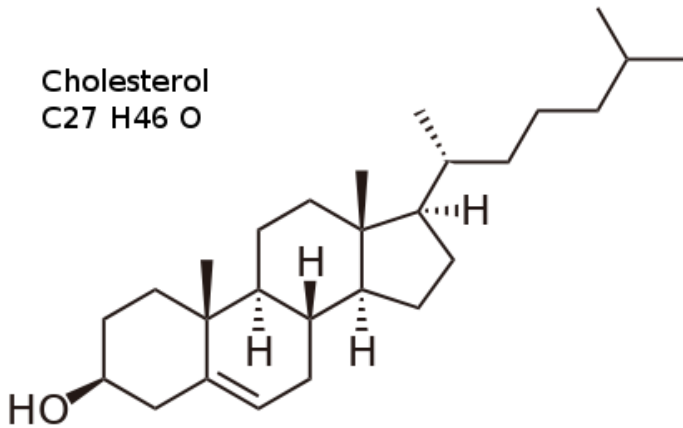


# Will the *Real* Cholesterol Please Stand Up!

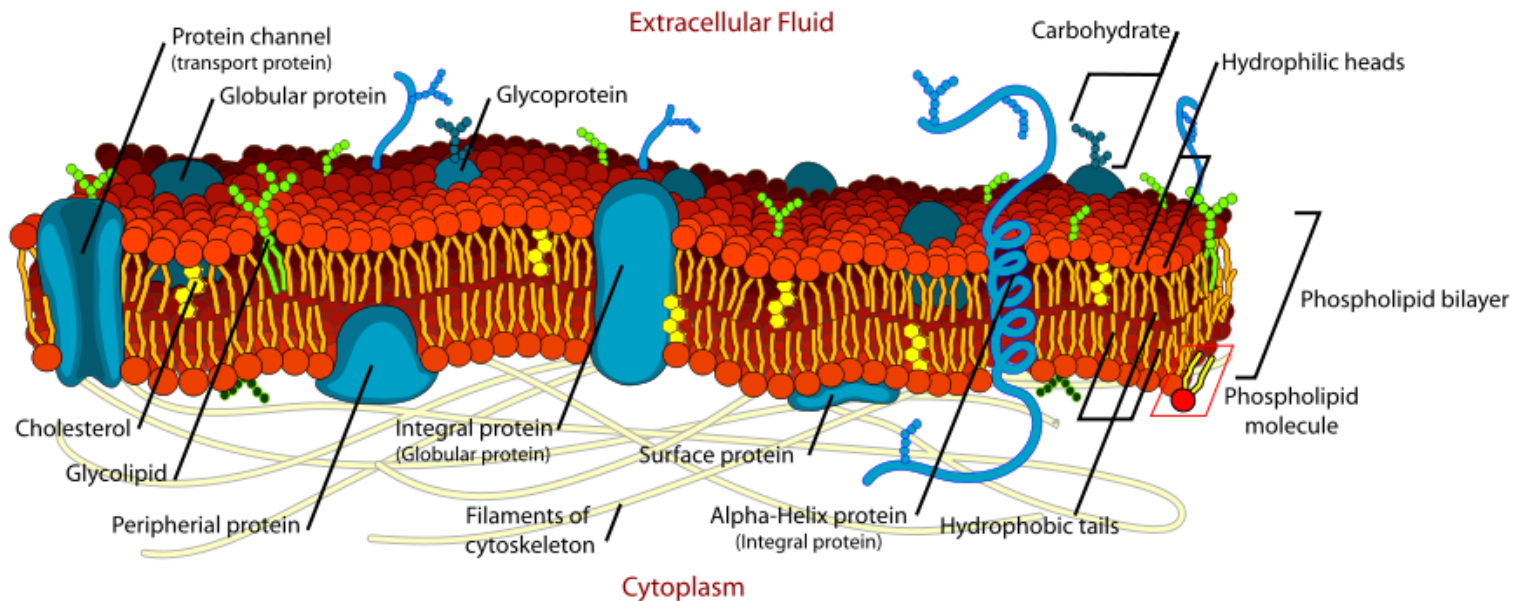
Edwin Cox, M.D.  
OLLI

# What is cholesterol, and why do we care?



Cholesterol is a complex lipid molecule vital to all animals cells

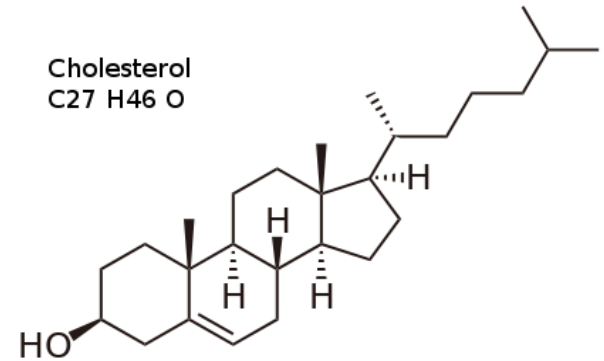
Essential component of cell membranes



# What is cholesterol, and why do we care?

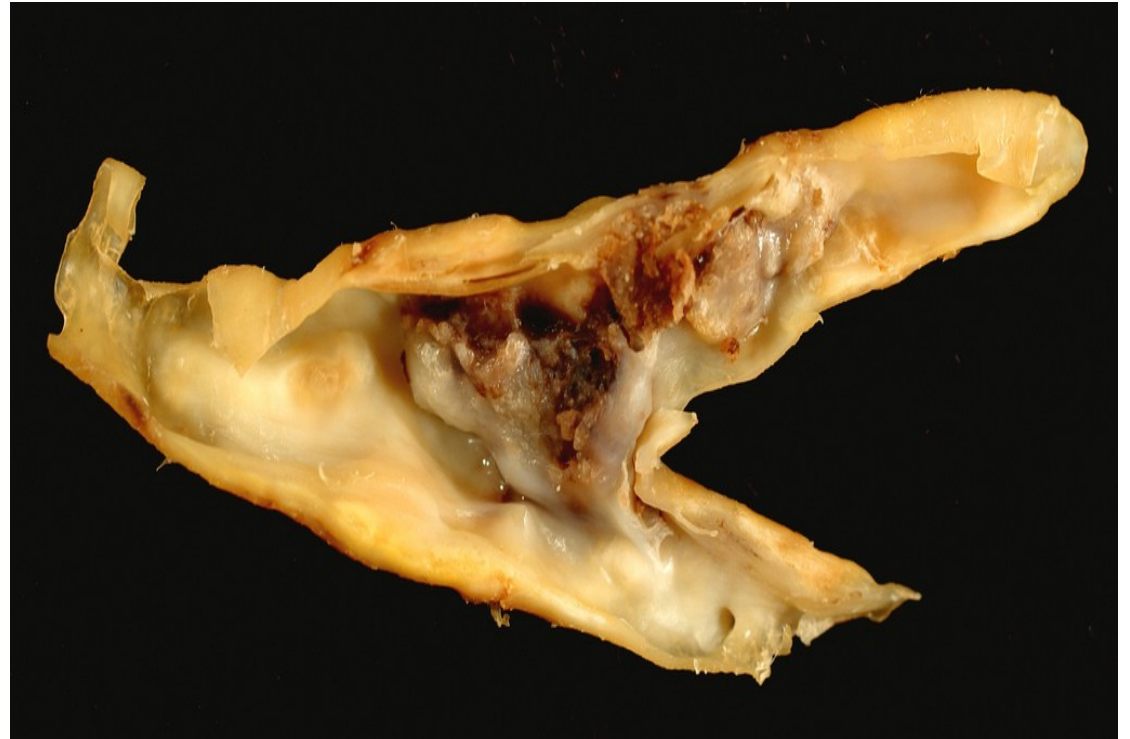
The main ingredient of  
many important compounds

- Hormones
- Steroids
- Bile acids
- Vitamin D



# What is cholesterol, and why do we care?

BUT... cholesterol is involved in our most common chronic disease, atherosclerosis, which is responsible for heart attacks, strokes and other vascular diseases



# From whence cometh cholesterol?

Cholesterol is made throughout the body

Every cell can manufacture it from scratch

- Complex 37 step process - mevalonate pathway

Total body cholesterol ~35 g (3 tbsp)

Daily synthesis ~1 gm (1000 mg)

Daily dietary intake ~300 mg

- Consumed cholesterol has little influence on total body cholesterol or blood cholesterol

# Where does cholesterol go?

Senescent (excess and damaged) cholesterol is picked up for recycling

- Some delivered to endocrine glands for making hormones
- Remainder brought to the liver for disposition
- Liver converts cholesterol into bile salts, secreted into gall bladder
- After a meal, gall bladder squirts bile into duodenum for fat digestion
- Some of bile passes in stool, eliminating it from the body; some is reabsorbed in colon

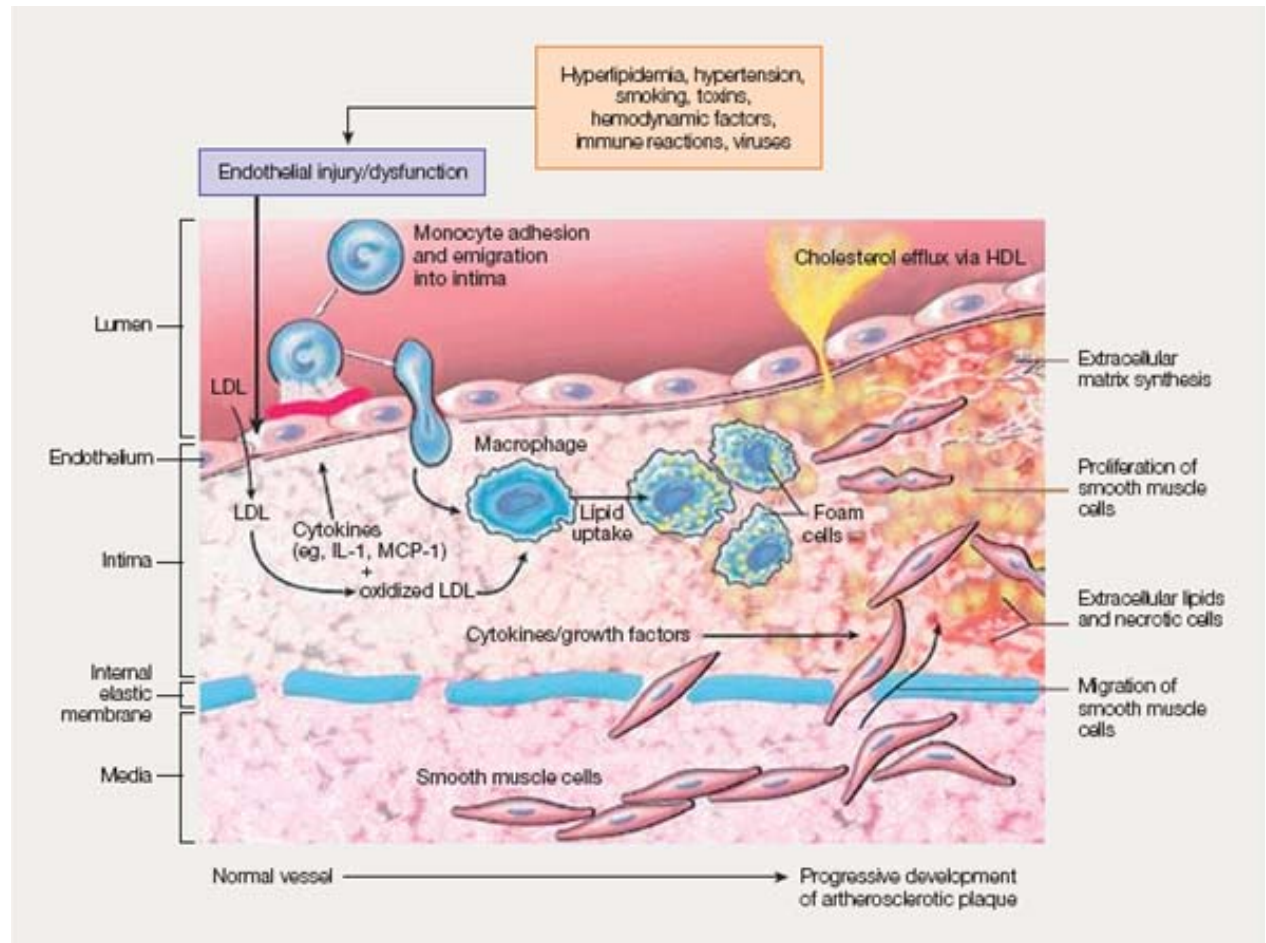
If cholesterol production and disposal are in balance, no cholesterol accumulates and all is well

# Where does cholesterol go?

In certain situations, more cholesterol is presented to recycling than it can process

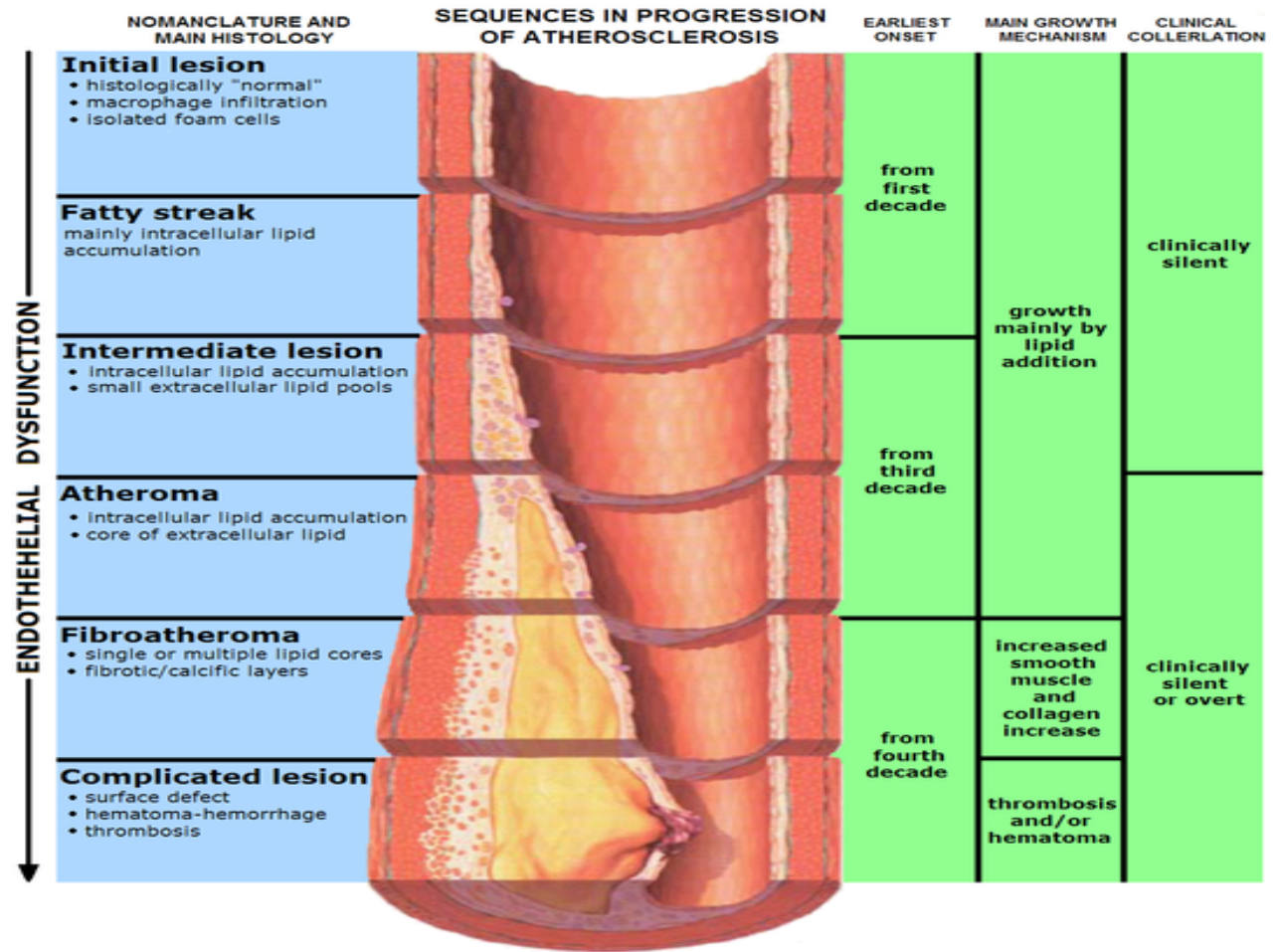
- Genetic mistakes in recycling mechanism
- Excessive production of cholesterol and other lipids, overloading the disposal system

The excess cholesterol is taken up by macrophages (white blood cells) in arterial walls, creating "foam cells"



# Where does cholesterol go?

Over decades, the accumulation of cholesterol and other lipids leads to damage, repair, scarring, disruption, rupture and eventually blockage of the artery (atherosclerosis)



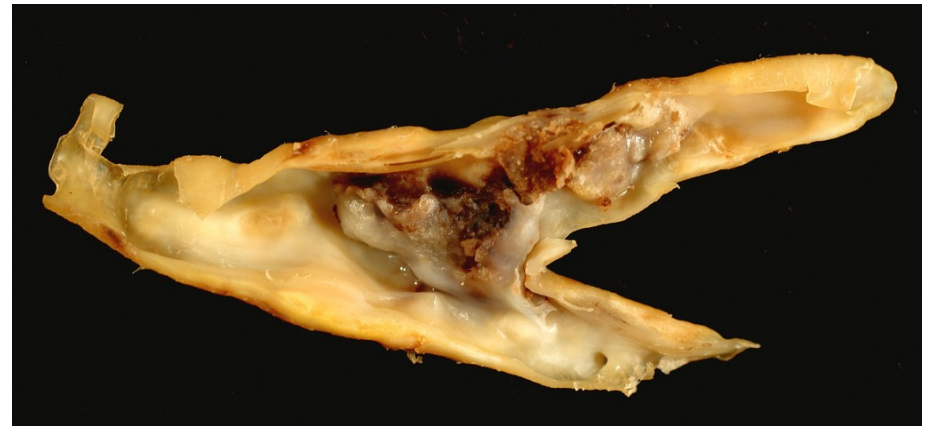


# Atheroma

The basic abnormality is a long-term vicious cycle of fat deposition, injury, repair, scarring, rupture, again and again

Etymology: Atheroma - "An abnormal mass of fatty or lipid material with a fibrous covering, existing as a discrete, raised plaque within the intima of an artery"

[G. athērē, gruel, + -ōma, tumor]



# Consequences of atherosclerosis

Atherosclerosis leads to blockage of arteries, with death of tissues supplied by those arteries ("infarction")

Consequences of AS are effects on specific organs

- Myocardial infarction (MI), or "heart attack" - ASHD, CHD (coronary heart disease), or IHD (ischemic heart disease)
- Cerebrovascular accidents (CVA), or "strokes" - AS cerebrovascular disease
- Infarction of extremities, organs - ASPVD (AS peripheral vascular disease)
- Known collectively as atherosclerotic cardiovascular disease (ASCVD, ASVD)

# ASCVD determinants

## Risk factors identified

- Lipoprotein ("cholesterol") metabolism - higher LDL and triglycerides, lower HDL
- Hypertension
- Obesity
- Cigarette smoking
- Metabolic syndrome → diabetes
- Sedentary lifestyle
- Stress

# ASCVD conundrum

Risk factors identified ...

Yet, in statistical analysis of populations, much of the disease risk remained unaccounted for by these factors

- Non-smokers with normal blood pressure who are not diabetic or obese and have "favorable" cholesterol profiles can have MI, CVA
- ASCVD becomes nearly universal in upper decades of life
- However, populations exist where ASCVD is extremely rare, pointing to responsible environmental and lifestyle factors not yet fully elucidated

# The lipid hypothesis (20<sup>th</sup> century)

Examination of atherosclerotic arteries revealed accumulation of lipids ("fat") as a major feature of the lesions

Patients with premature heart disease often had high blood cholesterol and/or triglycerides

Diets high in fats, especially saturated fats, were associated with high blood cholesterol

Therefore, dietary fats (especially saturated fats) were considered an important factor in causation of ASCVD, and reduction of (saturated) fat consumption should lead to reduction in ASCVD

This point of view became established in the 1970s and held sway until after 2000 as the official position of USDA, American College of Cardiology, etc.

# Beyond the lipid hypothesis

## Current understanding of the role of diet in ASCVD

- Saturated fats are not the sole, or even main, dietary factor responsible for ASCVD risk
- Replacing saturated fats with carbohydrates, especially sugars and refined grains, leads to elevated ASCVD risk on par with saturated fats
- Replacing saturated fats and carbohydrates with moderate amounts of monounsaturated and polyunsaturated fats dramatically reduces ASCVD risk
- Low dietary fiber is an independent risk factor for ASCVD
- Red meat and processed meats are independent risk factors for ASCVD, separate from saturated fat content

Obesity, diabetes and metabolic syndrome remain important risk factors for ASCVD, and are exacerbated by foregoing dietary factors

# Blood cholesterol and cardiovascular disease - history

Assays of blood cholesterol in some families with early onset coronary heart disease (CHD) showed incredibly high levels

- 1938 - Norwegian physician Müller

Population-based blood cholesterol surveys linked increased incidence of CHD with higher levels of blood cholesterol

- 1950s - Seven Countries Study
- 1960s - Framingham Heart Study

# Blood cholesterol and cardiovascular disease - history

Many, many studies since then have confirmed the relationship between total blood cholesterol level and ASCVD

Total cholesterol is relatively inexpensive and straightforward, so can be done universally

However, it became clear that total cholesterol was not the total answer to understanding and predicting ASCVD risk



# Cholesterol units

U.S. - mg/dl (milligrams per 0.1 liter)

International - mmol/l (millimoles per liter)

Conversion:

- 1 mmol/l = 40 mg/dl (approx.) HDL, TC, LDL
- 1 mmol/l = 90 mg/dl (approx.) TG

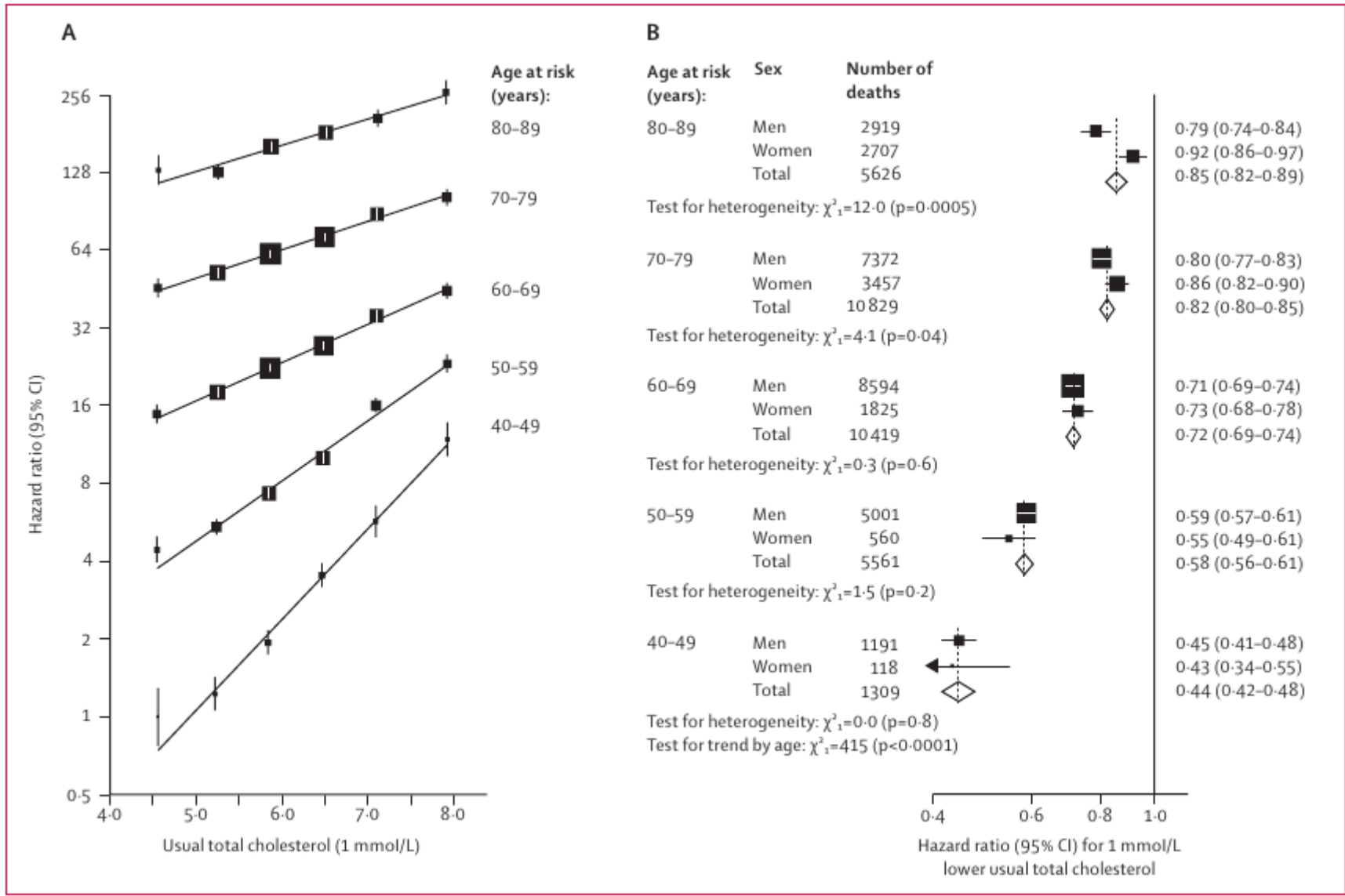


Figure 1: IHD mortality (33 744 deaths) versus usual total cholesterol

# So *which* cholesterol is it that really matters?

Is it the total cholesterol?

Or is it the LDL, HDL, VLDL, non-HDL?

- And what the "L" are they, really?

Is cholesterol some kind of Dr. Jekyll and Mr. Hyde?

- Model citizen by day, monster at night?

# L is for Lipoprotein

VLDL = very low-density *lipoprotein*

LDL = low-density *lipoprotein*

HDL = high-density *lipoprotein*

A lipoprotein is a bundle of lipids (cholesterol and triglycerides), surrounded by a shell of phospholipids, tied up with a string of protein

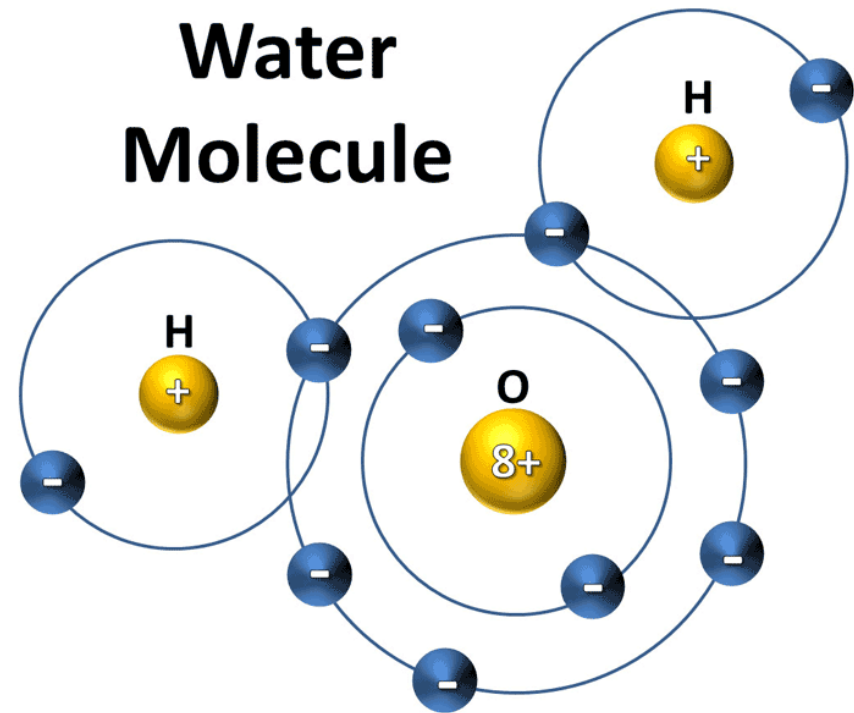
# Water (H<sub>2</sub>O)

Molecule of water is one atom of oxygen and two of hydrogen

The bonds are due to sharing of electrons

Electrons are negatively charged

Nucleus (protons) is positively charged

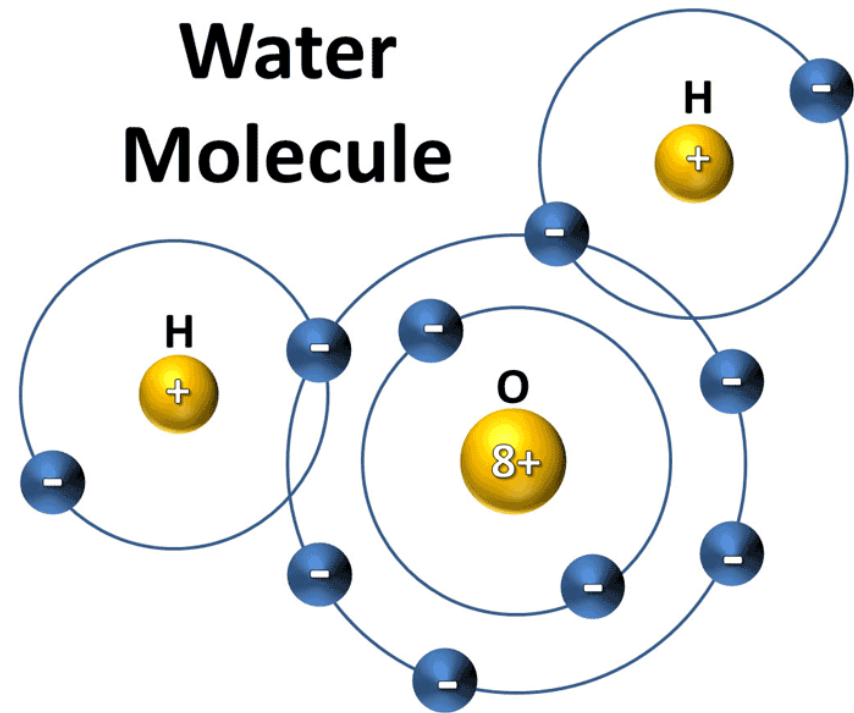


# Water (H<sub>2</sub>O)

Electrons stay (on average) closer to the oxygen nucleus

Oxygen "region" has slight excess negative charge

Hydrogens have slight excess positive charge



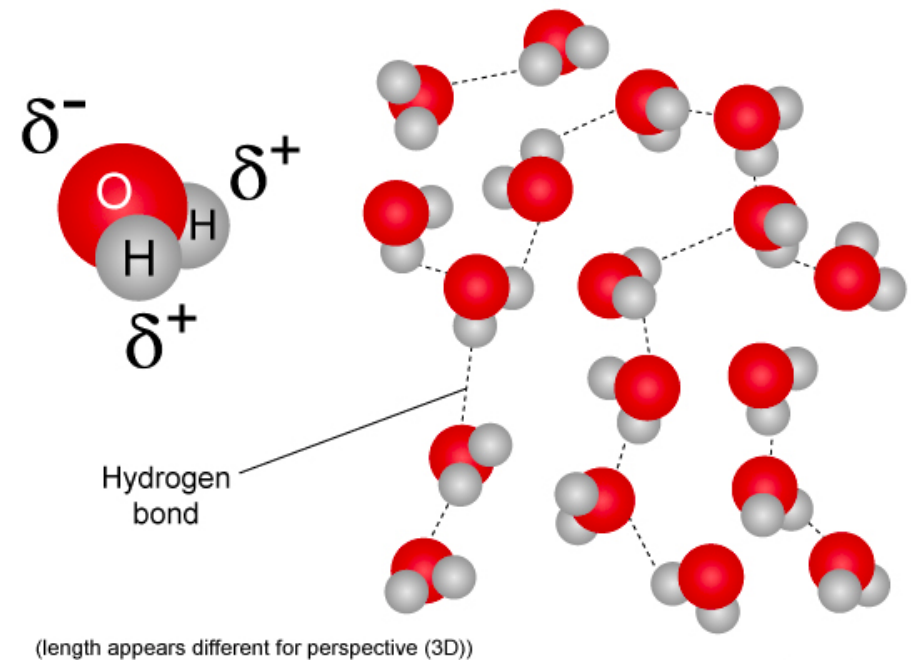
# Attraction between H<sub>2</sub>O molecules

Opposite charges attract

Positive H of one H<sub>2</sub>O is attracted to negative O of a neighbor H<sub>2</sub>O

H<sub>2</sub>O molecules form chains between 0° and 100° C, crystals below 0, and separate completely above 100

Electrostatic forces that bind H<sub>2</sub>O molecules together are called hydrogen bonds

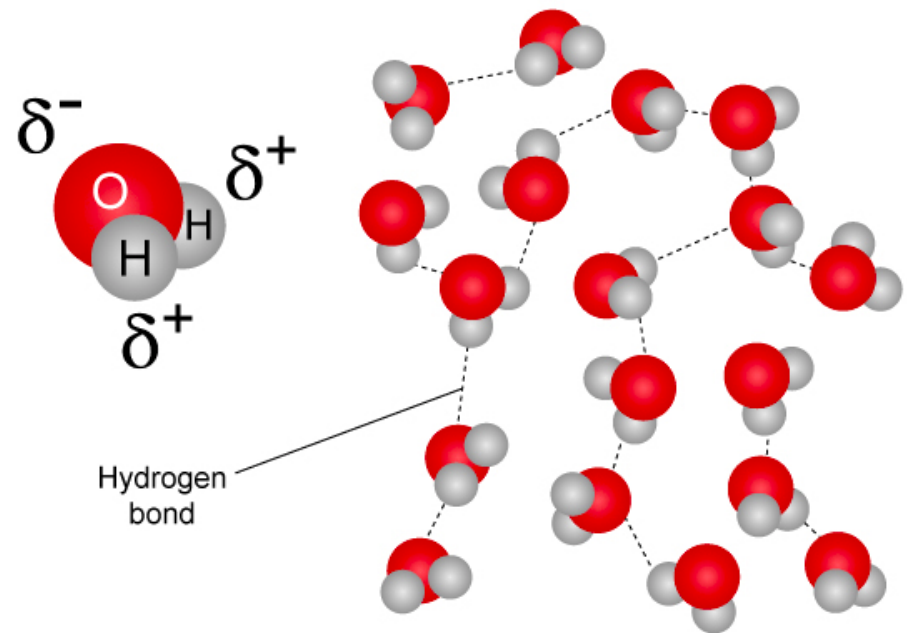


# Attraction between H<sub>2</sub>O molecules

Water molecules are very "tribal"; they stick together avidly

Molecules with asymmetric charge distribution are called "polar"

Water will be attracted to other polar molecules



(length appears different for perspective (3D))

Dept. Biol. Penn State ©2002



# Lipids

"Lipids are a group of *naturally* occurring molecules that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids, and others"

- Wikipedia

Cholesterol is a lipid

# Most common lipids

## Cholesterol

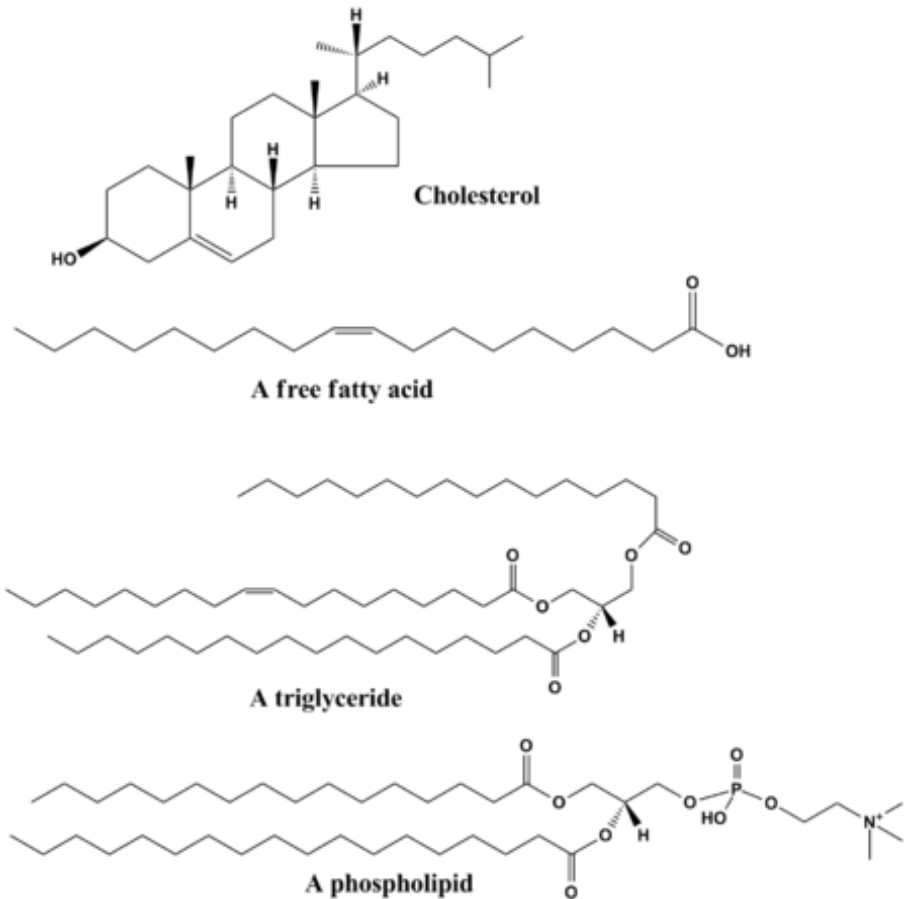
## Fatty acids (FA)

- Hydrocarbon with carboxylic acid at one end (-COOH)
- Saturated, mono-unsaturated, poly-unsaturated

## Triglyceride

- Glycerol backbone
- Three FA esterified
- TAG (triacylglycerol)

## Phospholipid



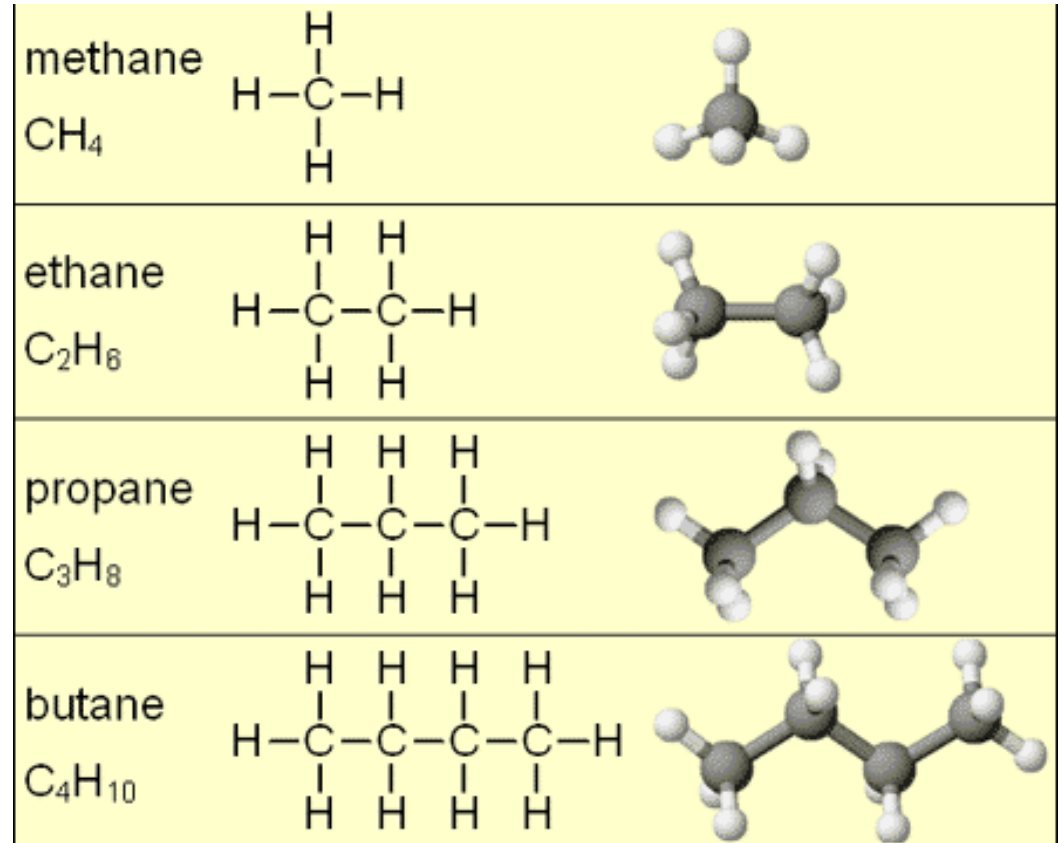
# Hydrocarbon compounds

Hydrocarbons are compounds made up of carbon and hydrogen

They are symmetric and have tiny net charges

They only attract one another and other hydrocarbon molecules weakly

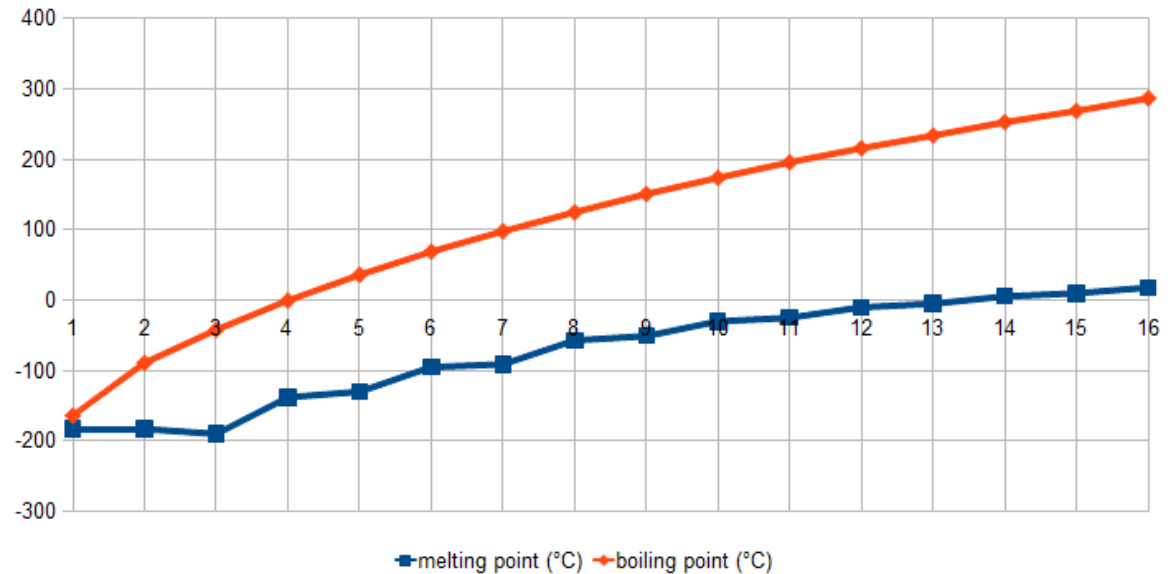
Such attraction that they do have are due to van der Waals forces (relatively weak)



# Hydrocarbon MP and BP

Total attraction between molecules increases with chain length, reflected in increasing melting points and boiling points with larger molecules

Liquids at room temperature (C<sub>5</sub>-C<sub>16</sub>) are oils; solids (C<sub>17</sub>+) are waxes (paraffins)



# Oil and water

"Oil and water do not mix"

Why?

The water molecules all get together and squeeze out oil molecules

The excluded oil molecules then get together among themselves



# Oil and water interaction

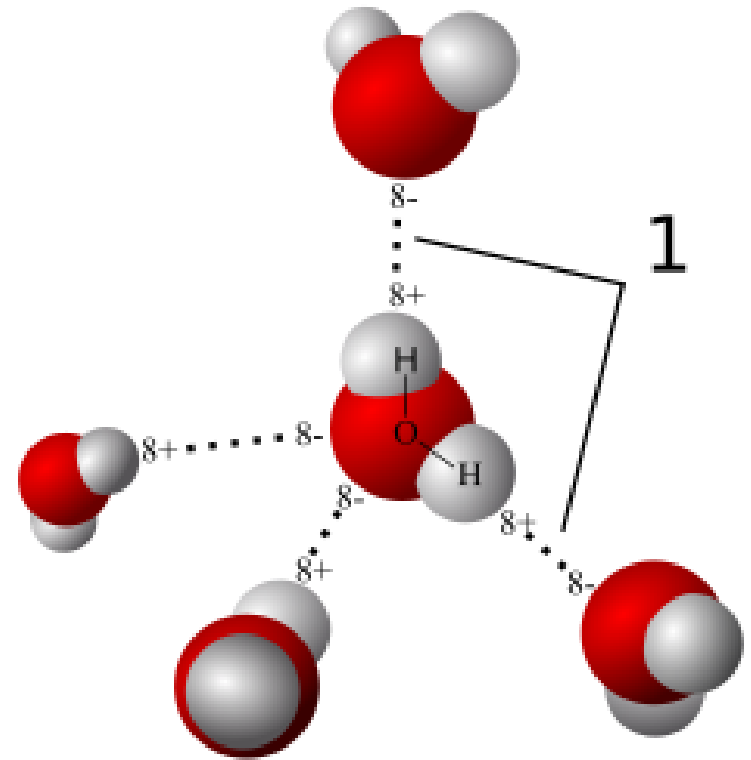
“Oil molecules and water molecules repel”

- Actually water molecules attract one another so tightly that they reject oil molecules
- $H_2O$  molecules' mutual attraction is based on electric charge
- Oil molecules have nowhere to go other than to hang out with other oil molecules

Oil is said to be *hydrophobic* (“water fearing”) or *lipophilic* (“fat loving”)

Other molecules that mix well with water, such as many proteins, are said to be *hydrophilic*

- They have atoms with net electric charges sticking out to “bond” with H and O



# Hydrocarbons: Alkanes

Hydrocarbons are compounds mostly comprised of carbon and hydrogen in various length chains and combinations with other elements



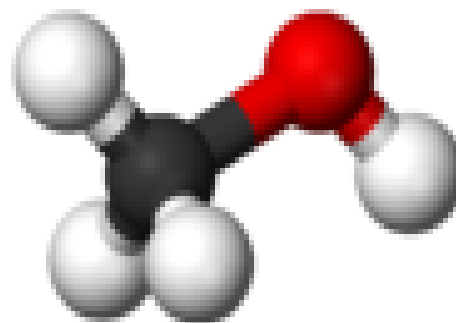
Alkanes are simplest - just C and H - but don't occur in living organisms, only as fossil fuels

- Octane - eight carbons, 18 hydrogens

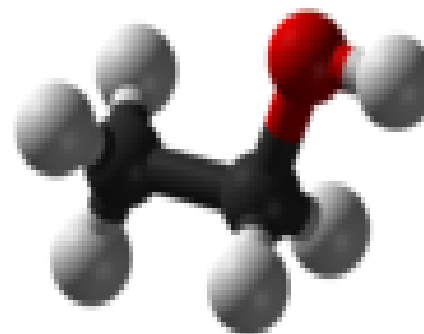
# Alcohols - Add OH

Replacing one hydrogen with -OH (hydroxyl group) converts alkane into alcohol

Methane, minus one hydrogen, plus -OH, is methyl alcohol, or methanol,  $\text{CH}_3\text{OH}$



Ethane, minus one hydrogen, plus -OH, is ethyl alcohol, or ethanol,  $\text{C}_2\text{H}_5\text{OH}$





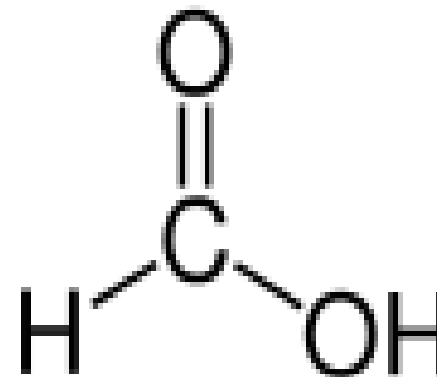
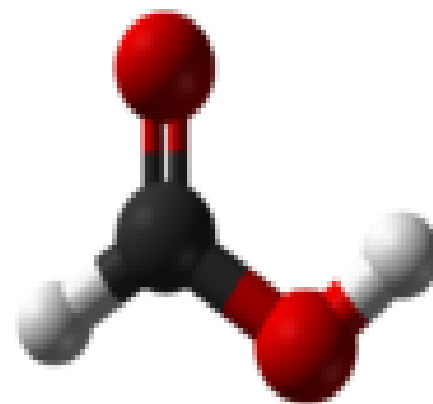
# Carboxylic acids - COOH

Carboxylic acids are alkanes in which a terminal carbon has an -OOH group replacing three hydrogens

In simple concept, one oxygen is double bonded to the carbon and an hydroxyl group shares the remaining electron

In actuality, the oxygens are symmetric about the carbon, and the hydrogen dissociates into an ion

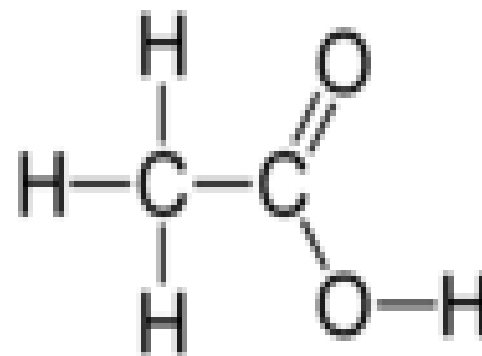
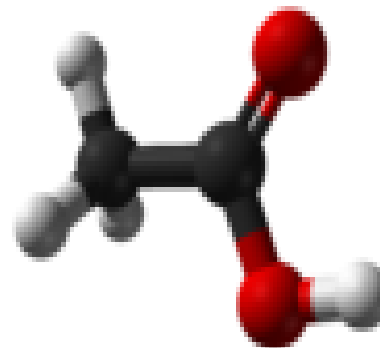
The simplest carboxylic acid is methanoic acid, better known as formic acid (the stinging chemical injected by ants and other insects when they bite)



# Ethanoic (acetic) acid

The second simplest carboxylic acid - ethanoic acid, or better known as acetic acid

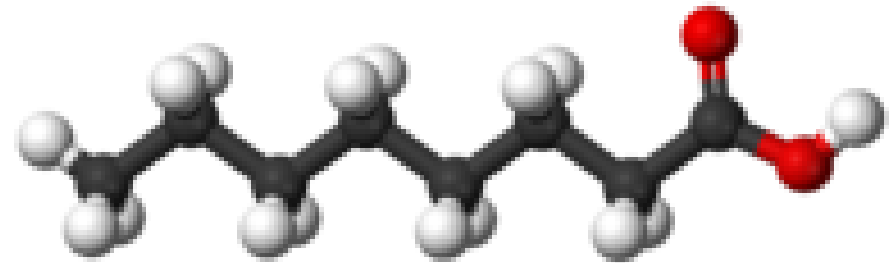
Vinegar is a mixture of water (95%) and acetic acid (5%)



# Fatty acids

Fatty acids are  
longer carboxylic  
acids

Caprylic acid  
(octanoic acid) -  
eight carbons, 16  
hydrogens, 2  
oxygens



# Fatty acids

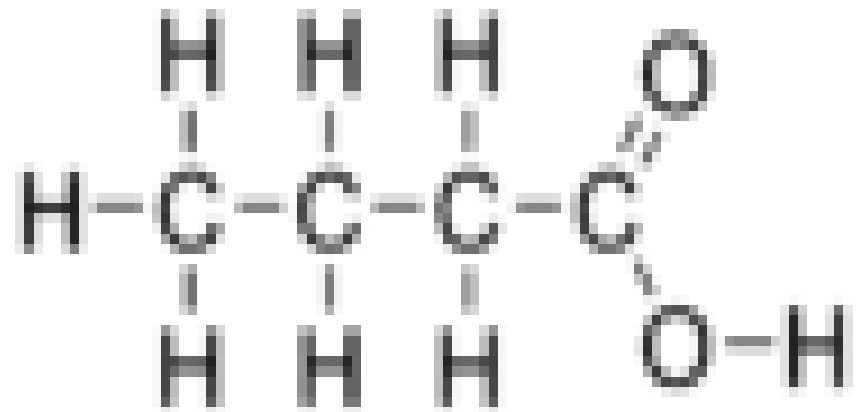
Fatty acids are nothing more or less than longer carboxylic acids

The shortest of biological interest is butanoic acid, better known as butyric acid

Butyric acid is the carboxylic acid from butane

It's found in dairy products and makes up about 3-4% of butter, where it is bound as a triglyceride

Free butyric acid has a very intense, unpleasant odor; it's the dominant smell in vomit



# Fatty acids

Naturally occurring fatty acids generally have an even number of carbons

Fatty acids are usually found incorporated into triglycerides (more later)

Short-chain fatty acids are up to 4 carbons, medium-chain FA are 6 to 12, and long-chain FA are longer than 12 up to 21.

Many fatty acids have common names that were used before the systematic chemical names came into use

Fatty acids may have no double bonds (saturated fats), one double bond (monounsaturated fatty acids) or more than one double bond (polyunsaturated fatty acids)

# Lipid number of fatty acids

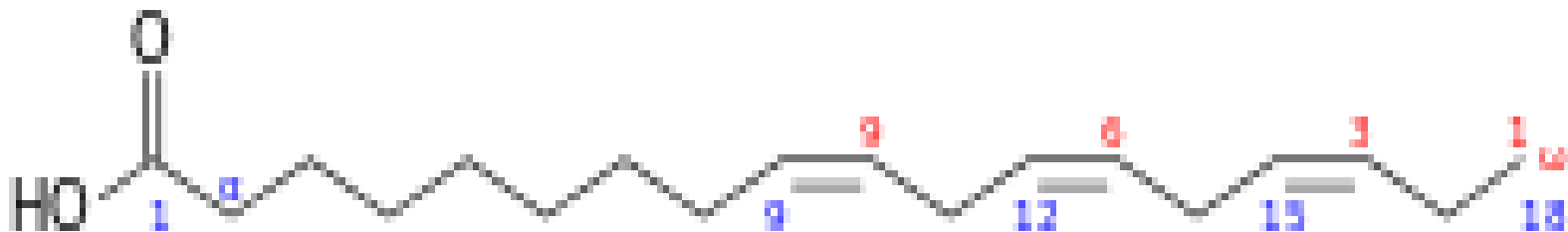
Lipid number is a shorthand for:

- a) the number of carbons in a fatty acid,
- b) the number of double bonds,
- c) the location of the first double bond from the non-acid end

Example: Alpha-linolenic acid (18:3 n-3) has:

- a) 18 carbons,
- b) 3 double bonds,
- c) first double bond at the third carbon from the non-acid end

The location of the double bond, designated n-3, is also referred to as "omega-3", or " $\omega$ -3"; these are spoken as "n minus three" and "omega minus three", though the "minus" is often left out in casual speech



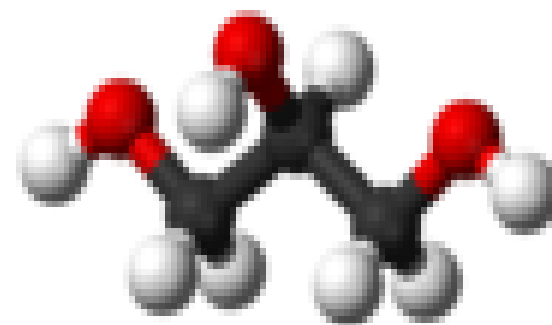
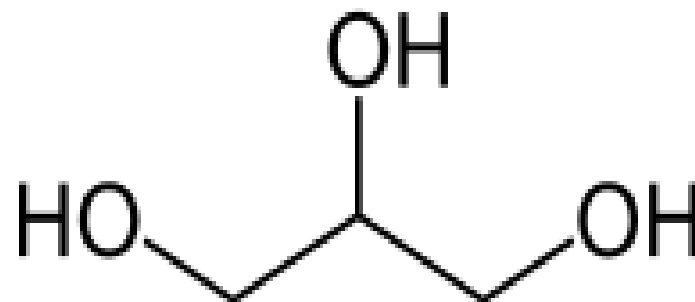
# Glycerol

Glycerol, perhaps more familiar by its common name "glycerin", is a poly-alcohol, or polyol

- Propane-1,2,3-triol

Based on propane, each carbon has an hydroxyl group

Serves as the backbone of a triglyceride, formed by attaching fatty acids at the hydroxyl locations by their acid end through a reaction called esterification



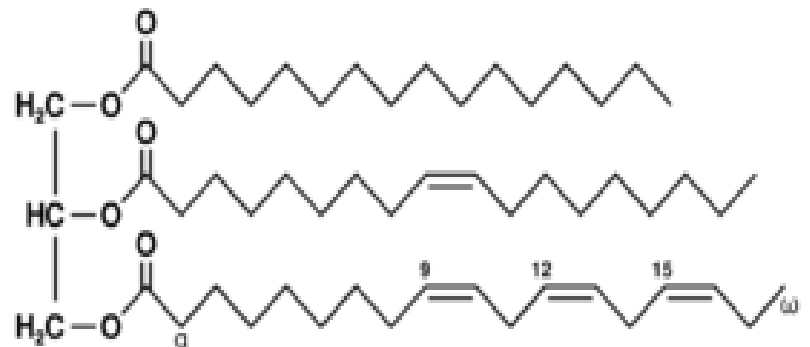
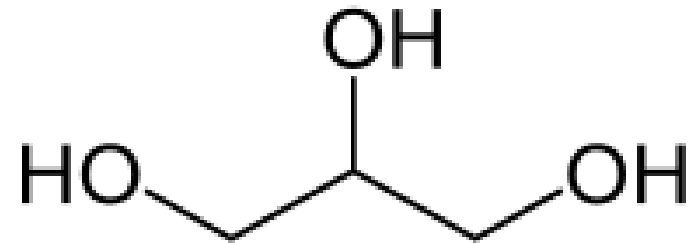
# Esterification

A reaction that brings together an alcohol and an acid

Take out  $H_2O$  and link the remaining ends

$R-CO-O-R$

Glycerol can esterify with three fatty acids to make a triglyceride





# Oil and water

"Oil [lipids] and water do not mix"

Blood is mostly water with other stuff, including lipids, floating around in it

Since lipids do not dissolve in water, moving them around in the blood stream efficiently requires special methods of making them soluble



# Lipid transport solution

Mother Nature found an elegant solution for immiscibility of water and lipids

Cholesterol (CHOL) and triglycerides (TAG) are packaged in specialized particles for blood transport

Particles contain lipids on the inside, but are polar on the outside to bond with, and dissolve in, water

# Water & oil

"He drew a circle that shut me out-

Heretic , rebel, a thing to flout.

But love and I had the wit to win:

We drew a circle and took him In !"

Edwin Markham - "Outwitted"

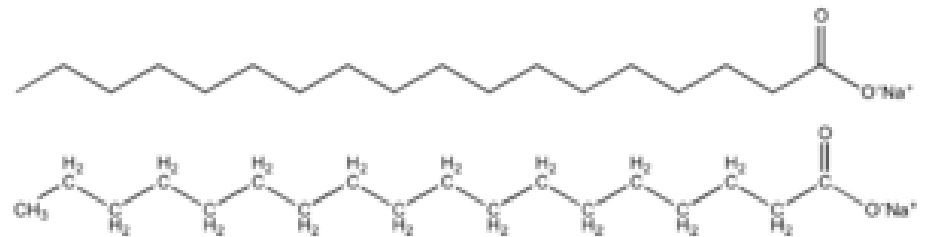
# Water-oil mediation

Molecules exist that can both attract water at one end and mix with lipids at the other

- Amphiphilic ("loving both")
- Polar at one end, non-polar at the other
- Soaps are examples

Sodium stearate, the salt of a saturated fatty acid, is a familiar amphiphilic molecule

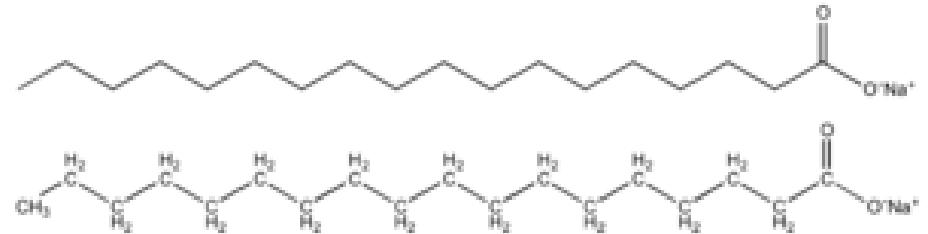
- React tallow (triglyceride from beef fat) with lye (sodium hydroxide) to get soap



# Micelles allow water and oil to mix

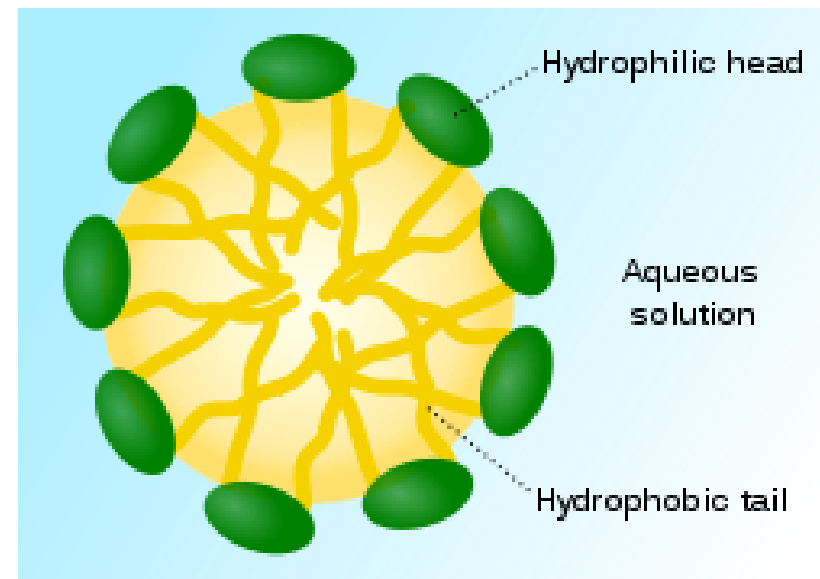
## Micelles

- Amphiphilic molecules orient in balls with non-polar tails to the inside and polar heads to the outside
- Structure known as a *micelle*



Micelles happen when you wash the dishes in detergent

- Grease bonds with non-polar regions, goes to the inside
- Polar heads form hydrogen bonds with water and one another on the outside



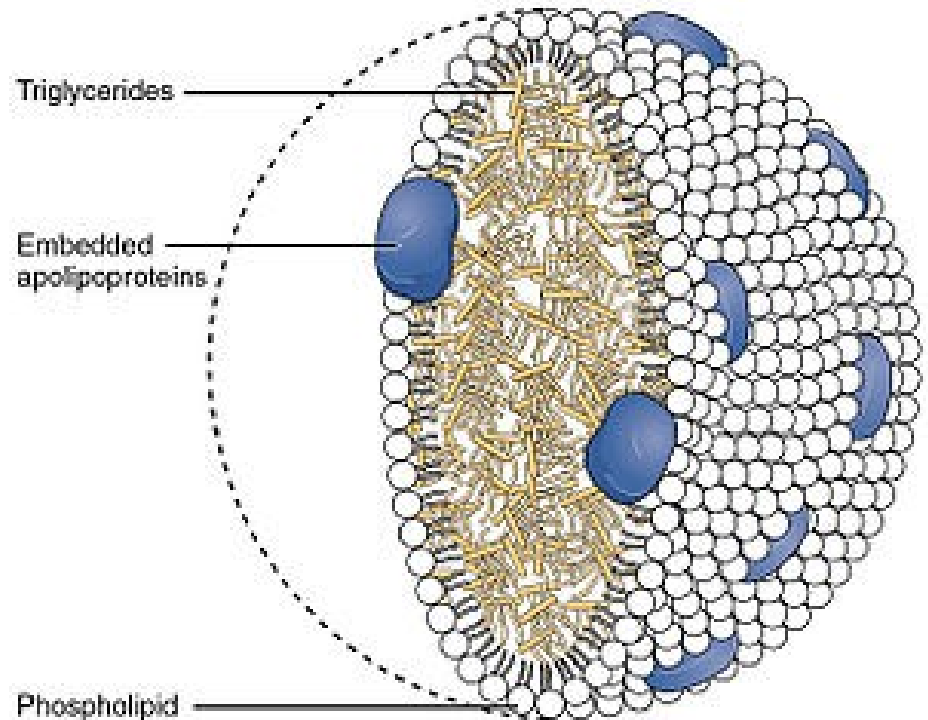
# Lipoproteins as micelles

Lipoproteins are just very large micelles

Amphiphilic molecules forming the shell are phospholipids

Large numbers of lipid molecules (TAG, CHOL) are stored in the interior

A large protein molecule is embedded in the shell



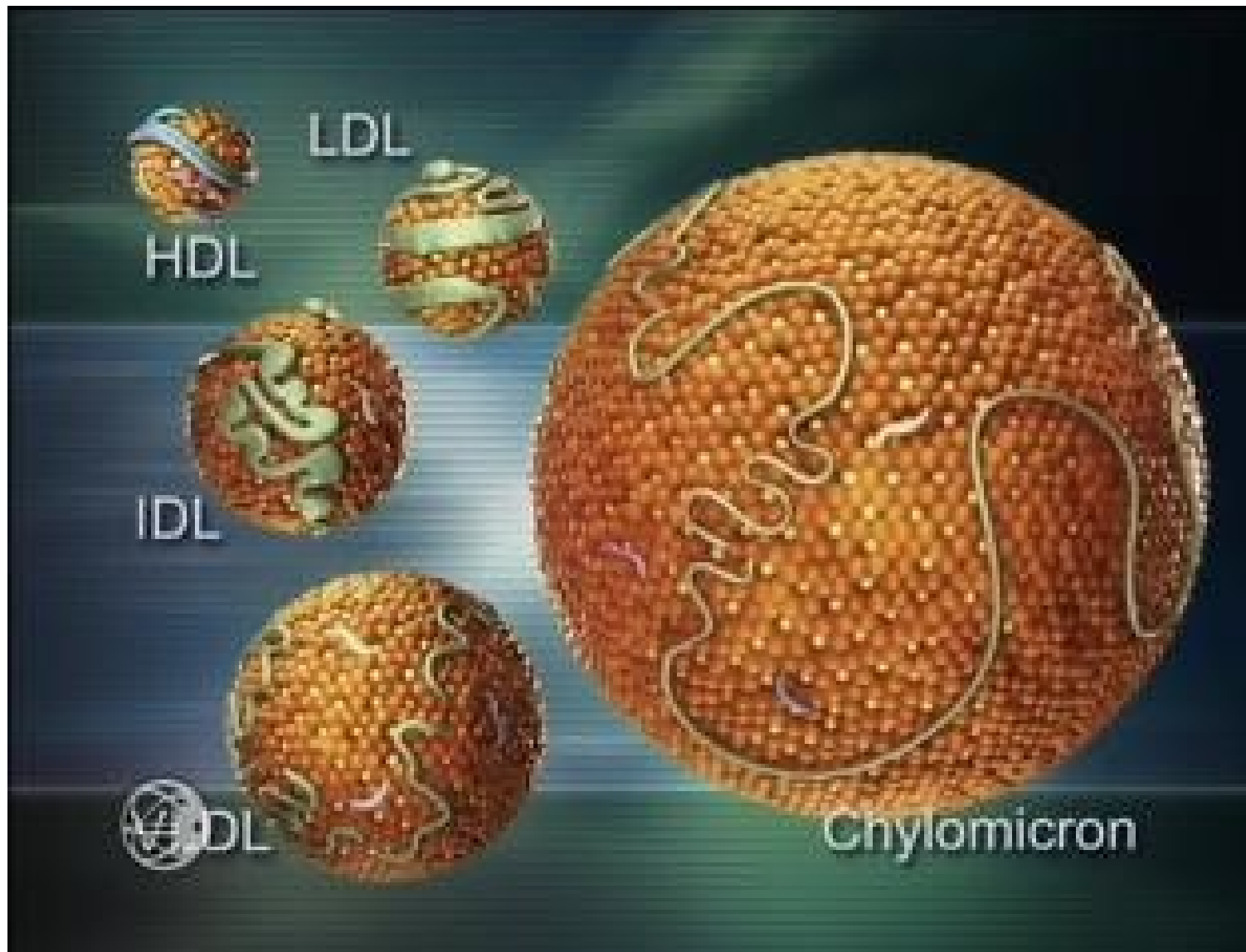
# Lipoprotein particles

Allow lipids to be carried efficiently and reliably through the blood stream

Have an amphiphilic shell of phospholipids and cholesterol, with hydrophilic heads outward into the water, and hydrophobic tails inward to mix with lipid cargo

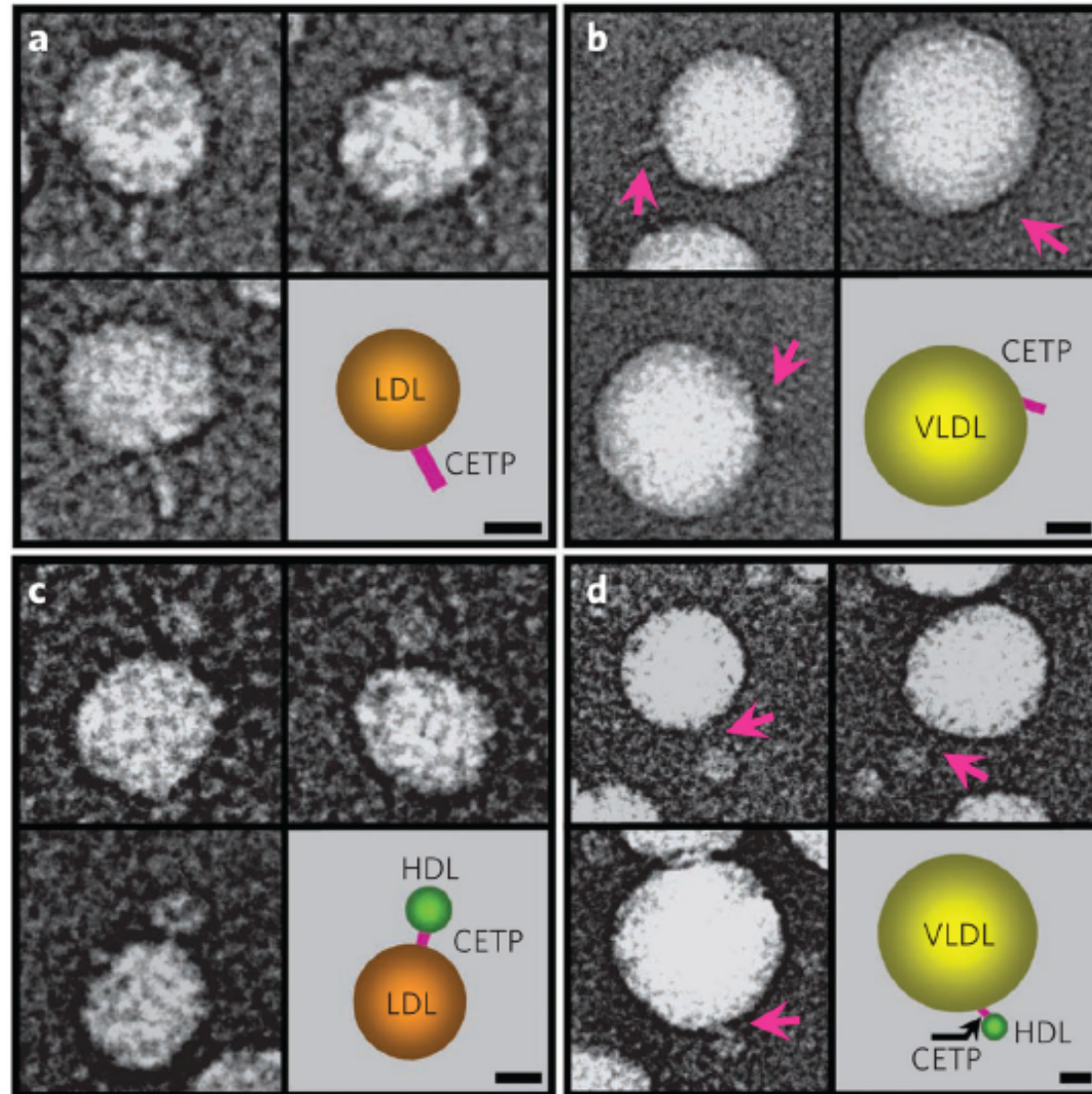
They have a large protein molecule encircling and incorporated into the shell that stabilizes the structure and interacts with receptors

# The Lipoproteins





# Portrait of a lipoprotein



# Lipoproteins: Two families

## B lipoproteins

- VLDL
- IDL
- LDL
- Chylomicrons

Contain the B  
protein

## A lipoprotein

- HDL

Contains the A  
protein

# Lipoprotein nomenclature

The *protein* component of a lipoprotein - what I am calling B or A protein - is technically referred to as the *apolipoprotein*

- For example, apolipoprotein B, or just apoB for short, is the *protein component* of the B family of lipoproteins

Other protein molecules affiliate with lipoproteins to act as enzymes, signals, attachments, etc. (e.g., apoC, apoE)

Nomenclature developed historically and unsystematically, so it is arcane, cumbersome and obscures relationships

# B particles

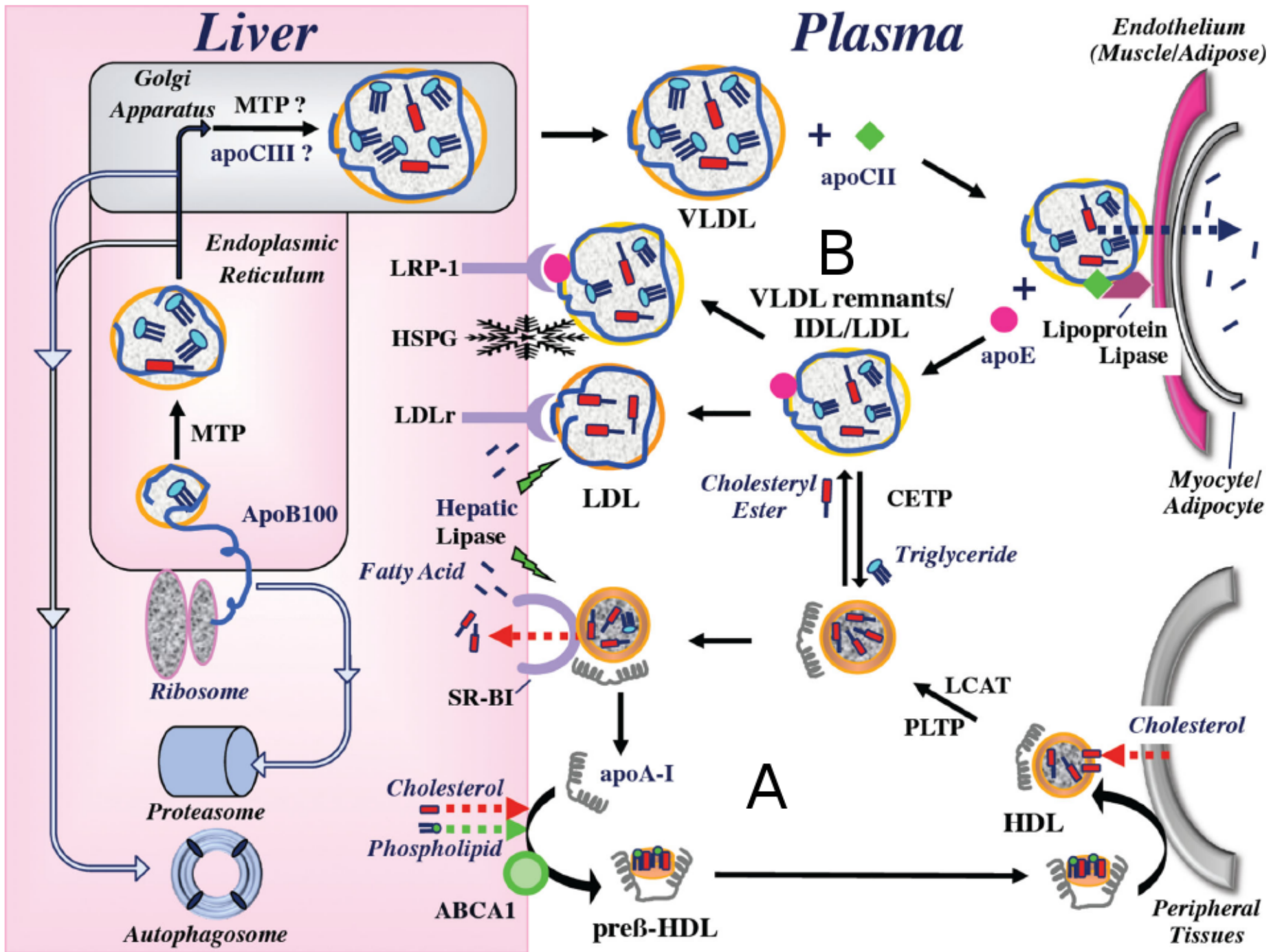
## B particles

- Include VLDL, IDL, LDL, chylomicrons
- Contain apoB as their primary protein component
- Carry TAG from small intestine to muscle, adipose and liver (B48) - chylomicron
- Carry TAG from liver to muscle and adipose (B100) - VLDL
- Collect cholesterol from A particles and deliver it to the liver - LDL

# A particles

## A particles

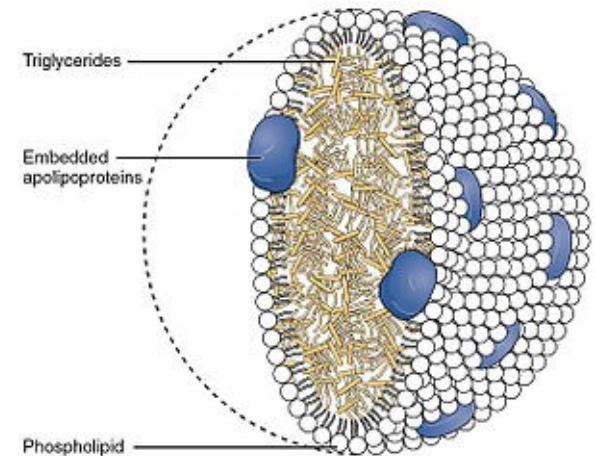
- Includes HDL
- Contain apoA-1 as their primary protein component
- Collect excess cholesterol from tissues (reverse cholesterol transport)
- Deliver cholesterol to endocrine gland as ingredient for hormones
- Deliver cholesterol to liver as ingredient for bile and storage
- Transfer cholesterol to B particles for delivery to liver



# B particle system - creation

B particles are made in the liver or small intestine

- ApoB protein "skeleton"
- Phospholipid/cholesterol "shell"
- FA + glycerol → TAG, to particle interior
- Cholesterol → CE, to particle interior
- Assembled particle released to blood



Complete particle ~30-80 (liver) up to 1200 nm (SI) diameter (vs. RBC 6000 nm)

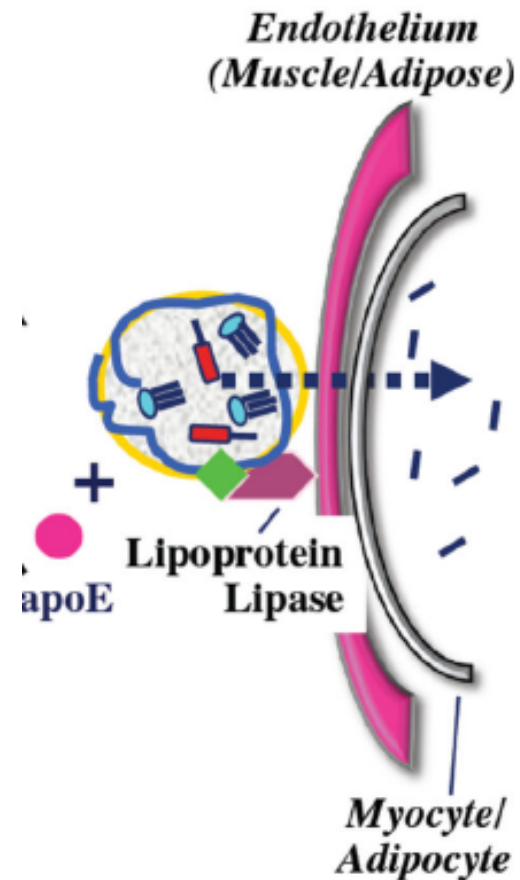
Protein 2-10%, TAG 83-50%, remainder Cholesterol, Cholesterol Esters, Phospholipids

# B particle - delivery

Arrives at destination to deliver TAG

- Attaches to LPL enzyme on blood vessel surface
- LPL removes TAG, releases FA
- FA diffuses to adjacent muscle or adipose
- B particle shrinks, detaches from LPL
- Repeat until TAG exhausted

"Remodeled" B particle ~30-20 nm

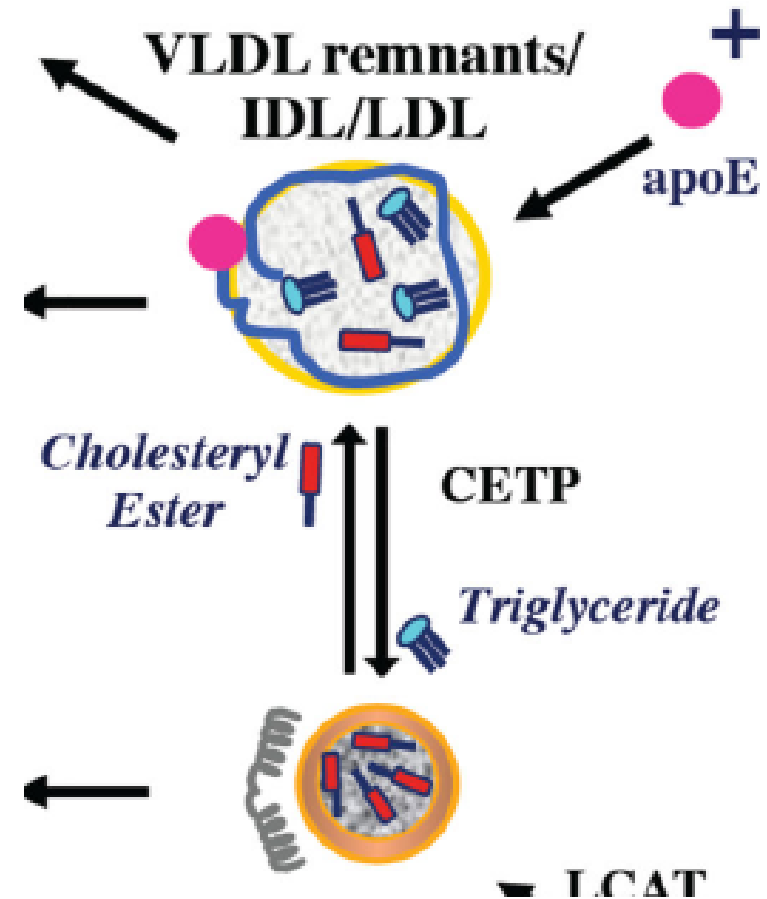




# B particle - repurposed

Donates remaining TAG to A particles in exchange for CE in exchange for CE

- Thus, one route of cholesterol retrieval from tissues back to liver

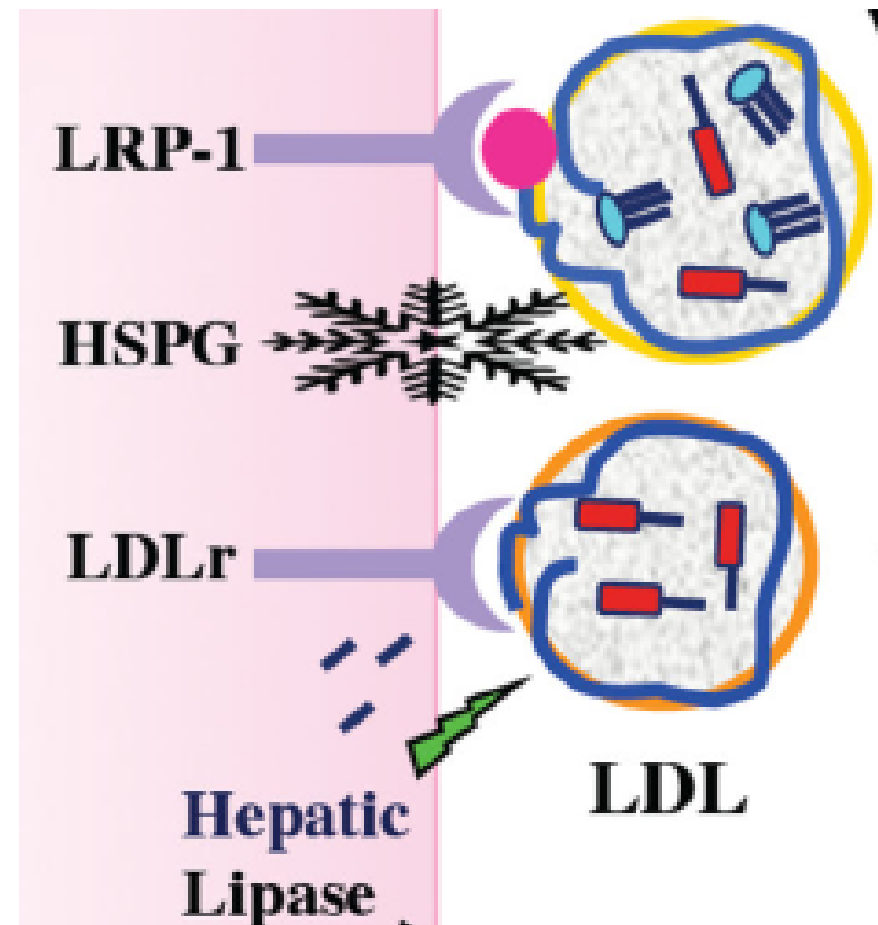


# B particle - repurposed

Gives up remaining  
TAG to liver LPL

Residual B particle  
with cholesterol taken  
up by liver

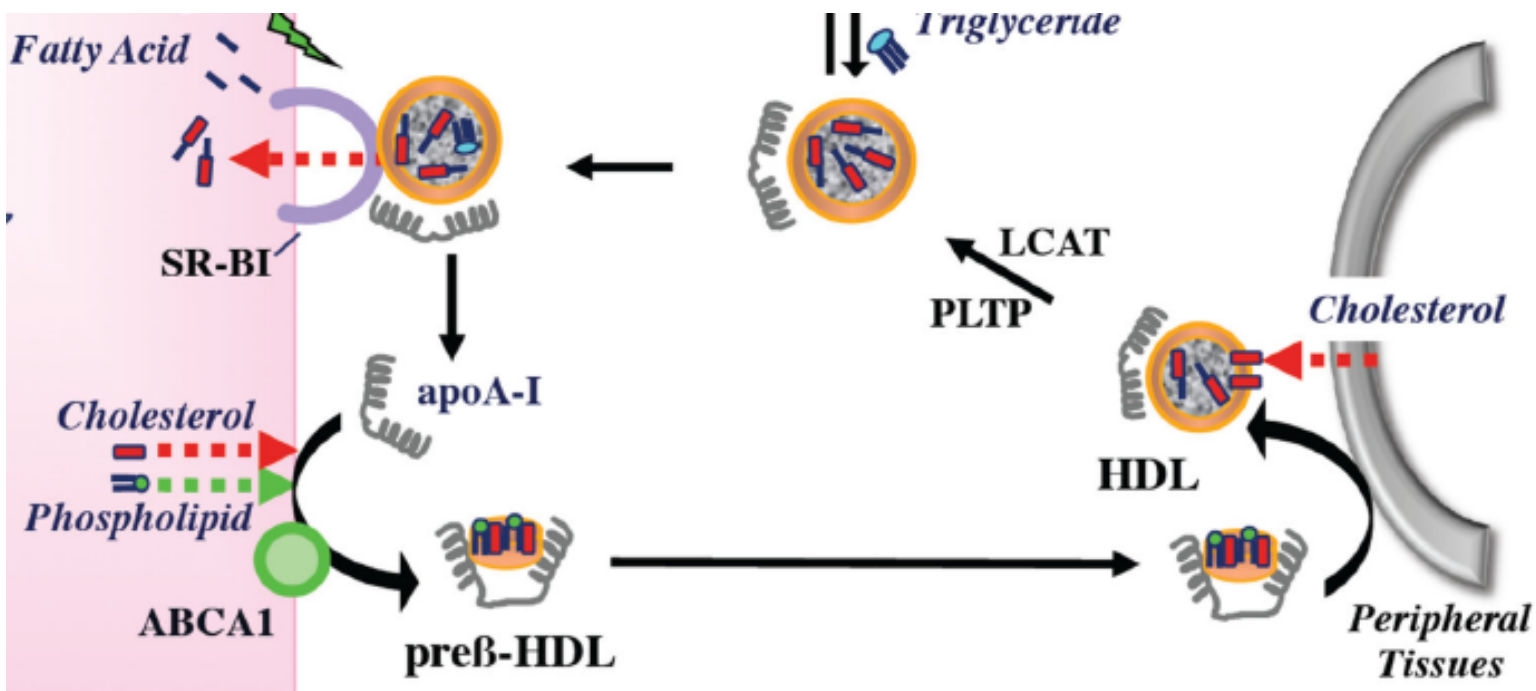
- Binds to LDL receptor  
and swallowed up by  
endocytosis



# A particle - creation

A particles are made by liver

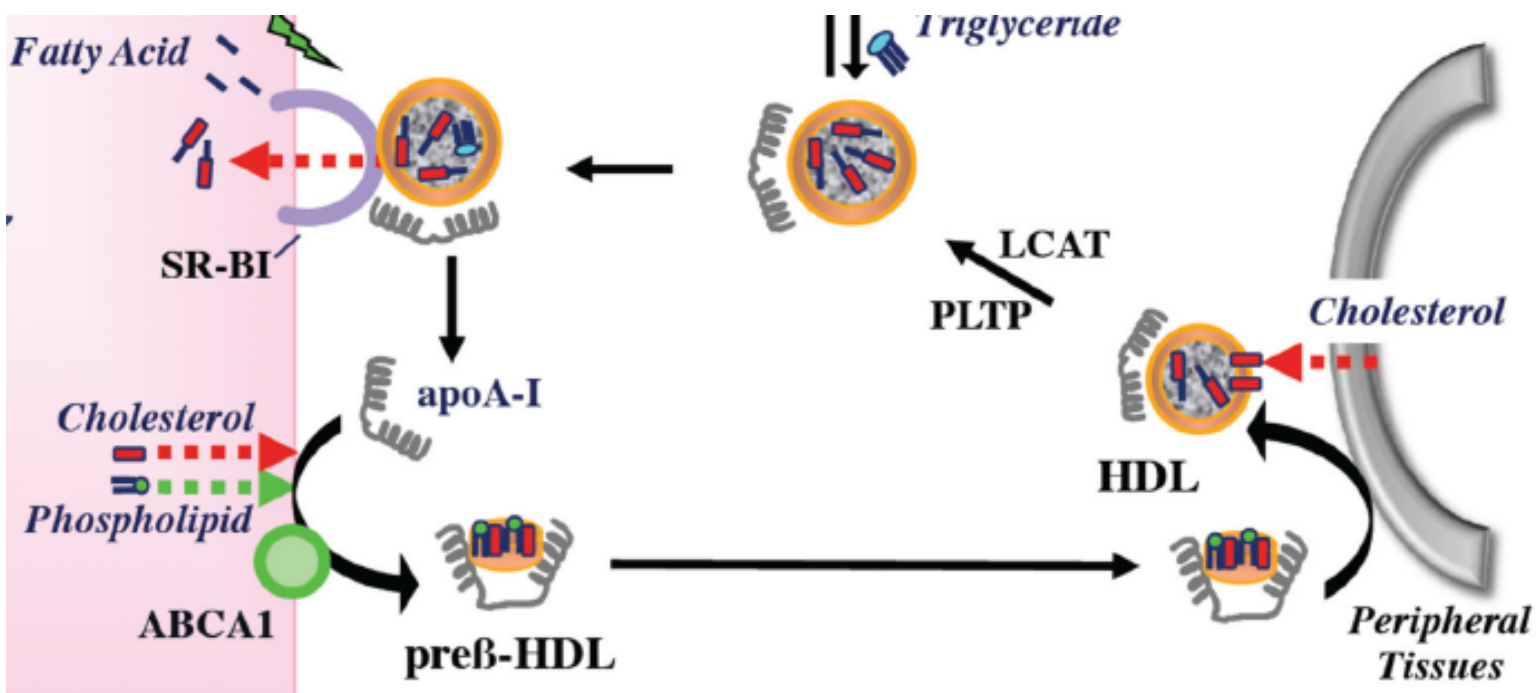
- ApoA skeleton released from liver cell
- Phospholipid and cholesterol derived from liver cell membrane by action of ABCA1 enzyme to form "shell"
- The new-born A particle is flat like a disc



# A particle - collecting cholesterol

Arrives at destination - cells containing excess cholesterol - to off-load their excess

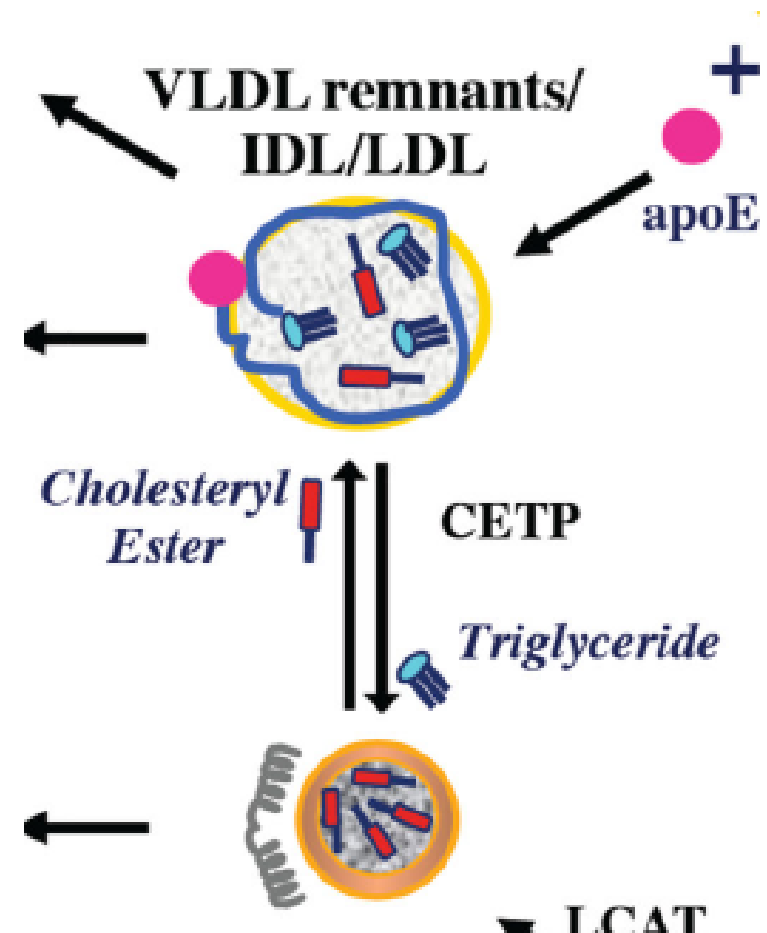
- Disc-shaped nascent A particle takes on cholesterol
- A particle gets "pumped up" into sphere by LCAT enzyme, increasing its capacity to accept more cholesterol
- Fatty acids esterified to cholesterol, so molecules will pack more efficiently
- A particle expands (~10 nm)



# A & B particle interaction

A particle passes cholesterol to B particle for transport back to liver

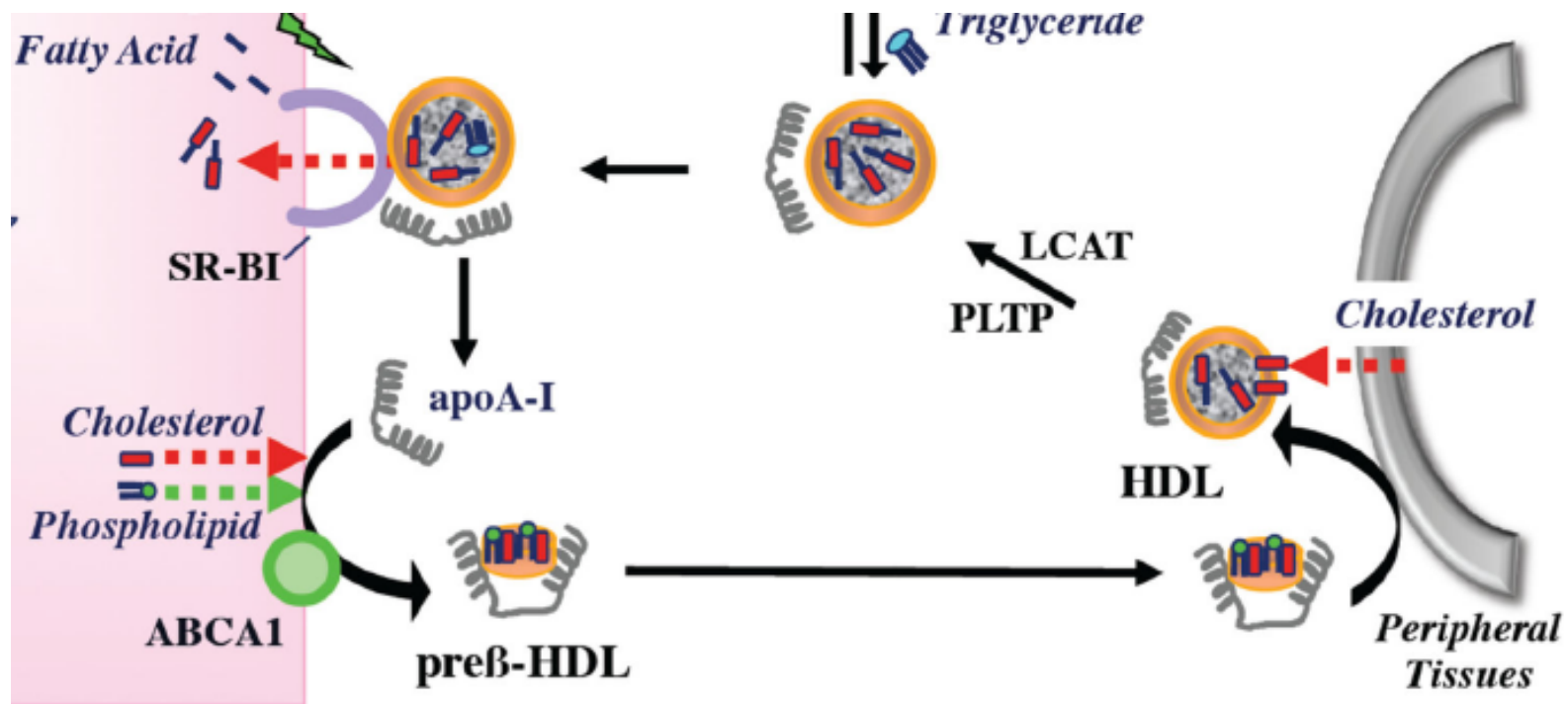
- Enzyme CETP attaches to A & B simultaneously
- Cholesterol ester (CE) migrates from A to B via CETP
- A gets TAG in return to obtain FA that will be used for esterification of cholesterol to CE



# A particle - delivery

Cholesterol carried to steroid producing tissues (adrenal glands, ovaries, testes)

Cholesterol carried to liver, where it is removed, and A particle remodeled for re-use as nascent A particle



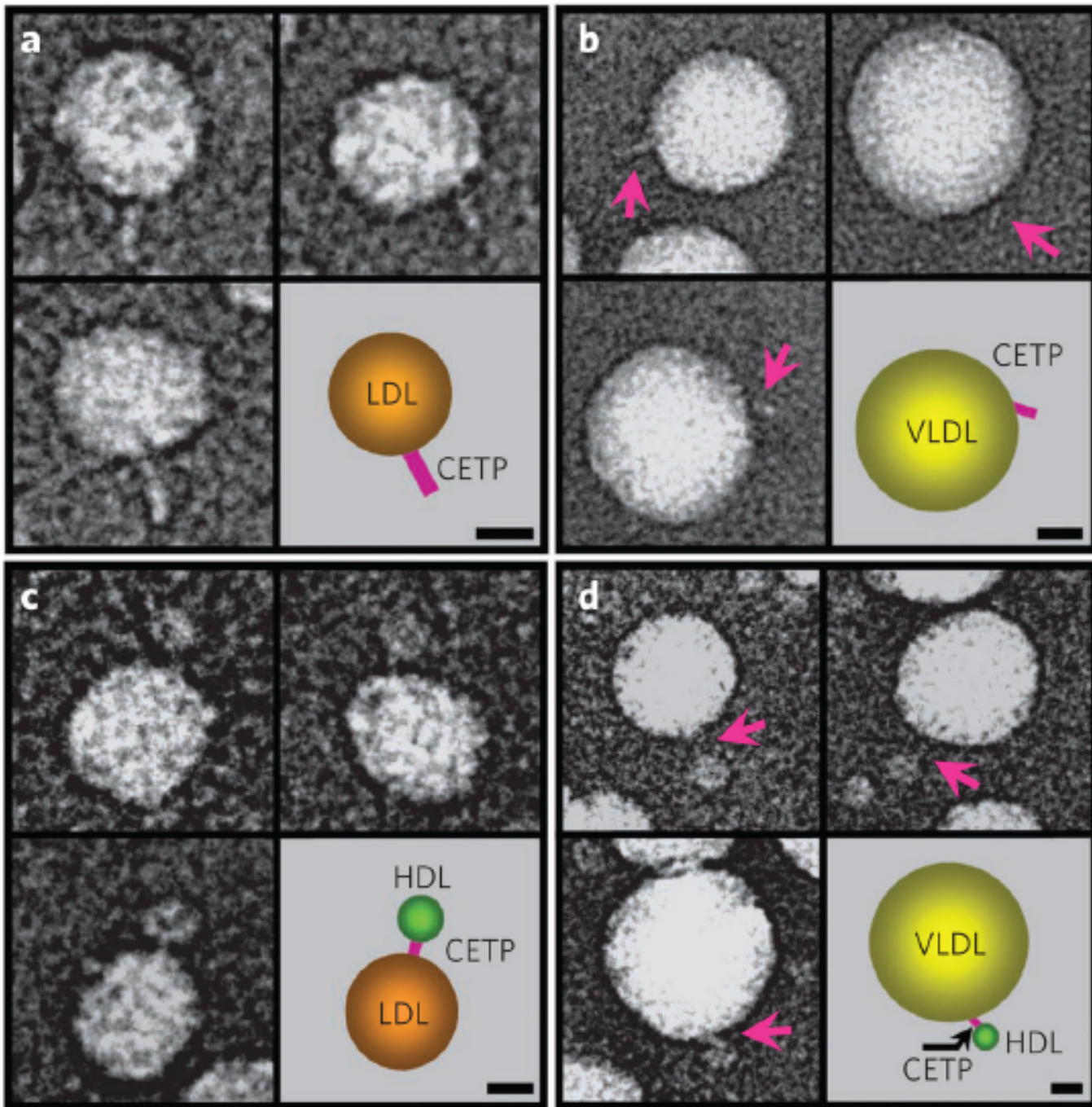
# What are these particles?

VLDL, IDL and LDL are B particles made in the liver, in different life stages

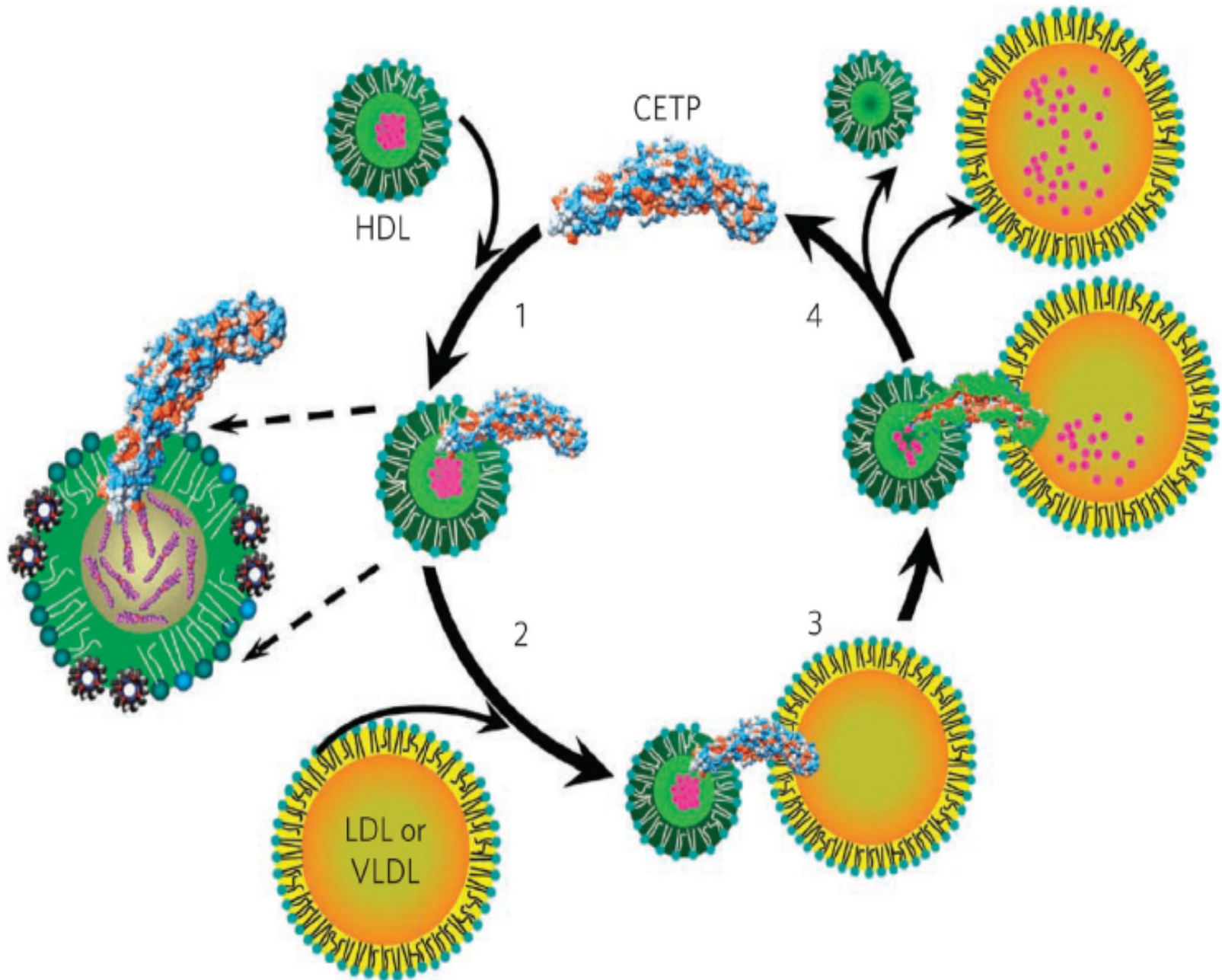
- Newly created B particles are VLDL (80 nm)
- Once they deliver their load and shrink down, B particles are IDL (intermediate density, 30 nm)
- Once IDL have been further remodeled, they shrink further into LDL
- Take up cholesterol from HDL particles and carry it to the liver (20 nm)
- No longer useful, B particles (LDL) are now removed from circulation by the liver by special receptors

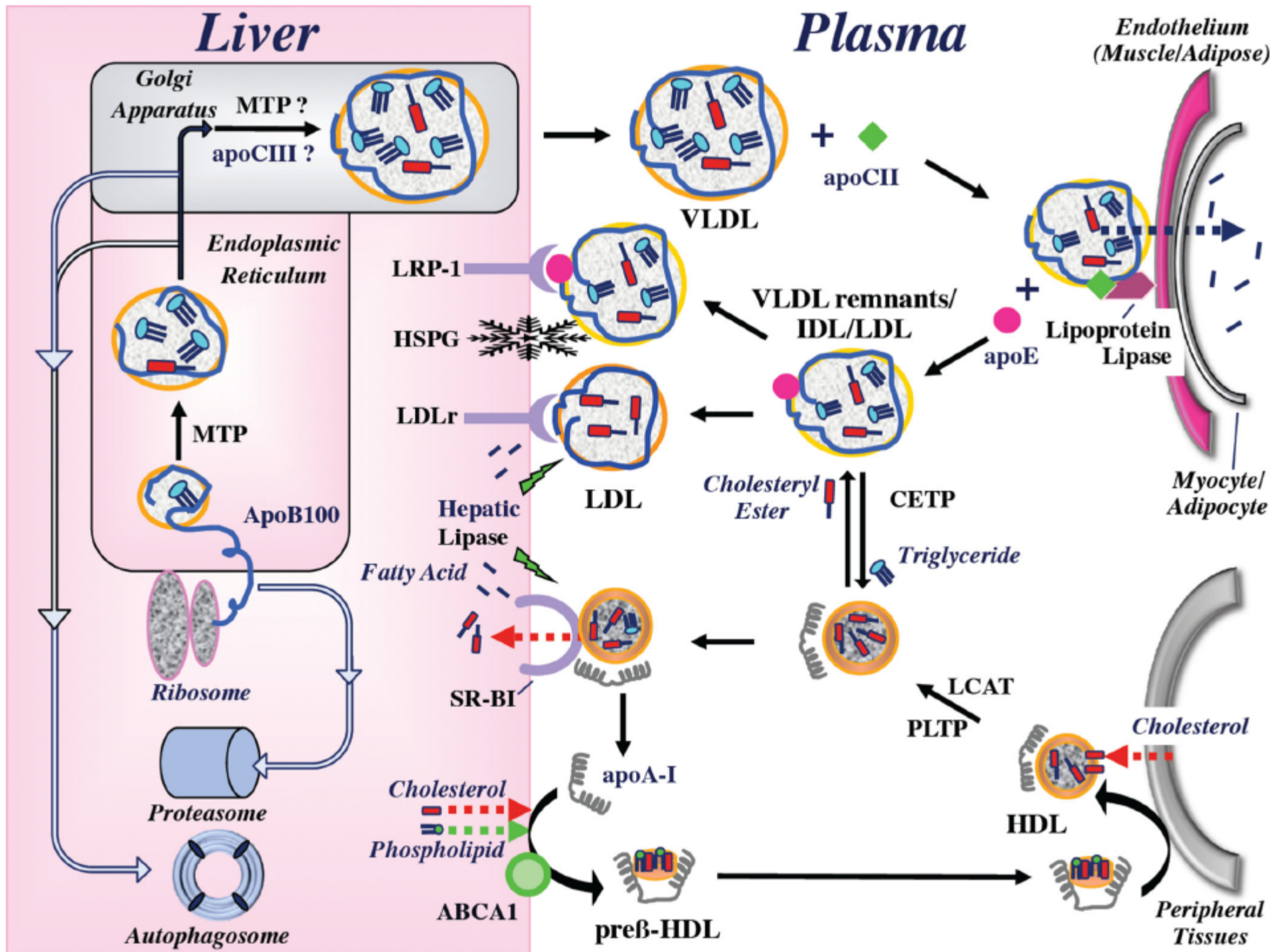
Chylomicrons are B particles made in the small intestine

- Travel in lymph vessels to the thoracic duct, where they empty into the subclavian vein
- Deliver their TAG load to muscles, fat cells and other organs, shrink down, and the remnants are removed from the circulation









# Blood cholesterol and CVD mortality

Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55 000 vascular deaths

*Prospective Studies Collaboration\**

Lancet, 2007    Prospective Studies Collaboration

# Meta-analysis: Blood cholesterol and CVD

## Prospective Studies Collaboration

- 892,000 subjects without previous disease
- 61 prospective observational studies; no treatment
- Ages 40-89
- Follow-up: 13 years
- CVD deaths: 55,000 (33,744 CHD, 11,663 stroke, 9,855 other CVD)
- All had total cholesterol; 154,000 had HDL-C
- 1 mmol/L  $\approx$  40 mg/dL

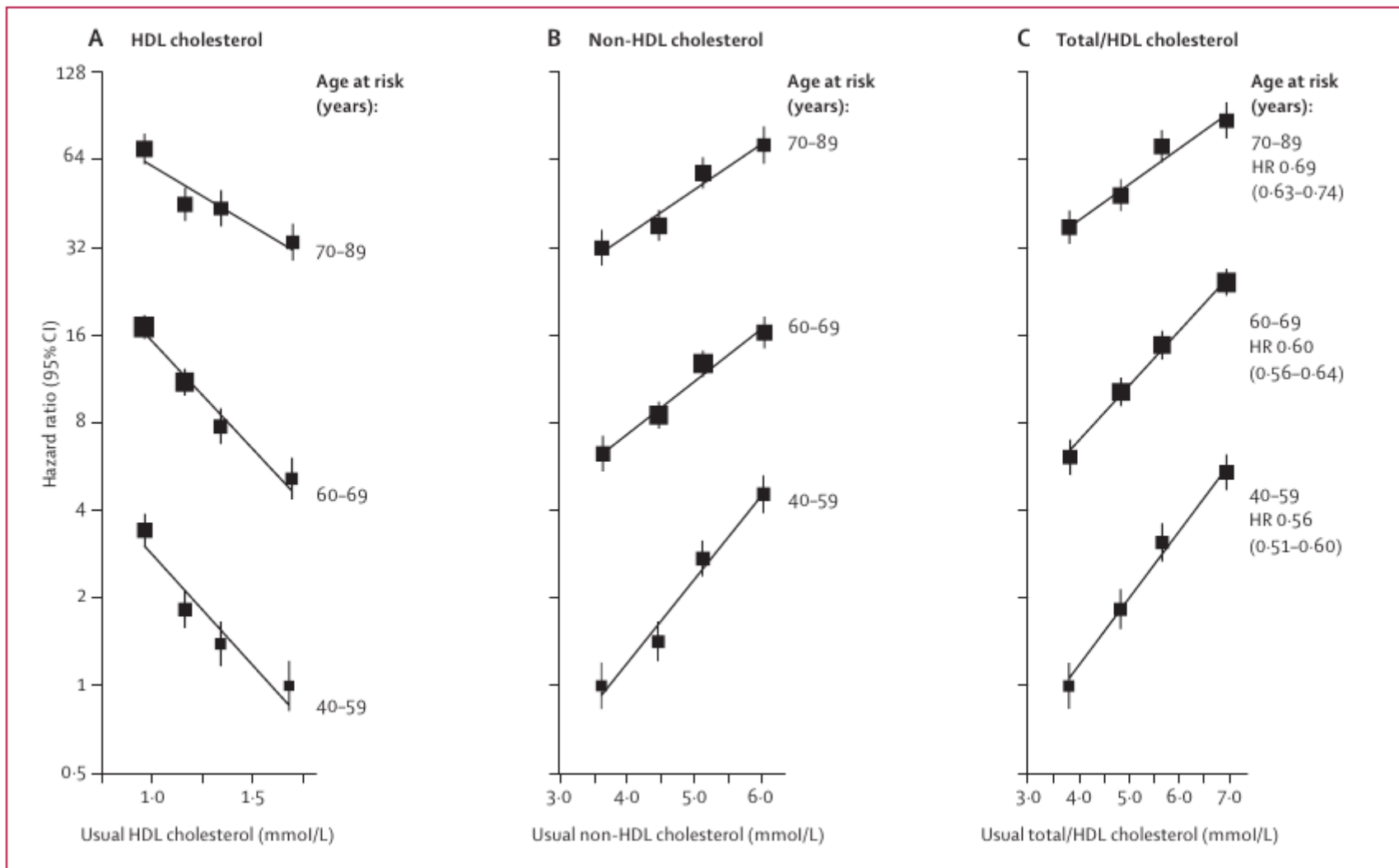
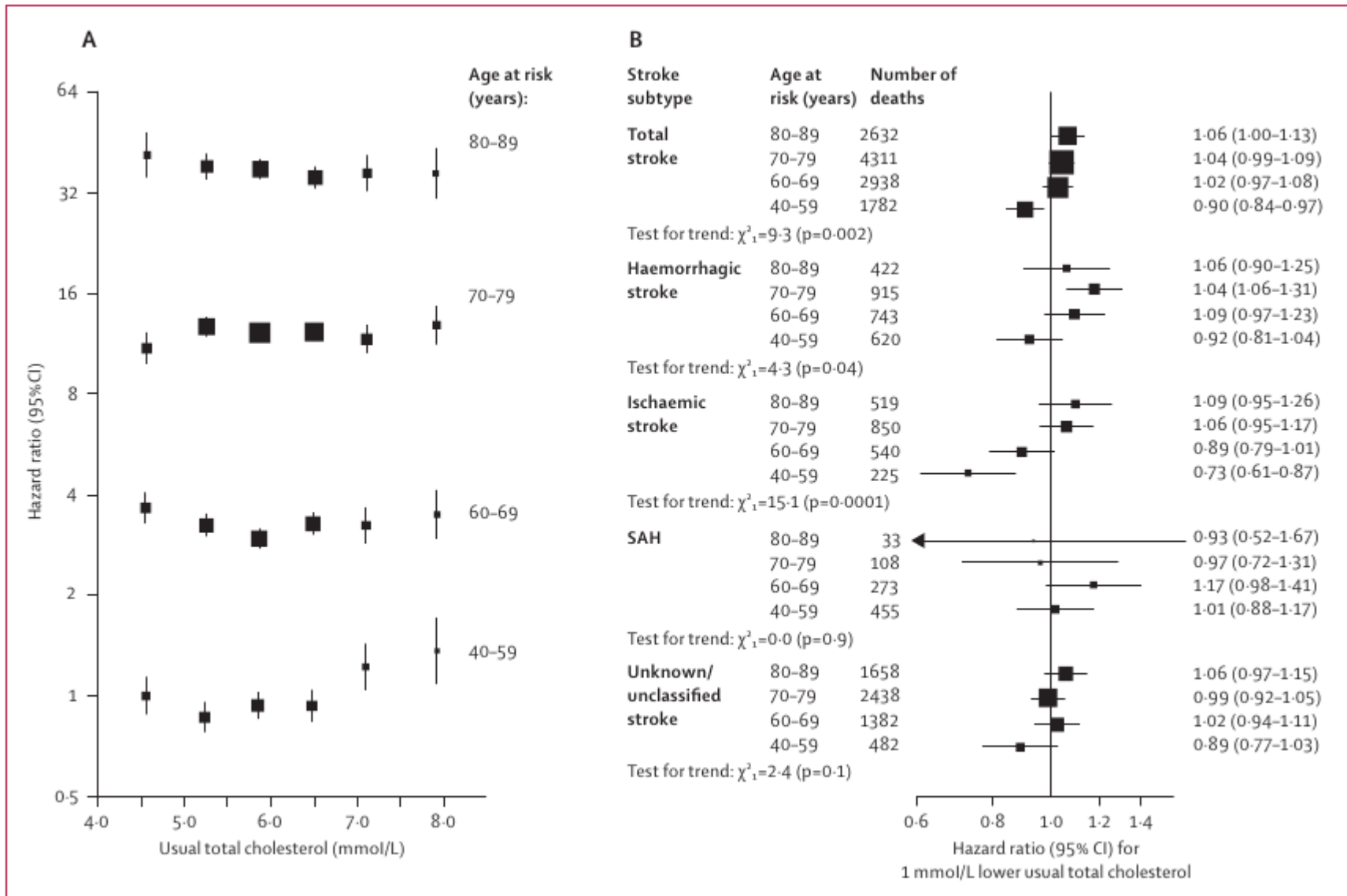
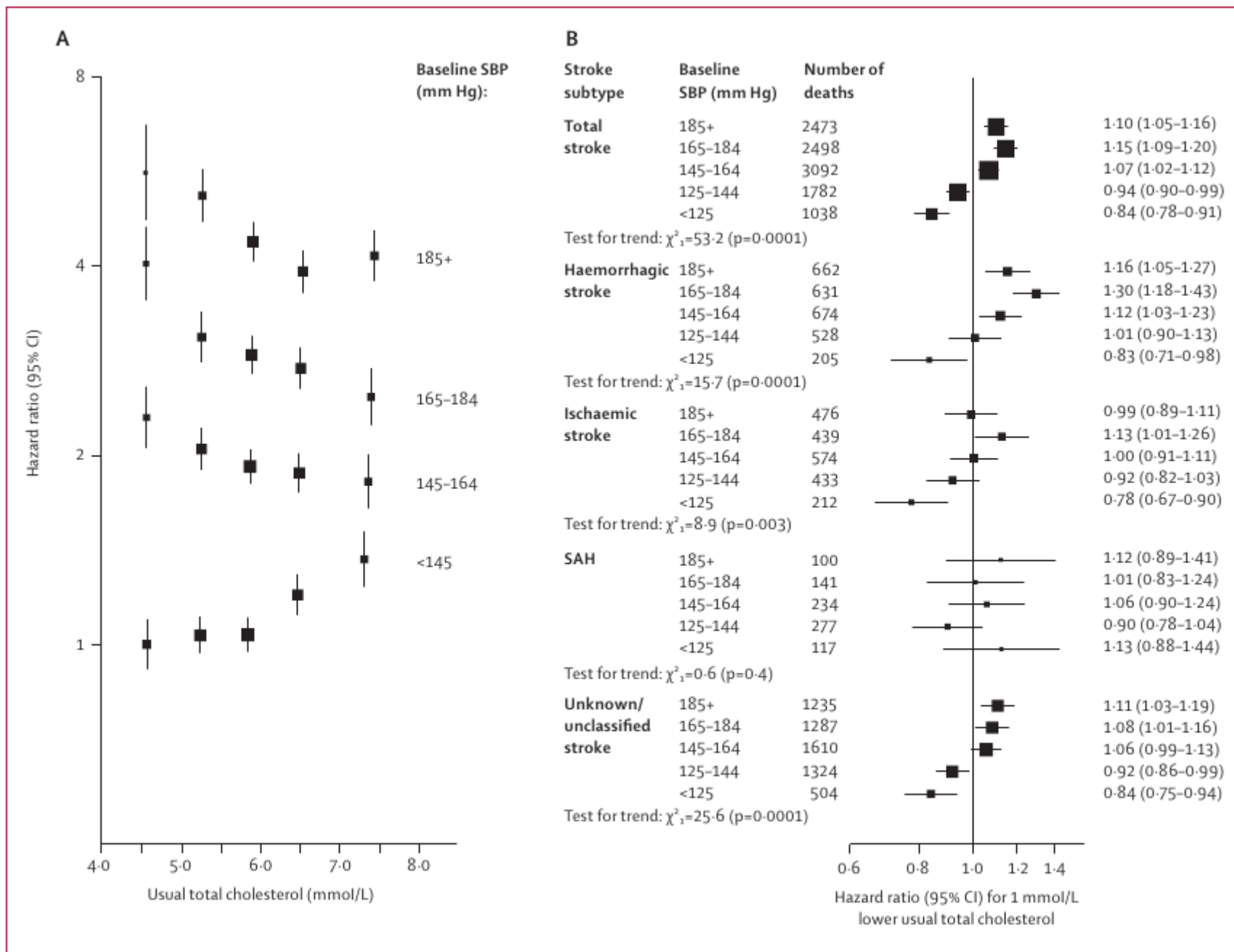
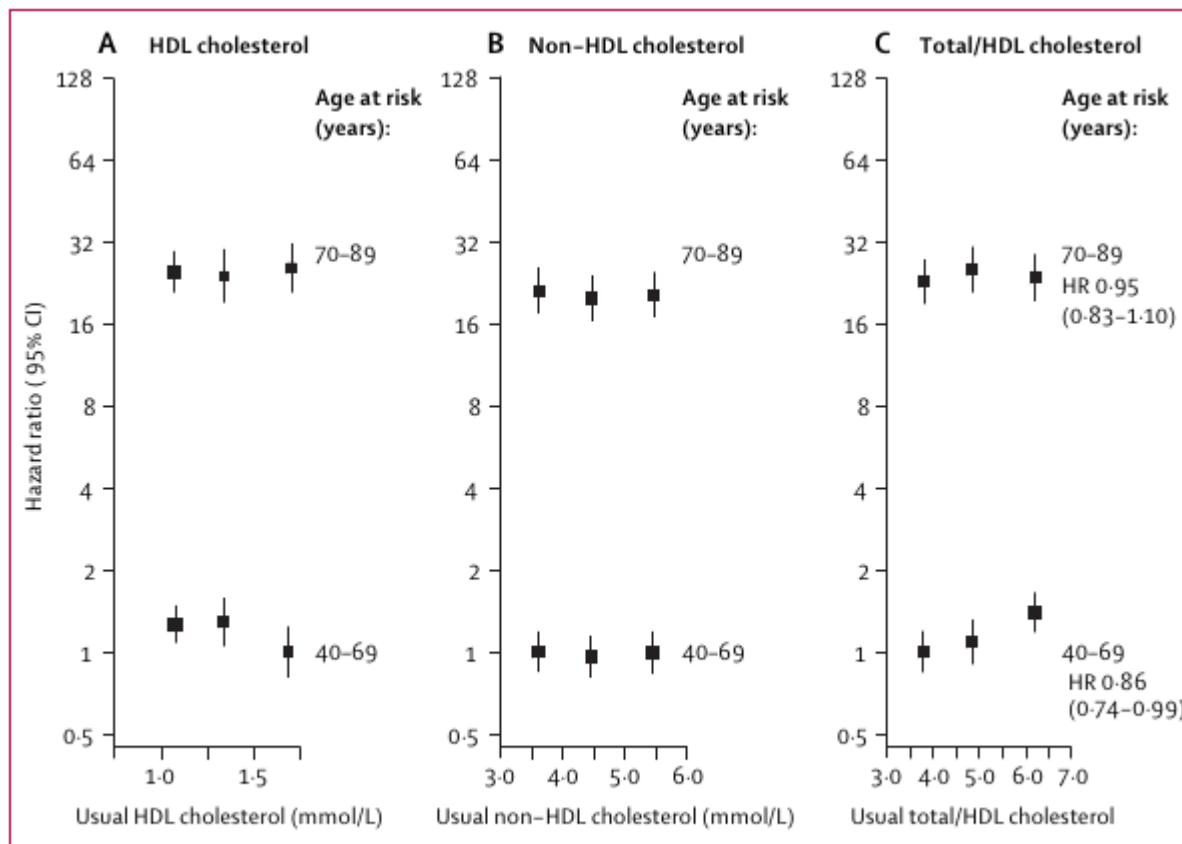


Figure 3: IHD mortality (3020 deaths) versus usual (A) HDL cholesterol; (B) non-HDL cholesterol; and (C) total/HDL cholesterol







**Figure 6:** Stroke mortality (914 deaths) versus usual (A) HDL cholesterol; (B) non-HDL cholesterol; and (C) total/HDL cholesterol



# Cholesterol and CVD: Conclusions

CHD mortality with each 40 mg/dl decrease in total cholesterol (TC)

- Falls by 56% in ages 40-49
- Falls by 17% in ages 80-89 (men 21%, women 8%)
- Proportional fall constant across range from 180-320

CHD mortality at lowest TC quadruples with each decade into 70s, doubles into 80s

CHD mortality at highest TC doubles with each decade

Results similar for men and women

# Efficacy of statin treatment in reducing CVD and mortality

Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90 056 participants in 14 randomised trials of statins

*Cholesterol Treatment Trialists' (CTT) Collaborators\**

Lancet, 2005 Cholesterol Treatment Trialists

# Statin treatment meta-analysis

Randomized controlled trials

90,056 subjects (24% women) in 15 trials

Ages: 19-90

No previous CVD: 46%

Diabetes: 21%

Followup: 5 years

LDL-C reduction: 40 mg/dl (mean)

Deaths: 8186 (4655 CVD)

CVD events: 7557

# Mortality during statin treatment

Lowering LDL by 40 mg/dl for 5 years:

- Decreases overall deaths by 12%
- Decreases CHD deaths by 19%
- Decreases CVD deaths by 17%
- Decreases major CHD events by 23%
- Decreases revascularization by 24%
- Decreases occlusive stroke by 19%
- Decreases all CVD by 21%

# Statin treatment benefit

Proportionally similar effects (~20% reduction)

- Pre-existing vs no pre-existing CVD
- Hypertensive vs normotensive
- Male vs female
- Diabetes vs non-diabetic
- Total cholesterol, LDL-C, HDL-C, TAG

Exception: Slightly less effect for age >65 vs age ≤65

# Absolute benefit of statins by pre-existing CHD status

Pre-existing CHD disease

- 14 fewer CHD deaths per 1,000 treated

No pre-existing CHD

- 4 fewer CHD deaths per 1,000 treated

# Statin side effects

## Cancer

- No effect on incidence of GI, GU, lung, or breast cancer, hematologic malignancies or melanoma
- No change in cancer incidence by number of years of treatment

## Rhabdomyolysis (muscle breakdown)

- 9/40,000 treated vs 6/40,000 controls (p=0.4)

# Statins: glass half-full?

Proponents say ~20% reduction in CHD deaths and major CVD events without significant adverse effects represents a major advance against CHD and other vascular disease

Skeptics point to the large number of people without pre-existing heart disease that must be treated (250) to prevent one CHD death

- With longer treatment, will some ugly side effect emerge to offset that small benefit?

Proportional benefits were equal regardless of HDL-C, LDL-C or TC

- Therefore, you can't define a low relative benefit group to exclude from eligibility for treatment
- "Let's put statins in the drinking water!"



# Raisin' H\*L

If HDL level is so strongly associated with CHD, why not try to increase HDL level?

- Decreasing simple carbs
- Exercise
- Alcohol consumption
- Omega-3
- Magnesium supplement
- Medium-chain fatty acid consumption
- Drugs

All of these have been shown to associate with higher HDL levels, or to raise them in experimental trials; however, efficacy as interventions to reduce CHD has yet to be demonstrated and cannot be assumed

# Increasing HDL does not always reduce CHD risk or mortality

Niacin and fibrates increase HDL-C levels, but their use in RCTs in patients taking statins is not associated with lower CHD

A CETP inhibitor (e.g., torcetrapib) raises HDL-C but does not lower CHD and had 50% increased mortality!

Individuals with a particular genetic trait associated with higher HDL-C did not have lower CHD in a "mendelian randomisation" study

Subclassifications of HDL exist (HDL2, HDL3, HDL efflux capacity, etc) that will need separation to fully understand and exploit the benefits of HDL

# Lipoproteins, not Cholesterol

Cholesterol is just the innocent bystander, that is involuntarily turned into a vascular terrorist, not the perpetrator

ASCVD has two main conventional causes:

- Genetic errors that perturb the delicate lipoprotein pathways
- Chronic excess energy consumption (fats and/or carbohydrates, primarily) that overwhelms and disrupts normal lipoprotein mechanisms

A recently discovered third major cause, TMAO from dietary carnitine and choline, may account for much of the previously unexplained ASCVD

Cholesterol got the rap because it is something we can readily measure

Drug treatments (i.e., statins) aimed at cholesterol do result in reduced ASCVD over the short term (5 years), especially in men and those with existing CVD

Lifestyle changes (diet, exercise, weight reduction) are likely to be much more effective over the lifespan and especially in upper age ranges

# The End of the Beginning

Questions?

Email me at Edwin Cox <[ebcox@yahoo.com](mailto:ebcox@yahoo.com)>

# Lipids are transported bound to protein

Nerking (1901) found that lipids in plasma were not extractable by solvents until the proteins were excised by proteolytic (protein-slicing) enzymes (pepsin)

Therefore, lipids travel bound to proteins in the blood

Gave rise to concept of the lipid-protein hybrid, or "lipoprotein"

# First particles discovered: the chylomicrons

Gage and Fish (1924) saw 1  $\mu\text{m}$  fat-laden particles by dark-field microscopy in blood taken after a fatty meal

These particles carry triglycerides (TAG) from small bowel, through lymph channels, into the blood stream, to muscles and adipose tissue

Later established that chylomicrons were lipoprotein particles

# Salt precipitation of lipoproteins

Macheboeuf (1929) isolated a lipoprotein by ammonium sulfate precipitation of plasma that could be redissolved in water to form a clear solution

The proportions of lipids and protein were consistent with what we now recognize as HDL

# Electrophoresis of plasma proteins

Blix (1941) separated plasma proteins by charge in an electric field (electrophoresis)

Four groups -  $\alpha$ ,  $\beta$ , and  $\gamma$  globulins, and albumin

Found substantial amounts of lipids associated with  $\alpha$  and  $\beta$  globulins



# Ultracentrifugation of plasma proteins

Cohn (1946) - Centrifugation of plasma proteins to derive albumin and gamma globulins for war effort yielded byproduct of lipid-containing globulins

Gofman (1949) - Ultracentrifugation of lipoproteins yielded high-density, low-density, very low-density fractions with quantification

Ultracentrifugation won out over electrophoresis as more practical and informative way of sorting out lipoproteins

Levels of different fractions had disparate implications regarding CHD; HDL "good", LDL "bad", VLDL ??

# Discovery of cholesterol

18<sup>th</sup> century: A new substance found in gallstones

19<sup>th</sup> century: Purified crystals produced; empirical formula determined; isolated from blood

# Cholesterol & heart disease connection

## Anitschkow (1913)

- Feed purified cholesterol to rabbits
- Rabbits develop blood vessel damage

## Ancel Keys (1950s)

- Countries with high intake of fat had high cholesterol levels and high rates of cardiovascular diseases

## Framingham Heart Study (1960s)

- Higher cholesterol was associated with higher rates of cardiovascular diseases

# Assaying blood cholesterol

Separate serum from blood cells

Chemically separate cholesterol, color it and measure concentration

- Abell-Kendall method (1951) for estimating total serum cholesterol level
- Saponification of cholesterol ester by hydroxide, extraction with petroleum ether, and color development with acetic anhydride-sulphuric acid
- Measure concentration by measuring blockage of colored light (photometry)
- Reference standard method against which new methods are calibrated

# Cholesterol fractions - two methods

## Gofman (1953)

- Ultracentrifugation to separate cholesterol components by density
- VLDL, LDL, HDL

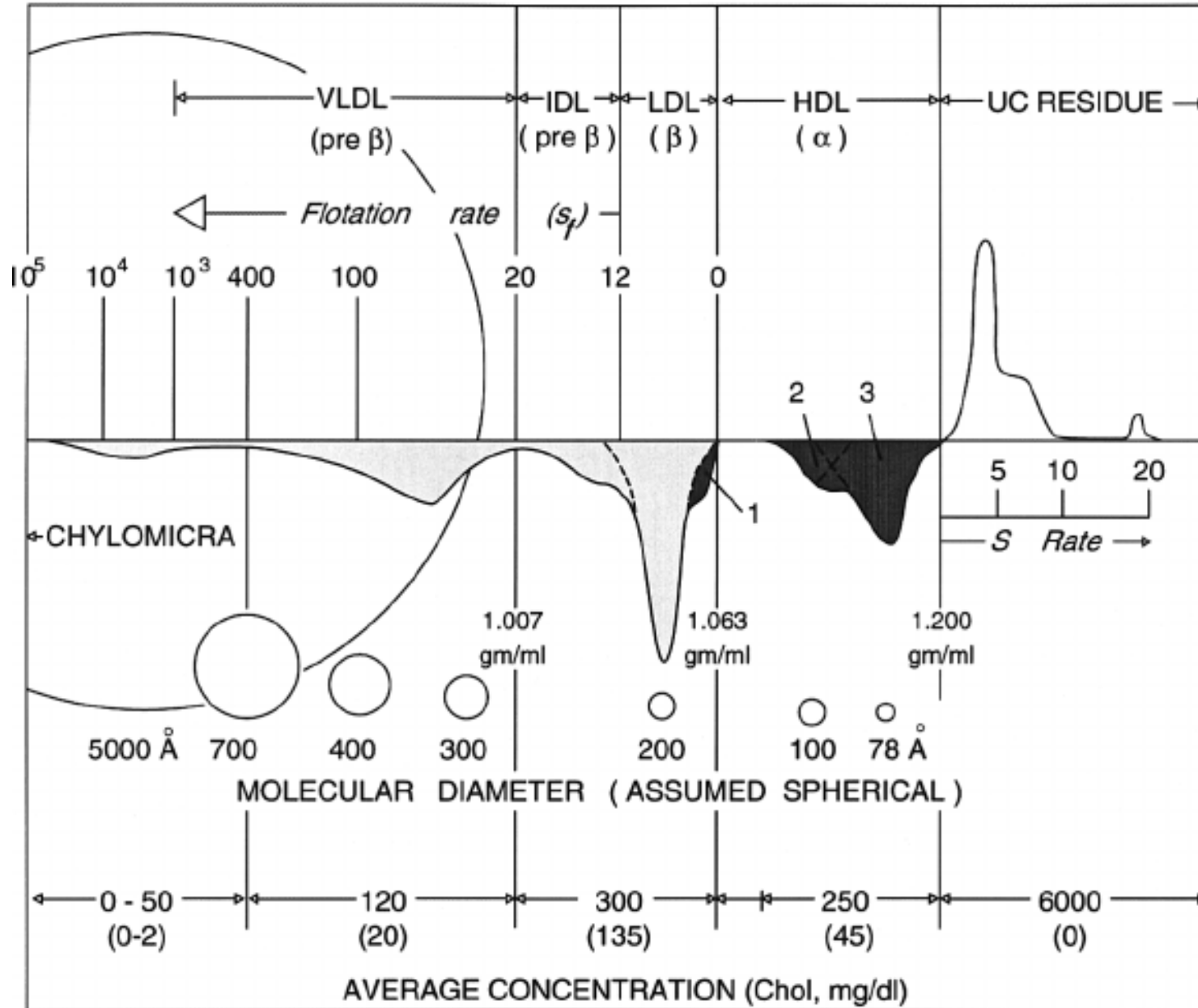
## Swahn (1953)

- Electrophoresis to separate cholesterol components by electric charge
- Alpha, beta lipoproteins

## Gofman method won out over time

- More feasible
- More informative
- But, many many studies were based on total cholesterol alone before cholesterol fractions became practical

# Lipoproteins



# Practical methods of determining cholesterol fractions

Assay total cholesterol (TC), HDL-C and triglyceride (TAG)

- Calculate "Non-HDL-C" as TC minus HDL-C
- Estimate "VLDL-C" as 0.2 times TAG
- Calculate LDL-C as "Non-HDL-C" minus "VLDL-C"

The numbers we get back on our lipid assay are not as "pure" as we might be led to believe

# Cholesterol fractions had opposite implications

Gradually it was feasible to assay the fractions

Higher concentration of high density lipoprotein cholesterol (HDL-C) was associated with better cardiovascular outcomes

Higher concentration of low density lipoprotein cholesterol (LDL-C) was associated with worse cardiovascular outcomes

Total cholesterol is sum of HDL-C and LDL-C and VLDL-C

- You would expect prognostic information of TC was clouded by the relative amounts of HDL and non-HDL, and it was
- New metrics developed: TC/HDL, non-HDL/HDL ratios



# Roles for lipoproteins

VLDL = very low-density *lipoprotein*

- Made in liver
- Delivers triglycerides (TAG) from liver to organs & tissues

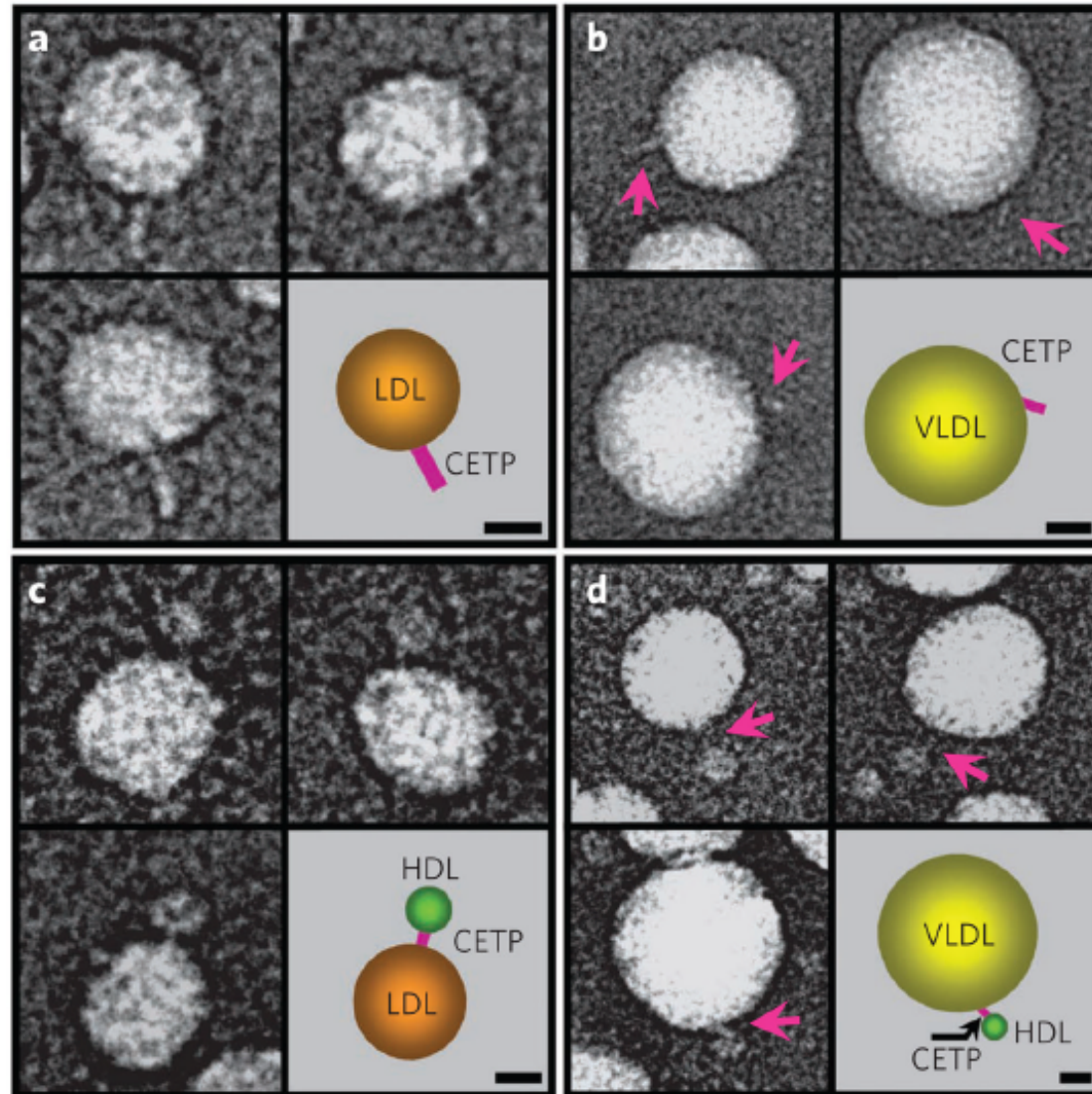
LDL = low-density *lipoprotein*

- Made from remodeled VLDL once it has given up TAG
- Accepts cholesterol from HDL
- Returns cholesterol to liver for disposition

HDL = high-density *lipoprotein*

- Picks up "worn-out" cholesterol from organs & tissues
- Transfers cholesterol to LDL

# Portrait of a lipoprotein



# Cholesterol is carried in lipoproteins

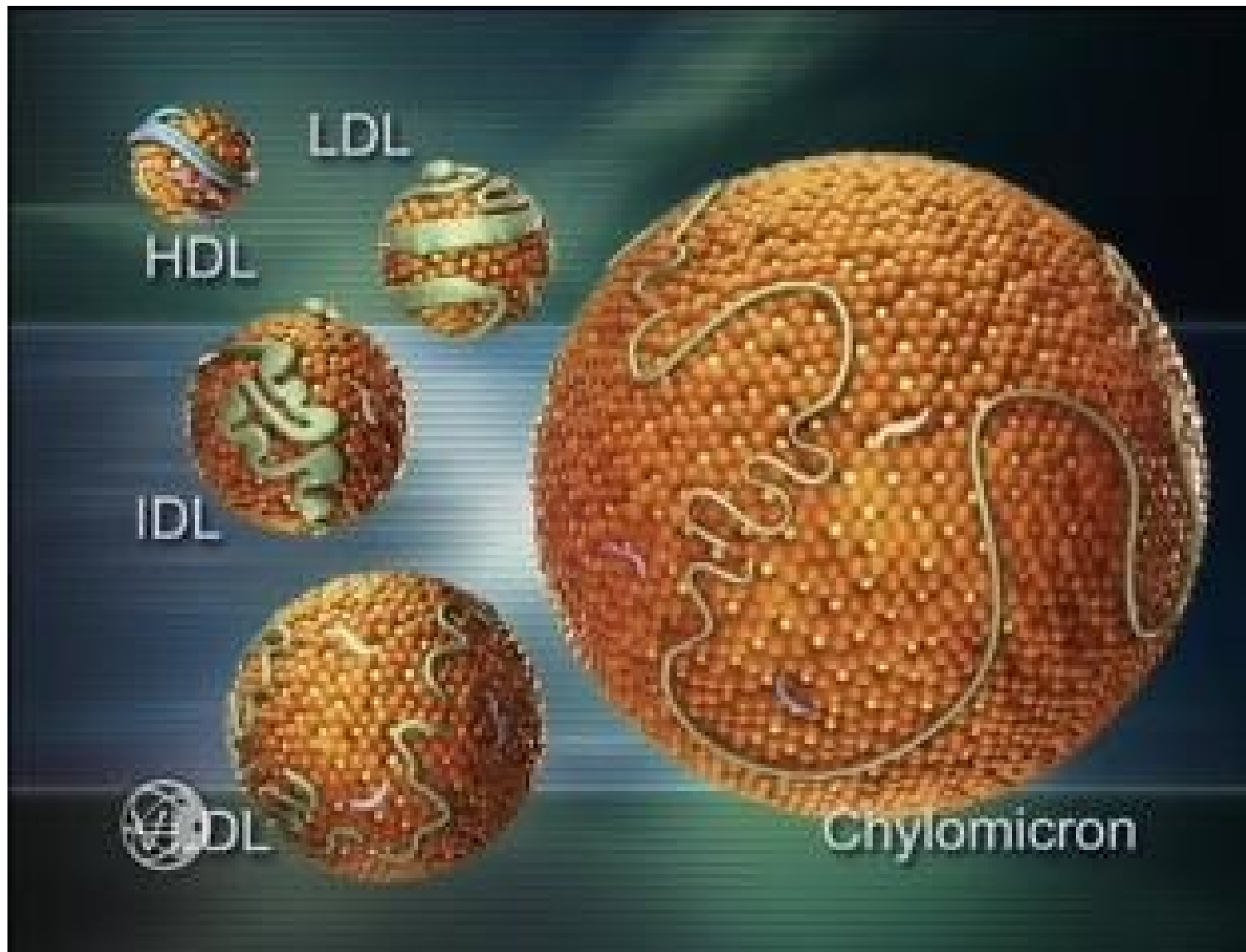
Lipids are not soluble in water and are not detectable in unprocessed blood

Adding a protease (protein-splitting enzyme) to blood releases lipids, which are now detectable (Nerking, 1901)

Lipids are carried in specialized packages

- Given the name "lipoproteins"
- These are particles that have a protein component as part of their makeup

# The Lipoproteins

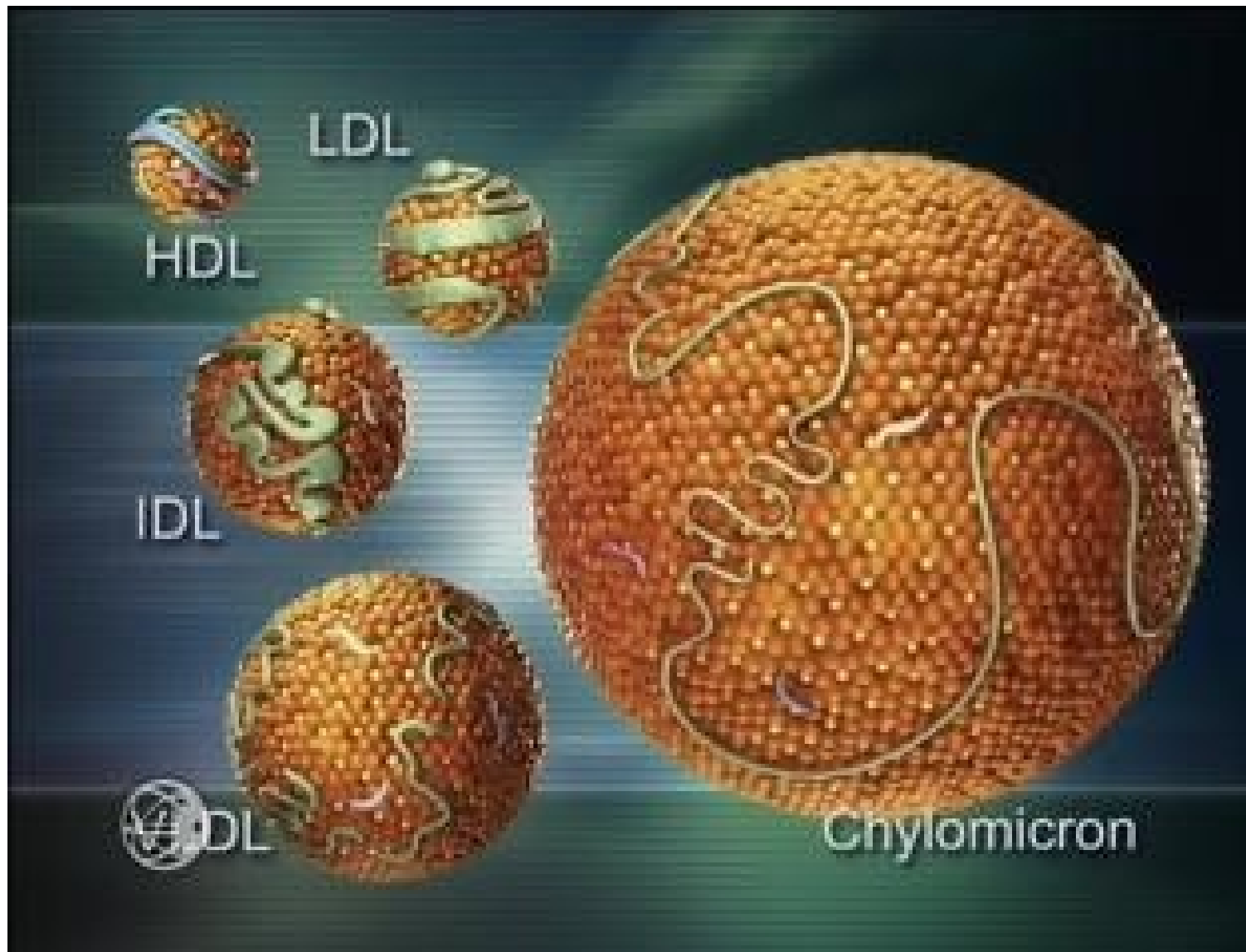


# Underlying causes of abnormal cholesterol disposition

Excess circulating cholesterol can occur for several reasons

- Most often, it's due to dietary indiscretion
- A small but important minority are genetically driven by errors in metabolic pathways (familial hyperlipidemias)

# The Lipoproteins



### Characteristics of Plasma Lipoproteins<sup>a</sup>

	<b>CM</b>	<b>VLDL</b>	<b>IDL</b>	<b>LDL</b>	<b>HDL</b>
Density (g/ml)	<0.95	0.95–1.006	1.006–1.019	1.019–1.063	1.063–1.210
Diameter (nm)	75–1200	30–80	25–35	18–25	5–12
Total lipid (% wt)	98	90	82	75	67
Composition, % dry weight					
Protein	2	10	18	25	33
Triglycerides	83	50	31	9	8
Unesterified cholesterol Cholesteryl esters	8	22	29	45	30
Phospholipids (% wt lipid)	7	18	22	21	29
Major Apolipoproteins	B48,A-I,A-IV, E,CI,CII,CIII	B100, E, CI,CII,CIII	B100,E, CI,CII,CIII	B100	A-I,A-II,CI, CII,CIII,E

Abbreviations: CM, chylomicron; VLDL, very low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein