Clinical Chemistry (CHE 221)
Week 11
Bilirubin and Cholesterol

Biomolecules and Solubility

• Water is the biological solvent.

• Water is vital to all forms of life and makes up about 70% to 85% of the weight of a typical cell.

• A study of biomolecules is not complete without an understanding of the extraordinary properties of water as a solvent.

• How do we know if a biomolecule is soluble in water?

Like Dissolves Like

• Fats, oils, grease, contain lots of non-polar C-H bonds

• Water is a polar substance
  - polar and non-polar substances are not soluble in each other
  - polar substances will dissolve in other polar substances
  - non-polar substances will dissolve in other non-polar substances
Surfactants
soaps, detergents, phospholipids are all said to be amphoteric.
- polar “head” group
  - C-CO₂⁻ or
  - C-O-PO₃²⁻ or
  - C-O-SO₃⁻
- non-polar CH chain

Micelles
- form when mixing “soapy” molecules with water
- polar head groups point out facing solvent water
- non-polar tails point in
- oils (dirt) dissolve in the non-polar interior

Liposomes
- double layer formed
- polar faces inside and out
- forms spontaneously
- resemble cell membrane

What is Bilirubin?
Why is it important?
FATE OF RED BLOOD CELLS

- Life span in blood stream is 60-120 days
- Senescent RBCs are phagocytosed and/or lysed

- Normally, lysis occurs extravascularly in the reticuloendothelial system (spleen, liver, and bone marrow) subsequent to RBC phagocytosis.
- Lysis can also occur intravascularly (in blood stream)

Bilirubin Metabolism

- Approximately 80% of the 200 to 400 mg of bilirubin formed daily is produced from hemoglobin released from old red blood cells (RBCs).

- Normally, bilirubin passes through the liver and is excreted as bile through the intestines. Elevated levels may indicate certain diseases.

DEGRADATION OF HEME TO BILIRUBIN

- Normal plasma concentrations are less than 1 mg/dL
- Unconjugated bilirubin is hydrophobic – so must be transported by albumin to the liver for further metabolism prior to its excretion.
**Bilirubin**

Two forms:
- **Unconjugated bilirubin** – carboxylic acids not derivatized to esters
  - NOT soluble in water
- **Conjugated bilirubin**
  - form of bilirubin with two ester groups with - Soluble in water

Direct bilirubin: Conjugated with glucuronic acid (water soluble)
Indirect bilirubin: unconjugated (insoluble in water)

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**Conjugated Bilirubin**

- Several types of conjugated (direct) bilirubin are found in the plasma:
  - Bilirubin monoglucuronide: has one glucuronic acid
  - Bilirubin diglucuronide: has two glucuronic acids
  - Delta bilirubin: conjugated bilirubin bound through a covalent bond with albumin
HYPERBILIRUBINEMIA

- Increased plasma concentrations of bilirubin (> 3 mg/dL) occurs when there is an imbalance between its production and excretion
- Recognized clinically as jaundice

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<th>Type</th>
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<td>Gastro-renal</td>
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Jaundice

1. Increased production of bilirubin by hemolysis or blood disease:
   - Increase in blood indirect bilirubin
   - Called pre-hepatic jaundice
   - Stool color remains normal.

2. Abnormal uptake or conjugation of bilirubin:
   - Leads to non-hemolytic unconjugated hyperbilirubinemia
   - Increased indirect bilirubin.
   - Stool color turns gray.
   - Caused by liver damage or disease.
Hyperbilirubinemia

- Unconjugated hyperbilirubinaemia in a newborn can lead to accumulation of bilirubin in certain brain regions with consequent irreversible damage to these areas manifesting as various neurological deficits, seizures, abnormal reflexes and eye movements. This type of neurological injury is known as kernicterus.

Prehepatic (hemolytic) Jaundice

- Results from excess production of bilirubin (beyond the liver's ability to conjugate it) following hemolysis.
- Excess RBC lysis is commonly the result of autoimmune disease; hemolytic disease of the newborn (Rh- or ABO- incompatibility); structurally abnormal RBCs (Sickle cell disease); or breakdown of extravasated blood.
- High plasma concentrations of unconjugated bilirubin (normal concentration ~0.5 mg/dL).

Intrahepatic Jaundice

- Impaired uptake, conjugation, or secretion of bilirubin.
- Reflects a generalized liver (hepatocyte) dysfunction.
- In this case, hyperbilirubinemia is usually accompanied by other abnormalities in biochemical markers of liver function.
Post hepatic Jaundice

- Caused by an obstruction of the biliary tree
- Plasma bilirubin is conjugated, and other biliary metabolites, such as bile acids accumulate in the plasma
- Characterized by pale colored stools (absence of fecal bilirubin or urobilin), and dark urine (increased conjugated bilirubin)
- In a complete obstruction, urobilin is absent from the urine

Methods of Bilirubin Detection

- Van den Bergh
- Evelyn-Molloy
- Jendrassik and Grof

Methods of Bilirubin Detection

- Ehrlich (1883) - described the reaction of bilirubin with a diazotized sulfanilic acid solution to form a colored product using urine samples. Since then, this type of reaction (bilirubin with a diazotized sulfanilic acid solution) has been referred to as the classic diazo reaction, a reaction on which all commonly used methods today are based.
- van den Bergh (1913) - applied diazo reaction to serum samples in the presence of an accelerator (solubilizer). This methodology had errors associated with it.
- Malloy and Evelyn (1937) - invented first clinically useful methodology for the quantitation of bilirubin in serum samples using the classic diazo reaction with a 50% methanol solution as an accelerator (solubilizer).
- Jendrassik and Grof (1938) - designed method using the diazo reaction with caffeine-benzoate-acetate as an accelerator. Today, all commonly used methods for measuring bilirubin and its fractions are modifications of the technique described by Malloy and Evelyn.
When using the several methods described earlier, two of the three fractions of bilirubin were identified: conjugated (direct) and unconjugated (indirect) bilirubin.

- Unconjugated (indirect) bilirubin is water-insoluble substance and found in plasma bound to albumin. Because of these characteristics, unconjugated bilirubin will only react with the diazotized sulfanilic acid solution (diazo reagent) in the presence of an accelerator (solubilizer).
- Conjugated (direct) bilirubin is water-soluble and found in plasma in the free state (not bound to any protein). This type of bilirubin will react with the diazotized sulfanilic acid solution directly (without an accelerator). Thus, conjugated and unconjugated bilirubin fractions have historically been differentiated by solubility of the fractions.

The third fraction of bilirubin is referred to as “delta” bilirubin. Delta bilirubin is conjugated bilirubin that is covalently bound to albumin. This fraction of bilirubin is seen only when there is significant hepatic obstruction. Because the molecule is attached to albumin, it is too large to be filtered by the glomerulus and excreted in the urine. This fraction of bilirubin, when present, will react in most laboratory methods as conjugated bilirubin. Thus, total bilirubin is made up of three fractions: conjugated, unconjugated, and delta bilirubin. The three fractions together are known as total bilirubin.

Measuring Total Bilirubin

- Total bilirubin and conjugated bilirubin are measured and unconjugated bilirubin is determined by subtracting conjugated bilirubin from total bilirubin.

Methods of Bilirubin Detection

- Feces: Excretion of bilirubin is assessed by inspection of stool. Clay colored stool due to absence of fecal excretion of bilirubin indicates obstructive jaundice.

- Urine: Conjugated bilirubin can be detected with the help of dipstick test, Fouchets test.

- New insights into bilirubin physiology create an increased demand for accurate measurement of bilirubin within the normal range of values and may place more stringent requirements on sample handling.

- Bilirubin is a substance absorbing light within the visible spectrum, and it is well recognized to undergo both isomerization and oxidation in serum exposed to visible light, resulting in decreased measured bilirubin values.
All of the following describe the direct bilirubin component except

- A. Insoluble in water
- B. Conjugated in the liver
- C. Conjugated with glucuronic acid
- Excreted in the urine of jaundiced patients

When measuring serum bilirubin the purpose of adding caffeine-sodium benzoate or methanol the reaction mixture is to

- A. Accelerate the reaction with conjugated bilirubin
- B. Accelerate the reaction with unconjugated bilirubin
- C. Destroy the excess diazo reagent
- D. Shift the wavelength absorbed by azobilirubin.
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A nonhemolyzed, nonlipemic serum specimen was collected in a doctors office. The instructions indicated that the specimen for the analyte should be protected from light following collection. If these instructions are not followed, what analyte cannot be accurately measured?

• A. Glucose
• B. AST
• C. Calcium
• D. Bilirubin
In the condition kernicterus, the abnormal accumulation of bilirubin occurs in what tissue?

• A. brain
• B. Liver
• C. Kidney
• D. Blood

Which of the following functions as a transport protein for bilirubin in the blood?

• A. Alpha₁-globulin
• B. Beta-globulin
• C. Gamma-globulin
• D. Albumin
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As a reduction product of bilirubin catabolism, this compound is partially reabsorbed from the intestine through the portal circulation for reexcretion by the liver. What is this compound?

- A. Vardohemoglobin
- B. Urobilinogen
- C. Urobilin
- D. Biliverdin

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As the red blood cells disintegrate, hemoglobin is released and converted to the pigment bilirubin. Which organ is primarily responsible for this function?

- A. Spleen
- B. Kidneys
- C. Intestines
- D. Liver

Where is bile stored?

- A. Spleen
- B. Gall bladder
- C. Intestines
- D. Liver
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What breakdown product of bilirubin metabolism is produced in the colon from the oxidation of urobilinogen by microorganisms?

- A. Porphobilinogen
- B. Urobilin
- C. Stercobilinogen
- D. Protoporphyrin
What condition is characterized by an elevation of total bilirubin primarily due to an increase in the conjugated bilirubin fraction?

- A. Hemolytic jaundice
- B. Neonatal jaundice
- C. Crigler-Najjar syndrome
- D. Obstructive jaundice

What is Cholesterol?

Why is it important?
Cholesterol Metabolism

OVERVIEW

• Cholesterol, a steroid alcohol in animal tissue, essential for functions in the body.
• Cholesterol is a structural component of all cell membranes, modulating their fluidity - a precursor of bile acids, steroid hormones, and vitamin D.
• Cholesterol levels are of critical importance.
• Clinical importance: An imbalance between cholesterol influx and efflux results in a potentially life-threatening occurrence when the lipid deposition leads to plaque formation, causing the narrowing of blood vessels (atherosclerosis) and increased risk of cardio-, cerebro- and peripheral vascular disease.

The liver plays a central role in the regulation of the body’s cholesterol homeostasis.

Cholesterol enters the liver’s cholesterol pool from a number of sources including dietary cholesterol, as well as cholesterol synthesized de novo by extrahepatic tissues and by the liver itself.

Cholesterol is eliminated from the liver as unmodified cholesterol in the bile, or it can be converted to bile salts that are secreted into the intestinal lumen. It can also serve as a component of plasma lipoproteins (VLDL) sent to the peripheral tissues.
**STRUCTURE OF CHOLESTEROL**

Cholesterol is a hydrophobic compound with a polar –OH group. It consists of four fused hydrocarbon rings (A-D) called the “steroid nucleus”, and it has an eight-carbon, branched hydrocarbon chain attached to carbon 17 of the D ring.

Ring A has a hydroxyl group at carbon 3, and ring B has a double bond between carbon 5 and carbon 6.

In animals, cholesterol is the key regulator of membrane fluidity. Cholesterol inserts into bilayers with its long axis perpendicular to the plane of the membrane. The hydroxyl group of cholesterol forms a hydrogen bond with a carbonyl oxygen atom of a phospholipid head group, whereas the hydrocarbon tail of cholesterol is located in the nonpolar core of the bilayer. The different shape of cholesterol compared with that of phospholipids disrupts the regular interactions between fatty acid chains.

Lipoproteins transport cholesterol and triacylglycerols throughout the organism

- This transport is important for a number of reasons.
  - triacylglycerols are delivered to tissues, from the intestine or liver, for use as fuel or storage.
  - fatty acid constituents of triacylglycerols are incorporated into phospholipid membranes.
  - cholesterol is also a vital component of membranes and a precursor to steroid hormones.
  - Since cells are not able to degrade the steroid nucleus, cholesterol must be used biochemically or excreted by the liver. Excess cholesterol plays a role in the development of atherosclerosis.
The plasma lipoproteins are spherical macromolecular complexes of lipids and specific proteins (apolipoproteins or apoproteins).

- The lipoprotein particles include chylomicrons (CM), very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). They differ in lipid and protein composition, size, density, and site of origin.

- Lipoproteins function both to keep their component lipids soluble as they transport them in the plasma and to provide an efficient mechanism for transporting their lipid contents to (and from) the tissues.

- Chylomicrons are the lipoprotein particles lowest in density and largest in size, and contain the highest percentage of lipid and the lowest percentage of protein. VLDLs and LDLs are successively denser, having higher ratios of protein to lipid. HDL particles are the densest.

In general, cells outside the liver and intestine obtain cholesterol from the plasma rather than synthesizing it de novo. Specifically, their primary source of cholesterol is the low-density lipoprotein (LDL).

The role of LDL is to transport cholesterol to peripheral tissues and regulate cholesterol synthesis at these sites. A different purpose is served by high-density lipoprotein, which picks up cholesterol released into the plasma from dying cells and from membranes undergoing turnover and delivers the cholesterol to the liver for excretion.

The effects of excess cholesterol. Cross section of (A) a normal artery and (B) an artery blocked by a cholesterol-rich plaque.
Plaque build-up over time

Metabolism of HDL

- HDL metabolism is not yet completely understood.
- HDL perform a number of important functions:
  - HDL functions as a shuttle that moves cholesterol throughout the body.
    - HDL binds and esterifies cholesterol released from macrophages and the peripheral tissues and then transfers cholesteryl esters to tissues that use cholesterol to synthesize steroid hormones or to the liver, where the cholesterol is converted into bile salts or excreted.
  - The ratio of cholesterol in the form of LDL to that in the form of HDL can be used to evaluate susceptibility to the development of heart disease. For a healthy person, the HDL/LDL ratio is 3.5.
- Since there is an inverse relationship between plasma HDL concentration and atherosclerosis, and thus HDL’s is designated as the “good” cholesterol carrier.

STEROID HORMONES

Cholesterol is the precursor of all classes of steroid hormones: glucocorticoids, mineralocorticoids, and sex hormones—androgens, estrogens, and progestins.

Synthesis and secretion occur in the adrenal cortex, ovaries and placenta, and testes. Steroid hormones are transported by the blood from their sites of synthesis to their target organs. Because of their hydrophobicity, they must be complexed with a plasma protein, plasma albumin or a specific steroid carrier plasma proteins.

A number of genetic diseases are caused by deficiencies in specific steps in the biosynthesis of steroid hormones.
Bile acids

• Bile acids are the main components of Bile
• oxidized forms of cholesterol such as cholic acid
• created in the liver and stored in the gall bladder
  - Bile acids are more “soapy” than cholesterol because carboxylic acids are more polar than alcohols
• Form micelles
  → aid in absorption of fats and fat-soluble vitamins
• contain the Heme breakdown product Urobilinogen

Cholesterol procedure

• Based on three enzymes in a single reagent:
  1) cholesterol esterase
     - hydrolyzes esters formed between cholesterol’s OH and CO₂H on fatty acids
  2) cholesterol oxidase – catalyzes oxidation of cholesterol:
     cholesterol + O₂ → cholest-3-one + H₂O₂
  3) peroxidase – catalyzes reaction of peroxide with organics added to form a pink colored quinoneimine product