O-GalNAc	-	-	++	+	++	++	+	-	+
O-Xyl									
HS	-	-	+	+++	-	-	-	+	+++
CS/DS	-	-	+	-	+++	-	-	+	+++
GSL	++	-	++	+	+	++	+	-	+
GPI	-	+++	+	+(GlcN)	-	+	-	-	-
<u>Minor pathways</u>									
O-Man	-	++	++	++		++	+	-	+
O-Glc	++	-	-	-		-	-	+	-
O-Fuc	-	-	+	++	-	+	++	-	-

The various major and minor pathways are shown on the left side. Across the top is the standard abbreviation and symbols used to represent the individual monosaccharides (Essentials of Glycobiology, Second Edition). ++ indicates high abundance of the monosaccharide; +, indicates presence; -, indicates absence from this class.

Glc

Man

Gal

GlcNAc

Fuc

Sia

Xyl

GlcA