Lecture #12

Lecture 12
3/1/04

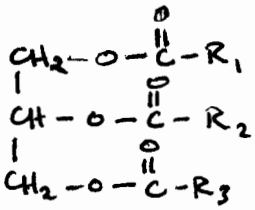
Cholesterol (Ch) Homeostasis
-Biosynthesis (last lecture)
-Regulation (synthesis or diet?)
-Receptor mediated endocytosis
-Sterol responsive element- binding protein (SRE-BP)
We get a lot of Ch from diet as well as synthesis, and excrete Ch every day-> dynamic process! So, how do keep CH levels constant?
If they fluctuate=Bad. Ch is insoluble and can deposit to from plaques

Overview of Ch homeostasis
See p. 7 of handout 2d and last lecture notes, also basic cycle on page 6 of handout 2d

Liver acts as "metabolic clearing house" –makes and distributes Ch and TAG
Adipocytes- fat storage
Intestine- absorbs TAG and Ch from diet, Ch sequestered by bile acids. Bile acids are almost 90% recycled

How do you sense the state of Ch in the body and regulate production in the liver? Ch is mostly in the plasma membrane, insoluble.

Solubilization of lipids: Fatty acids (FA), Cholesterol (Ch), triacylglycerols (TAG)

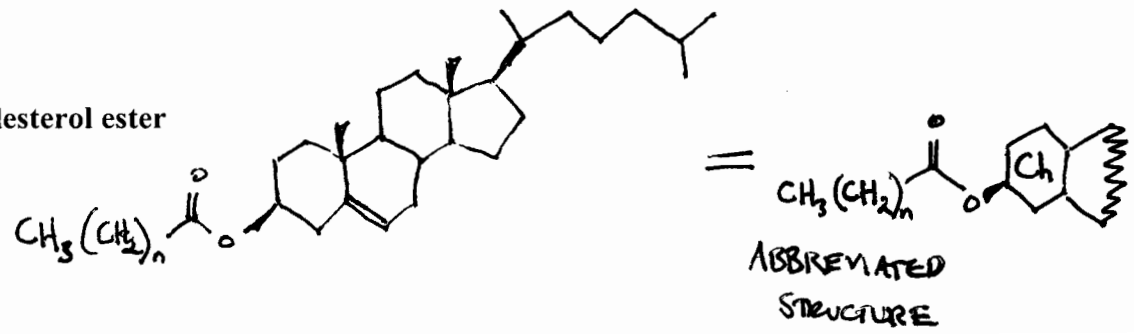


R= FATTY ACID CARBON CHAIN

Structure of **TAG**
Albumin is the carrier of FAs in the blood
In intestine, Fatty Acid Binding Protein binds Fas
TAG and Ch are packaged in lipoprotein particles

READ BROWN & GOLDSTEIN review article Science **232**, 34-47 (1986)
p. 6 of handout 2d, figure from B&G review shows structure of LDL lipoprotein particles
Globular, micelle-like particles, nonpolar-cores (TAG, Ch –esterified, usually with a FA)
Core coated with monolayer of phospholipid and Ch with hydroxyl group pointing out into media. LDL also has a protein, apo protein B (400kDa) that is ultimately recognized by the receptor (LDL-R)

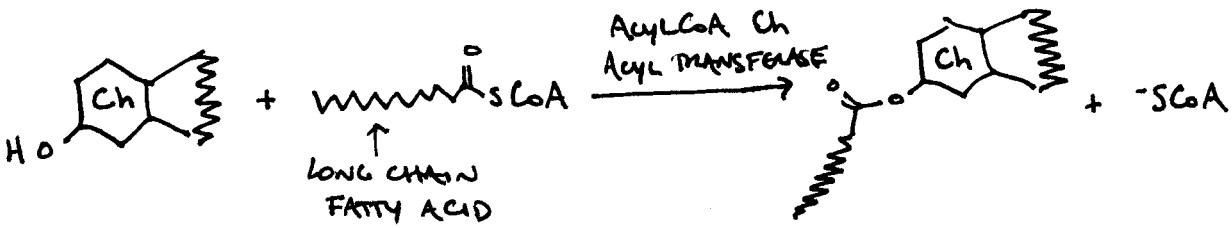
CE= Cholesterol ester



p.5 handout 2d – Breakdown of composition of different human lipoproteins (chylomicrons, VLDL, LDL, IDL, HDL)
chylomicrons package Ch from diet, recognized by remnant chlyomicron receptor in liver
LDL is the major carrier of Ch in the blood
Notice each lipoprotein has a different composition of proteins, lipids (average #s given)

Ch homeostasis
-at least 4 levels of regulation
we will only focus on two of these, paradigms for mechanisms of regulation that were discovered by studying Ch homeostasis

1) HMG-CoA reductase ("rate determining step" in Ch biosynthesis)
-target of statin drugs
-p.6 of handout 2d has the structure of the soluble domain. HMG-CoA reductase is a transmembrane protein. Transmembrane region in the ER
Is this the location of a Ch derivative sensing device?
HMG-CoA reductase also post-translationally modified by phosphorylation
2) regulation of the level of Ch esterification



Ch + Acyl-CoA w/ long chain FA -> CE (via acylCoA Ch Acyl transferase)

Ch esters (CE) can precipitate inside cell, forming CE droplets
CE production indicates high levels of Ch

3) (major focus) Regulation of LDL-receptor
paradigm for receptor mediated endocytosis
READ B&G review article, see ref. above

4) (major focus) Regulation at transcriptional level
SRE-BP= sterol responsive element-binding protein
Sterol= hydroxylated form of Ch
SRE-BP binds to sterol responsive element (sequence of DNA)

"THE LDL STORY"
TAKE HOME MESSAGE: LDL receptor is a key regulator of Ch homeostasis

Discovery of LDL-receptor by Brown and Goldstein – both M.D.s interested in the disease familial hypercholesterolemia (FH) –associated with very high levels of Ch (patients have 6-10 times the normal amount of Ch in the blood)
Most patients with FH die in childhood of heart attack

See p. 6 of handout 2d Endocytosis and Recycling of the LDL receptor
For diagram of the
WORKING MODEL

Liver cells with LDL-receptors
Apo protein B on LDL particle has a patch recognized by the LDL-receptor
Receptors MUST BE CLUSTERED!

-Each receptor has a "zip code" or amino acid tag –NPVY, that attracts the protein clathrin
-Membrane pinches off to form clathrin coated vesicle
-Uncoating, clathrin is removed
-Vesicle fuses with endosome (compartment with pH~6), the receptor stays in the membrane while LDL is left in the interior of the endosome
-Receptor can bud off and return to outer membrane (recycled)
-endosome fuses with lysosome (compartment with low pH ~5)
-lysosome is a bag of proteases and hydrolytic enzyme that break down the apo protein into amino acids and de-esterify Ch esters
-release free Ch

How do we know this process is occurring?
Experiments that give evidence for the working model:

Patients w/ disease vs. normal patients
B&G studied fibroblasts (skin cells), not a given that skins cells would faithly replicate hepatocytes given their different functions
liver cells are more difficult to study

p8. handout 2d "Regulation of HMG-CoA reductase"
-grow cells, with lipoprotein particles in the media
-replace w/ media w/ no lipoprotein particles
-monitor HMG-CoA reductase activity
(HMG-CoA reductase uses NADPH, easy reaction to monitor, absorbs at ~340nm)
Figure A: normal patients HMG-CoA reductase activity increases when lipoproteins are taken away, the cells make more Ch
No response is observed from FH patients
Figure B: addition of lipoprotein particles back to the media, providing a source of Ch
Normal patients decrease HMG-CoA reductase activity in response to higher levels of Ch coming in from media. No response from FH patients

Conclusion:
FH patients cannot sense Ch levels at all!
HMG-CoA reductase activity responds to changes in conc. Of lipoprotein particles in the media in normal patients