Introduction to the Science of Glycobiology

**GLYCOBIOLOGY**

Glyconutrients

- ANGIE LAW -

WWW.ANGIESOPTION.COM

**8 ESSENTIAL BIOLOGICAL SUGARS**

- Glucose (from table sugar)
- Mannose
- N-Acetyl glucosamine
- Mannose
- N-Acetyl glucosamine
- Xylose
- N-Acetyl galactosamine
- Galactose (from milk products)
- Fucose
- N-Acetyl neuraminic acid

Typically, only these two are found in our modern diet.
CME

- www.Proevity.com
Continuing Medical Education site

QUALITY

- Good Manufacturing Practices (GMP)
- NSF International Certification
- Patents
  - U.S.
  - International

Introduction to the Science of Glycobiology
MIT’s Technology Review
February 2003

“Glycomics one of the top 10 technologies that will change the world”

The Study of Biological Sugar Chains

- LARRY A. LAW -

INFORMATIONAL SOURCE: ROBERT K. MURRAY, MD, PHD
**DOES SUGAR MAKE A DIFFERENCE TO YOUR CELLS?**

- The only difference between Type A (GalNAc) and Type B (Gal) is one sugar, but it can make a life/death difference in a transfusion.

**TERMS**

- **Glyco** — Sweet

- **Glycoprotein** — Protein molecule with one or more sugars attached. Found in all cells, tissues, and body fluids. 50% of all human proteins have sugars attached.

- **Glycosylation** — Attachment of sugars to proteins and lipids via enzymatic attachment.
CHANGING PARADIGMS

- It’s not what we don’t know that prevents us from succeeding; it’s what we KNOW that just ain’t so.
  — Mark Twain

CARBOHYDRATES

- Old: Sugar only used for energy production
- New: The cell utilizes 8 essential sugars to glycosylate proteins and lipids within the Golgi Apparatus and Endoplasmic Reticulum.

These glycoforms cover the cell membrane and interact with other cells for specific biologic functions including:

- Cell to cell communication and identification
- Immune system function
- Cell/tissue/organ repair and function
GLYCOPEPTIDE CELL RECEPTORS

Glycoprotein Cell Receptors are surface sugars that serve as points of attachments for cells, bacteria, viruses, and many other molecules.

CARBOHYDRATES

- **Old:** Glucose only pathway—all sugar changed to glucose in the body
- **New:** Study in France demonstrated radioactive galactose and mannose were directly absorbed into the body without being broken down into glucose. Scientists conclude that specific dietary sugars could represent a new class of nutrients (Dec 1998).

- **Old:** Stomach enzymes cannot break down beta bonds holding sugar chains together
- **New:** Bacteria in the intestinal tract break these beta bonds so sugars can be absorbed directly by the body (Jan 2007)
**FUNCTION OF LIFE**

- **Old:** Proteins serve as the communication signaling molecules at the cell level. But 20 amino acids do not provide enough different chemical combinations to support a vocabulary large enough to account for cellular communication.

- **New:** The hexagonal, 3-D structure of the 8 sugars and their multiple linking possibilities provide a potential of $1 \times 10^{14}$ (100,000,000,000,000) chemically unique “letters” in this sugar code of biologic information.

**COMMUNICATION CODE OF LIFE**

- Undermodulated Immune System
  - Cancer

- Overmodulated Immune System
  - Multiple Sclerosis (MS)
  - Rheumatoid Arthritis (RA)
HEALTH STATE

RELEVANT FACTORS

DIET

- Six of eight NOT found in our modern diet
- Cooked/Processed/Refined foods
- Green harvesting
- Lower nutritional value of fruits and vegetables (Food Pyramid & Soil Depletion)
- Increased oxidative stress in modern world
  - Antibiotics, drugs, alcohol
  - Chemicals, plastics, pesticides, perfumes
- Primary versus backup system
SICKNESS

- Autoimmune disease exponential increase in last 30 years
  - Chronic Fatigue Syndrome, Lupus, Fibromyalgia
  - Diabetes 30 million to 246 million in last 3 decades
    - Type I: 5-10% of cases
    - Type II: 90-95% of cases (formerly adult onset)

<table>
<thead>
<tr>
<th>1990 – 1998</th>
<th>Ages</th>
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<tbody>
<tr>
<td>70%</td>
<td>30-39</td>
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<tr>
<td>40%</td>
<td>40-49</td>
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- Heart Disease
- Cancer

SICK CARE

- American medical system #1 in trauma care

- But World Health Organization ranks U.S. 72\textsuperscript{nd} in chronic disease
  - U.S. spends in excess of $7,290/person on health care (#2 Switzerland - $4,417)
  - Britain spends $2,992 but Brits are healthier than Americans in every major health category
GLYCOPROTEINS

WHAT DIFFERENCE DO THEY MAKE?

GLYCOPROTEIN FUNCTIONS

- Cell to Cell Adhesion—how cells align and stick together
- Cell to Cell Communication—glycoprotein receptors and how hormones (eg. insulin) affect destination cells
- Fertilization
- Antibody Functions—all antibodies are glycoproteins
- Structural Support—collagen and the connective tissue between cells are glycoproteins
- Lubricants—mucins
- Immunoglobulins
- Transport—plasma proteins (eg. Transferrin)
- Some Enzymes, Hormones—Alkaline phosphatase, TSH, hCG
DISEASE STATES RELATED TO GLYCOPROTEINS

- **Inflammation**—Attachment of neutrophils and lymphocytes to walls of small blood vessels
- **Platelet Aggregation**—Attachment of platelets to walls of blood vessels and each other. Hemostasis (clotting) and thrombosis (heart attacks and strokes)
- **Influenza**—Attachment and release of virus
- **AIDS**—Attachment of HIV-1 to cells
- **Bacterial Infections**—Urinary Tract Infections, Peptic Ulcer
- **Rheumatoid Arthritis**—Altered glycosylation of IgG
- **Cancer**—Altered cell surface glycoproteins, metastasis
- **Genetic diseases**—Cystic Fibrosis, Muscular Dystrophy, congenital disorders of glycosylation

HARPER’S ILLUSTRATED BIOCHEMISTRY

(27th Edition)

Chapter 46 “Glycoproteins” (p. 523-544)

- “About 200 monosaccharides are found in nature, however, only eight are commonly found in the oligosaccharide chains of glycoproteins.” (p. 524)
- 1996 edition was first time a medical textbook devoted an entire chapter to the biological significance these 8 sugars have in cellular communication.
“There are enzymes in human tissue that can make these sugars from glucose. However, there is evidence that the other sugars may be beneficial in some circumstances when added to the diet. This has led to the development of glyconutrient supplements containing either members of the sugars listed in Table 46-4 (excluding glucose) or precursors of them. The efficacy of such supplements is under study.” (p. 524)

Why doesn’t your doctor know about this?
- Wasn’t in medical school text book until 1996, so if they graduated before 2002...
- 7,000 articles printed in medical literature every day. If they read 2 articles each day, at the end of the year, they’d be 35 centuries behind in their reading! (Dr. Fouts)
- Who funds continuing education credit seminars for doctors? Pharmaceutical companies. Who funds research studies? Not broccoli farmers!
Multiple studies have documented breast fed babies have stronger immune systems, better intestinal health, brain development, and cognitive abilities than formula fed babies.

Human breast milk contains 5 of the 8 biologically active beta bonded sugars (critically different from cow or goat’s milk):

- Lactose (galactose/glucose), glucosamine, sialic acid, fucose

Chains of beta-bonded sugar structures were originally thought to be of no benefit to the body.

1/3 of milk solids are these sugars (higher % than protein).

**INDIVIDUAL SUGARS**

- A LEAGUE OF THEIR OWN
**MANNOSE**

- Extracted and stabilized from aloe vera plant
- Aloe mentioned in literature for over 5,000 years as having healing and curative properties
- Aloe vera craze
- What grandma knew but couldn’t explain

**BIOLOGICAL FUNCTION**

- Incorporated into glycoprotein receptors on cell surfaces.
- Modulates the immune system primarily by activating macrophages.
  - Macrophages phagocytize bacteria and cellular debris associated with tissue inflammation.
    - Stahl PD Am J Respir Cell Mol Biol. 1990 Apr;2:317-318
MANNOSE BACTERIAL INFECTIONS

- Mannose prevents bacterial infections by binding competitively to bacterial lectins. Therefore, it occupies the sites that would otherwise bind to host cell receptors.

- By preventing bacterial adhesion, infection is prevented.

MANNOSE BACTERIAL INFECTIONS

Free sugar molecules bind to lectins preventing adhesion.
**MANNOSE**

**ANIMAL STUDIES**

- Blocks Salmonella adherence to chicken intestine in vitro
  - Reduces incidence of Salmonella infections in vivo by 50-100% when added to drinking water for chickens
    - Oyoto BA Poult Sci. 1989;68: 1357-1360
    - Oyoto BA Avian Dis. 1989 Jul’33:531-534

- Decreases E. coli bacteria in newborn mice when applied topically to maternal vaginas prior to delivery
  - Cox F J Infect Dis. 1990 Oct; 162:978-981

**FUCOSE**

- Abundant in seaweeds and sea urchins/starfish
- Exhibits considerable anticoagulant and antithrombotic activity
- Topical administration of L-Fucose increases skin thickness and density of collagen
- Increases free radical scavenging
**FUCOSE**

**FUCODIANS AND STEM CELLS**

- Stimulates adult hematopoietic stem cell formation.
- Healthy adult volunteers ingested 3 grams per day of Undaria fucoidans for 12 days with a significant increase in stem cells seen in peripheral blood smears.
  - Irhimeh MK et al. Fucoidan and Cxck4 and hemopoietic progenitor stem cell population, 2004 Nov. The Sydney Convention Centre North, Darling Harbour, Australian Stem Cell Centre

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**GLUCOSAMINE AND N-ACETYL GLUCOSAMINE (NAG)**

- Important in cell-to-cell communication
- Widely distributed in body tissue
- Thyroid gland has NAG receptor indicating a role in transport of thyro-globulin within the gland
  - Thibault V Endocrinology. 1993 Jan;132:468-476
- Glucosamine is substrate for glycosaminoglycan (building block of damaged cartilage)
  - Inhibits metabolic breakdown
  - Directly involved with repair of damaged cartilage
  - Talent JM Clin Ther. 1996 Nov; 18:1184-1199
## N-ACETYL GLUCOSAMINE (NAG)

- Findings of Dr. Demetriou of the University of California Irvine Center for Immunology published in the *Journal of Biological Chemistry*, 16 Nov 2007

- NAG suppresses the growth and function of abnormal T-cells responsible for autoimmune attack in MS and Type 1 Diabetes

- NAG prevented T-cell hyperactivity that results in autoimmune attack on brain myelin and the insulin-producing cells of the pancreas. Preventing this attack of the body on itself protects against the development of paralysis in MS as well as elevated glucose in diabetes.

## N-ACETYL GLUCOSAMINE (NAG)

- “This finding shows the potential of using a dietary supplement to help treat autoimmune diseases. Most importantly, we understand how this sugar-based supplement inhibits the cells that attack the body, making metabolic therapy a rational approach to prevent or treat these debilitating diseases.”

- Another recent study found improvement in 75% of children with treatment-resistant autoimmune inflammatory bowel disease after a two year course of NAG.
N-ACETYL NEURAMINIC ACID (SIALIC ACID)

- Affects viscosity of mucous membranes in respiratory, reproductive, and intestinal tracts.
- Protects against colonization/infection bacteria in respiratory epithelial cells.
  - Mason CM J Lab Clin Med. 1992 Nov; 120: 740-745

N-ACETYL NEURAMINIC ACID (SIALIC ACID)

- Researchers at the University of New Hampshire Glycomics Center led by immunologist Jeffrey Ravetch of Rockefeller University reported in the 13 May 2008 edition of the journal Science that they had identified a specific carbohydrate structure that confers anti-inflammatory activity to a glycoprotein antibody that could lead to improved treatment of autoimmune diseases like lupus or rheumatoid arthritis.
- “The work revolves around immunoglobulin G (IgG), the most abundant antibody in blood plasma. Intravenous immunoglobulin (IVIG) has trace amounts of this very active material which effectively relieves inflammatory affects of lupus, rheumatoid arthritis, asthma, and other autoimmune diseases. But because of the trace amounts of active material, effective doses of IVIG need to be very high leading to unwanted side effects.”
- “This study involved rebuilding the human IVIG into a fully active molecule with a slight modification to a carbohydrate residue. These carbohydrate structures are linked to the immunoglobulin and referred to as glycans, and on the tip of this glycan is a specifically linked Sialic Acid. All the Sialic Acid on IVIG was converted to the active linkage that confers anti-inflammatory properties.”
The Glycomics Center helped Ravetch pinpoint exactly how Sialic Acid was linked, which let the researchers engineer a synthetic human antibody that mimicked the linkages in IgG, providing an IVIG with enhanced activity for treatment of autoimmune diseases. In the Science paper, the researchers report that when given to arthritic mice, the engineered IgG was about 30 times more effective than IVIG.

“Understanding and analyzing the exact structure of Sialic Acid was the contribution of the UNH Glycomics Center, headed by director and research professor Vernon Reinhold. The center has developed tools and protocols using multidimensional mass spectrometry to determine the structure and functional relationships of these carbohydrates. Reinhold notes that while most biopolymers are linear and thus relatively easy to sequence, bush-shaped carbohydrates have proved challenging.”

“With sequential mass spectrometry, we systematically untangle this bush,” says Reinhold. “We take it down to the trunk then try to put it back together to determine its structure.”

Beyond this work with IgG, the Glycomics Center is demystifying the carbohydrate connections in cancer that contributes to metastatic growth and in avian flu where Sialic residues on airway surface tissues serve as doorways for viral entry.

“Carbohydrates are the glue that pulls things together, the cell surface matrix in which cells communicate and they provide the connections for signal transduction. It’s only been within the last decade that we’ve realized that such structures are critical for all kinds of biological function,” says Reinhold. “Now that we can define precise structures, we can begin to understand their function. This structure-functional relationship will have a huge impact on our health in respect to immune regulation.”
SUPPLEMENTATION

- Take enough (amount)
- For long enough (time)
  - Cell apoptosis:
    “A time to live and a time to die”
    —programmed cell death

CANCER RESEARCH

THE PATH OF DISCOVERY
**ONCOLOGY STUDY**

“The difference in oligosaccharide chains could affect adhesive interactions between cancer cells and their normal parent tissue cells, contributing to metastasis.”

—Harper’s Illustrated Biochemistry (p 535)

- Nutraceutical dietary supplements:
  - Do not inhibit tumor cell destruction by radiation and chemotherapy—actually enhances it!
  - Protect normal cells from radiation and cytotoxic damage.
  - Induce reductions in tumor mass in malignancies resistant to all treatments.
  - Improve quality of life for patients by reducing toxicity and side effects from radiation and chemotherapy.
  - G. Hyland, M.D., D. Miller, M.T., Medcenter One, Dept. Radiation Oncology, Bismark, North Dakota

**NATIONAL CANCER INSTITUTE**

- National Institute of Health (NIH) Press Release, 21 Aug 2007, announced new $15.5 Million, Five-Year Initiative to study the Glycobiology of Cancer
- Seven Tumor Glycome Laboratories projects funded to discover, develop, and clinically validate cancer biomarkers by targeting the carbohydrate (glycan) part of a molecule.
- "Scientists have long recognized that certain sugar structures, which are attached to protein and lipid molecules, may be important as markers for cancer development,” said NCI Director John E. Niederhuber
- "While this area has compelling scientific interest, its biological and chemical complexities have often discouraged investigation. Today with the advent of advanced technologies to conduct protein and carbohydrate chemistry, research into this intriguing area has experienced renewed interest.” (Niederhuber)
- Analysis may improve the value of tests such as prostate-specific antigen (PSA), CA-125, and carinoembryonic antigen used in prostate, ovarian, and colon cancer detection respectively
Seven NCI-funded projects searching for glycan-based biomarkers for melanoma, breast, ovarian, lung, prostate, colon, and pancreatic cancers:

- Discovery and clinical validation of cancer biomarkers (Margaret Huflejt, PhD, Cellexicon, Inc, La Jolla, CA)
- Immunogenic sugar moieties of prostate cancers (Denong Wang, M.D., PhD, Stanford University, Palo Alto, CA)
- Early cancer detection and prognosis through glycomics (Milos Novotny, PhD, Indiana University, Bloomington, IN)
- Glycan markers for the early detection of breast cancer (William Hancock, PhD, ScD, Northeastern University, Boston, MA)
- Tumor glycomics laboratory for discovery of pancreatic cancer markers (J. Michael Pierce, PhD, University of Georgia, Athens, GA)
- Autoantibodies against glycopeptide epitopes as serum biomarkers of cancer (Michael Hollingsworth, PhD, University of Nebraska, Lincoln, NE)
- Neu5Gc (Sialic Acid) and AntiNeu5Gc antibodies for detection of cancer (Ajit Varki, M.D., University of California, San Diego, CA)

If you would like to further investigate this science or if you have any questions, please contact the person who shared or recommended this class.