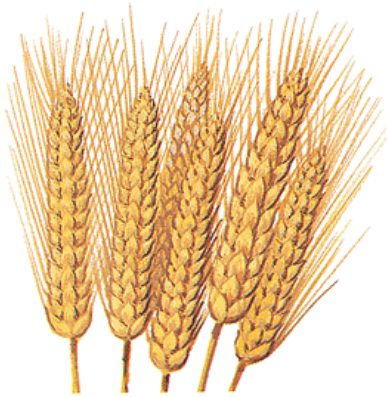


# Nutrition, Toxins and Health: Facts and Speculation

Stephanie Seneff

Wise Traditions Workshop

Weston Price Foundation

Monday, Nov. 12, 2012

# Part II: Biology

Download the slides from
http://people.csail.mit.edu/seneff/

# Outline

- Introduction
  - Big Ideas: Dysfunction and Consequences
- Dysfunction
  - Cholesterol Transport
  - Sulfate Deficiency
  - Obesity
  - Endothelial Nitric Oxide Synthase (eNOS)
  - SiNiC
- Consequences
  - Blood Clots and Hemorrhages
  - Cardiovascular Disease
  - Impaired Gut Bacteria
  - Infection
  - Impaired Autophagy
- The Environment
  - Environmental Toxins
  - Polyphenols
- Summary

# Introduction

# Modern Diseases

cancer

cardiovascular disease

Alzheimer's

asthma

depression

multiple sclerosis

allergies

fibromyalgia

ADHD

diabetes

Parkinson's disease

obesity

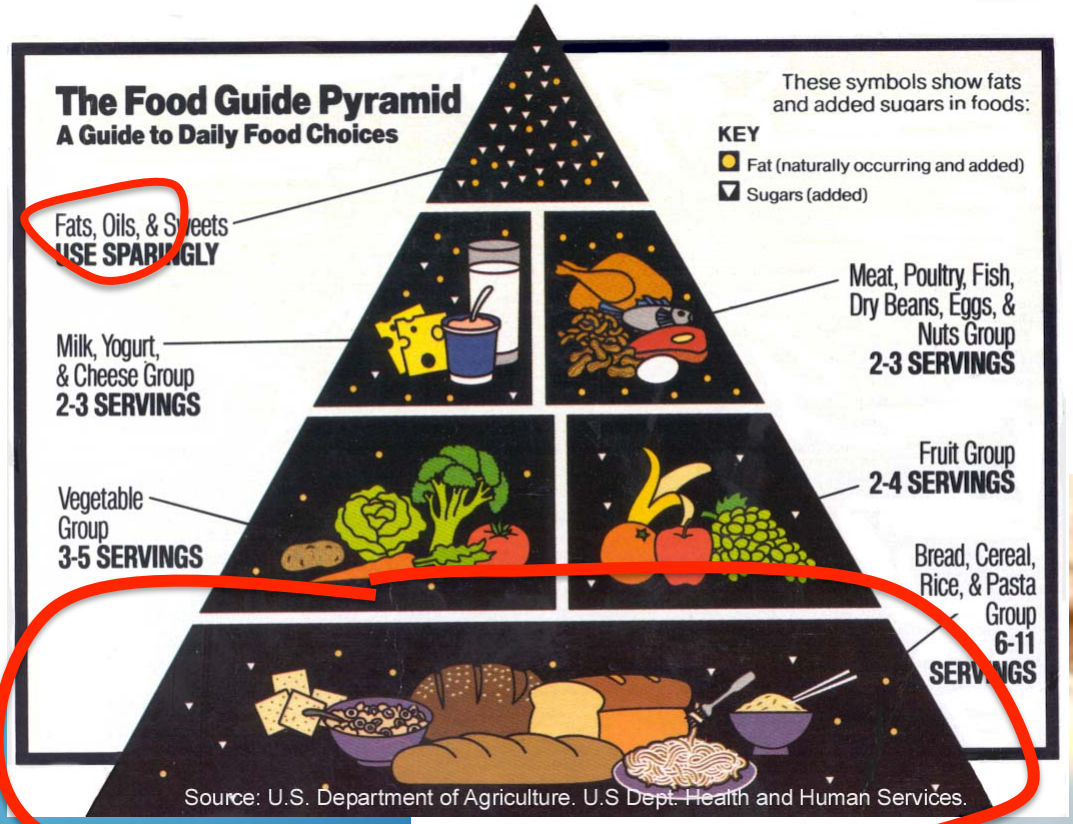
GERD

heart failure

autism

seizures

# Why We are Sick


# We need to worry about sulfur!

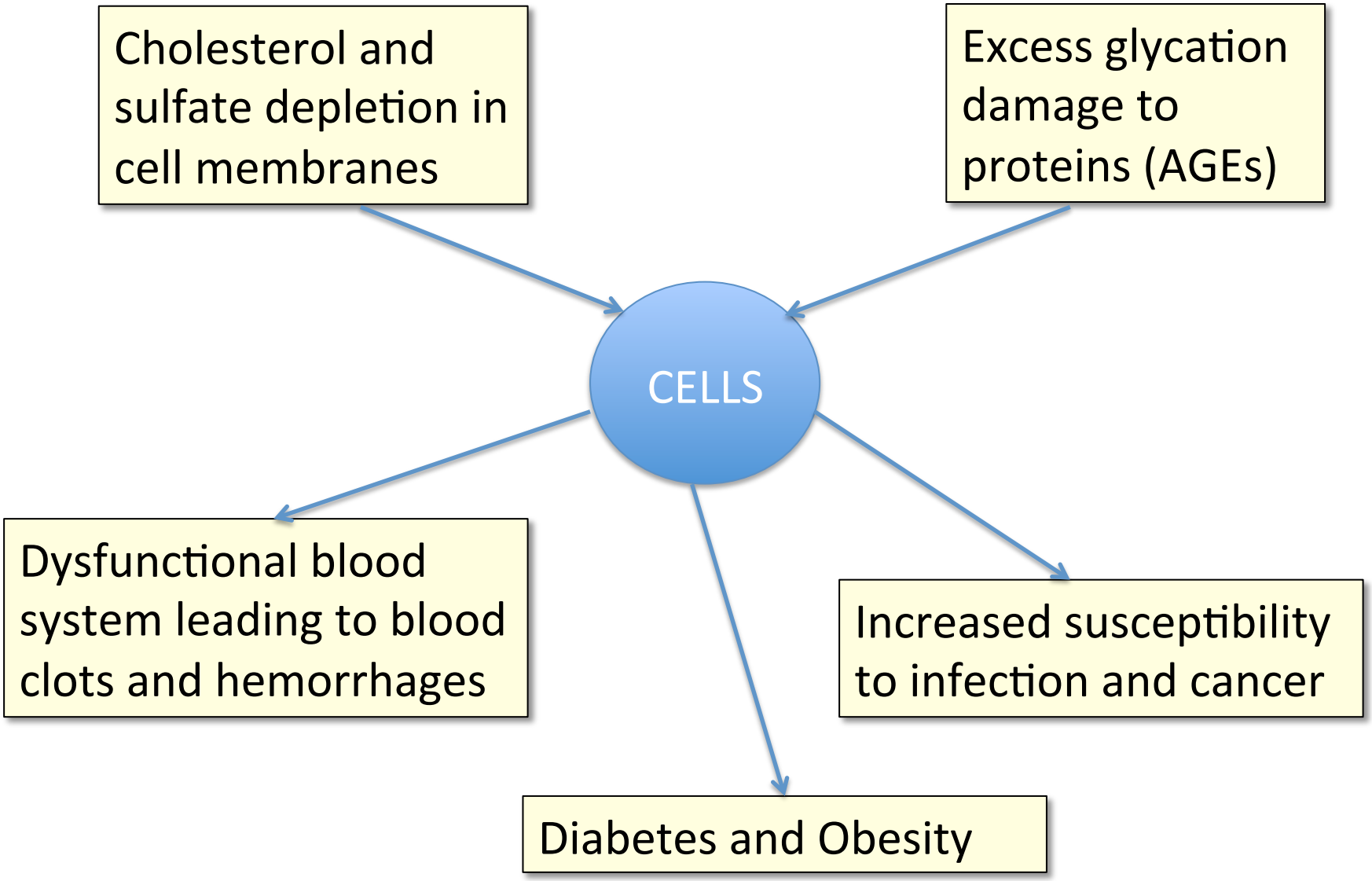
Current agricultural practices are depleting sulfate from the soil

This leads to insufficient dietary sulfur, especially for vegetarians

Environmental toxins deplete sulfur

Sulfate depletion in the body has widespread consequences

# A Biological Definition of Aging



**Dysfunction**

Cholesterol Transport
Sulfate Deficiency
Obesity
eNOS
SiNiC

**Consequences**

Blood Dysfunction
Diabetes
Heart Disease
Cancer
Impaired Gut Bacteria
Infections
Impaired Autophagy

# Dysfunction

# Cholesterol Transport

Under healthy conditions, abundant cholesterol sulfate is synthesized in the skin following sun exposure, and this results in generous supply of cholesterol and sulfate to the tissues.

Inadequate sun exposure and sunscreen impair this process.

# Sulfate Deficiency

The body depends upon heparan sulfate proteoglycans (HSPGs) as a temporary storage bin for glucose

Impaired sulfate synthesis in the skin disrupts this process and leads to diabetes

# Obesity

Insufficient HSPGs impairs muscles' ability to utilize glucose as a fuel

Fat cells insinuate themselves into the loop by transforming glucose to fat for later release to supply safe usable fuel to muscles

# eNOS

eNOS (endothelial Nitric Oxide Synthase) is a "moonlighting" enzyme: it makes sulfate upon sunlight stimulation, and switches to nitric oxide (nitrate) under stress

# SiNiC

The slide from Sulfur into Nitrogen into Carbon
as we age

Oxygen transport in the blood depends upon
these three atomic elements (S, N, C)
which can react with oxygen and form anions
that stabilize the blood and safely carry oxygen

Sulfur is the healthiest choice;
nitrogen is associated with many pathologies;
carbon acting alone will lead to
acidosis and cancer

# Consequences

# Blood Dysfunction

The stability of the blood colloidal system depends upon adequate cholesterol sulfate.

When sulfate is depleted, suspended cells and particles become deficient in negative charge, and start to stick together.

This leads to blood clots and hemorrhaging

# Heart Disease

Cardiovascular plaque develops as an alternative mechanism to produce cholesterol sulfate from damaged LDL and homocysteine

# Arthritis

Arthritis, and other chronic diseases like Alzheimer's and multiple sclerosis, result because the body robs an organ system of sulfate in order to keep the blood from coagulating

# Cancer

Cancer develops as a mechanism to massively convert glucose to lactate, which is a much safer fuel – especially when cells are insulin resistant

# Infection

Susceptibility to infection is a consequence of impaired cholesterol sulfate synthesis, which introduces widespread pathology

Infection serves a useful role in resupplying critical nutrients such as cobalamin, folate, and heparan sulfate

# Impaired Autophagy

Autophagy is the process by which cells dispose of the garbage that accumulates with living – they can recycle misfolded proteins into new proteins and replace broken mitochondria with fresh ones.

Excess nitric oxide leads to impaired autophagy, which results in accumulation of debris and busted mitochondria. Over time, the cell becomes so impaired that it has to shut down.

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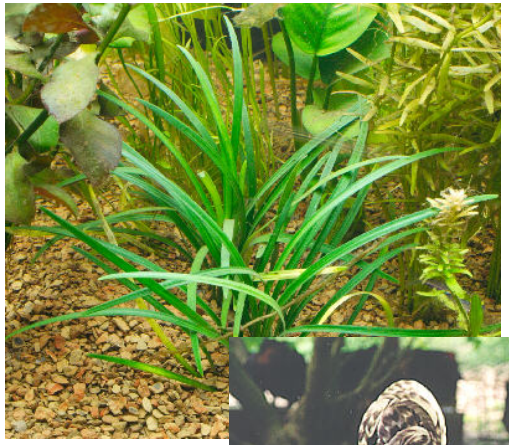
# Cholesterol Transport

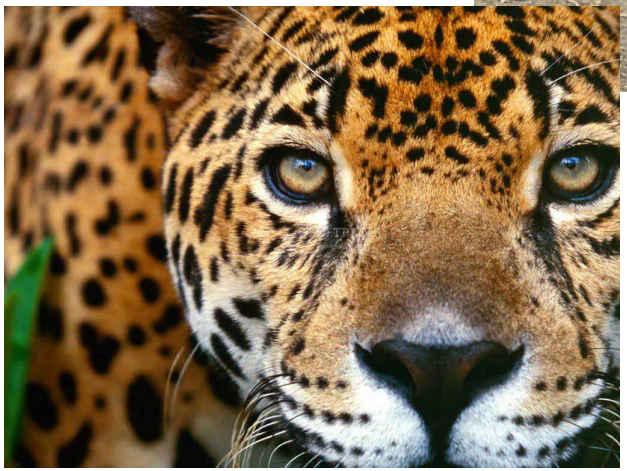
Under healthy conditions, abundant cholesterol sulfate is synthesized in the skin following sun exposure, and this results in generous supply of cholesterol and sulfate to the tissues.

Inadequate sun exposure and sunscreen impair this process.

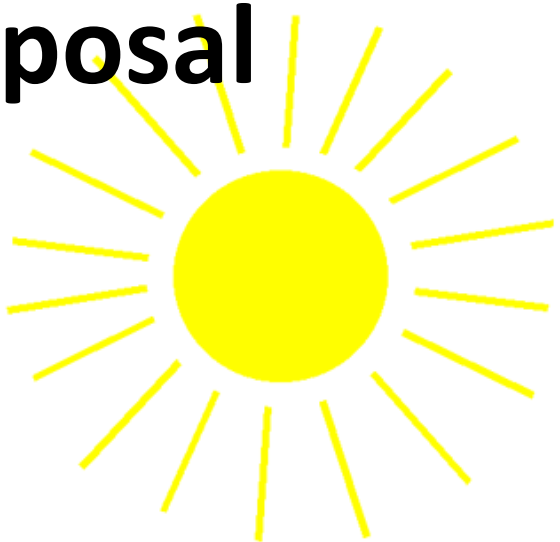
# Cholesterol is Essential for Mobility and a Nervous System

- Plants contain no cholesterol
- Plants can't move
- Plants don't have a nervous system
- In a sense, cholesterol is to animals as chlorophyll is to plants

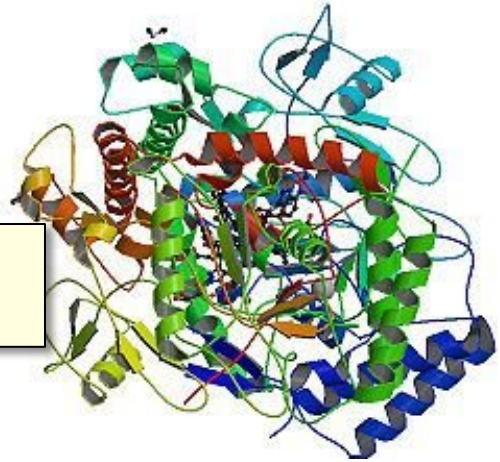




# A Provocative Proposal



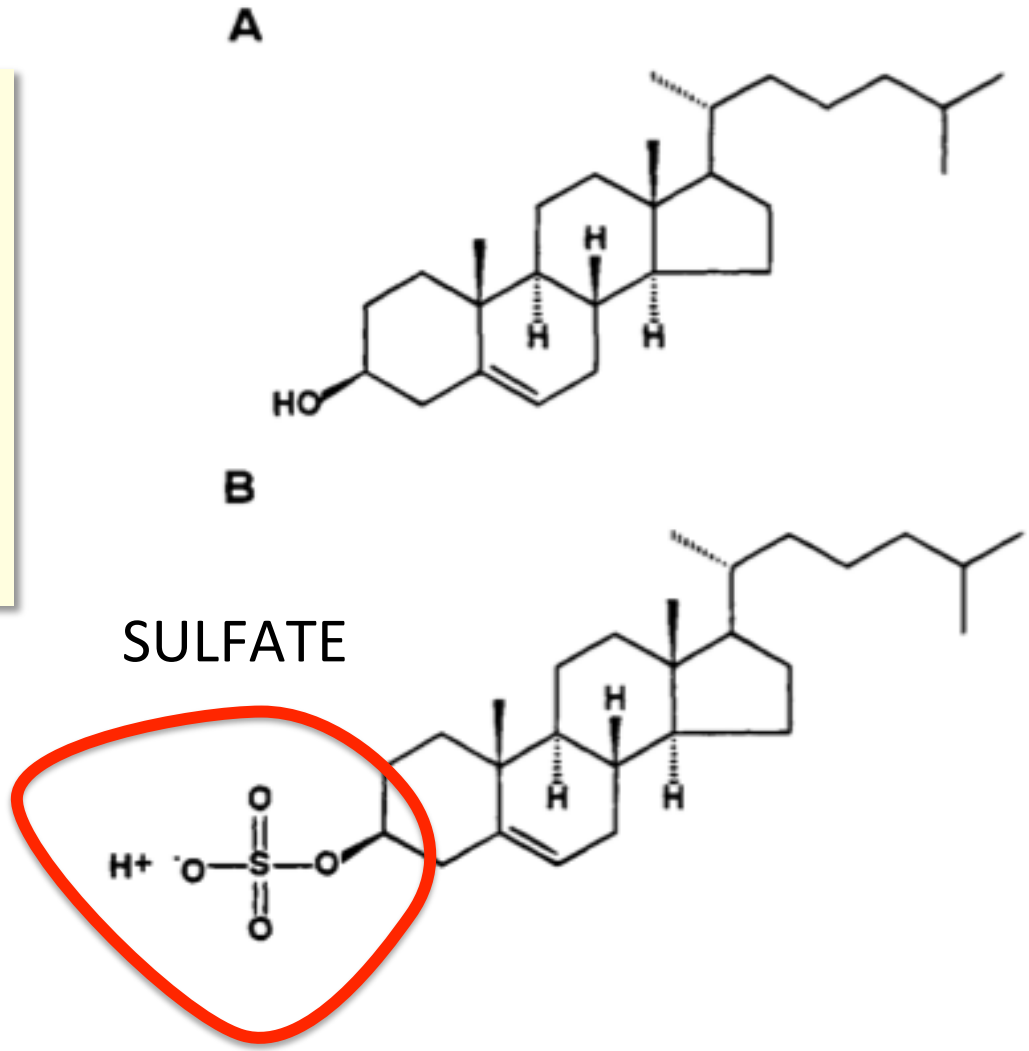
- Cholesterol sulfate supplies oxygen, sulfur, cholesterol, energy and negative charge to the tissues
- Sulfate is synthesized from sulfide in skin and blood stream utilizing energy in sunlight
  - Protects from UV damage and keeps microbes out
- Endothelial Nitric Oxide Synthase (eNOS) performs the magic



The skin is a solar powered battery!

# Cholesterol and Cholesterol Sulfate

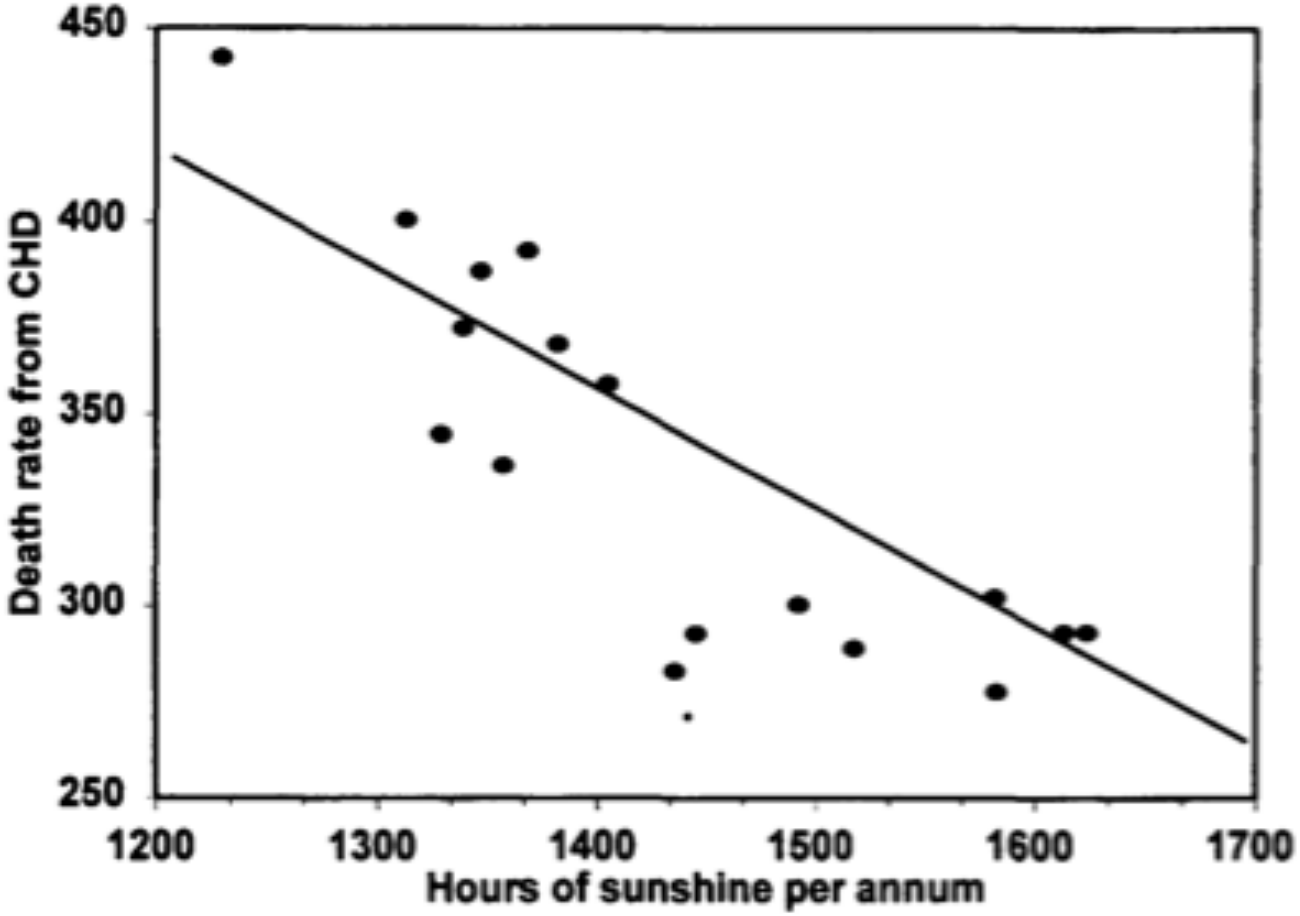
Sulfation makes cholesterol water-soluble and therefore much easier to transport



# Think about Sulfate!

- Cells in the skin produce vitamin D3 *sulfate* upon exposure to the sun
  - The precursor to vitamin D3 is cholesterol
- Cells also produce an abundance of *cholesterol* sulfate
  - I believe this is the more important molecule!
- Many of the alleged benefits of vitamin D3 are actually benefits of cholesterol sulfate
  - Protection against cancer, diabetes and cardiovascular disease; improved immune function

# Heart Disease Mortality and Sunlight*




*Grimes et al., Q. J. Med. 1996; 89:579-589

# Vitamin D3 Supplements are Not the Answer!!*


- 151 people w/ diagnosed vitamin D deficiency and heart disease risk profile
- 50,000 IU vitamin D3 supplement for 8 weeks
- Negative results
  - Increased serum calcium levels
  - Reduced serum parathyroid hormone levels
  - Increased serum LDL levels

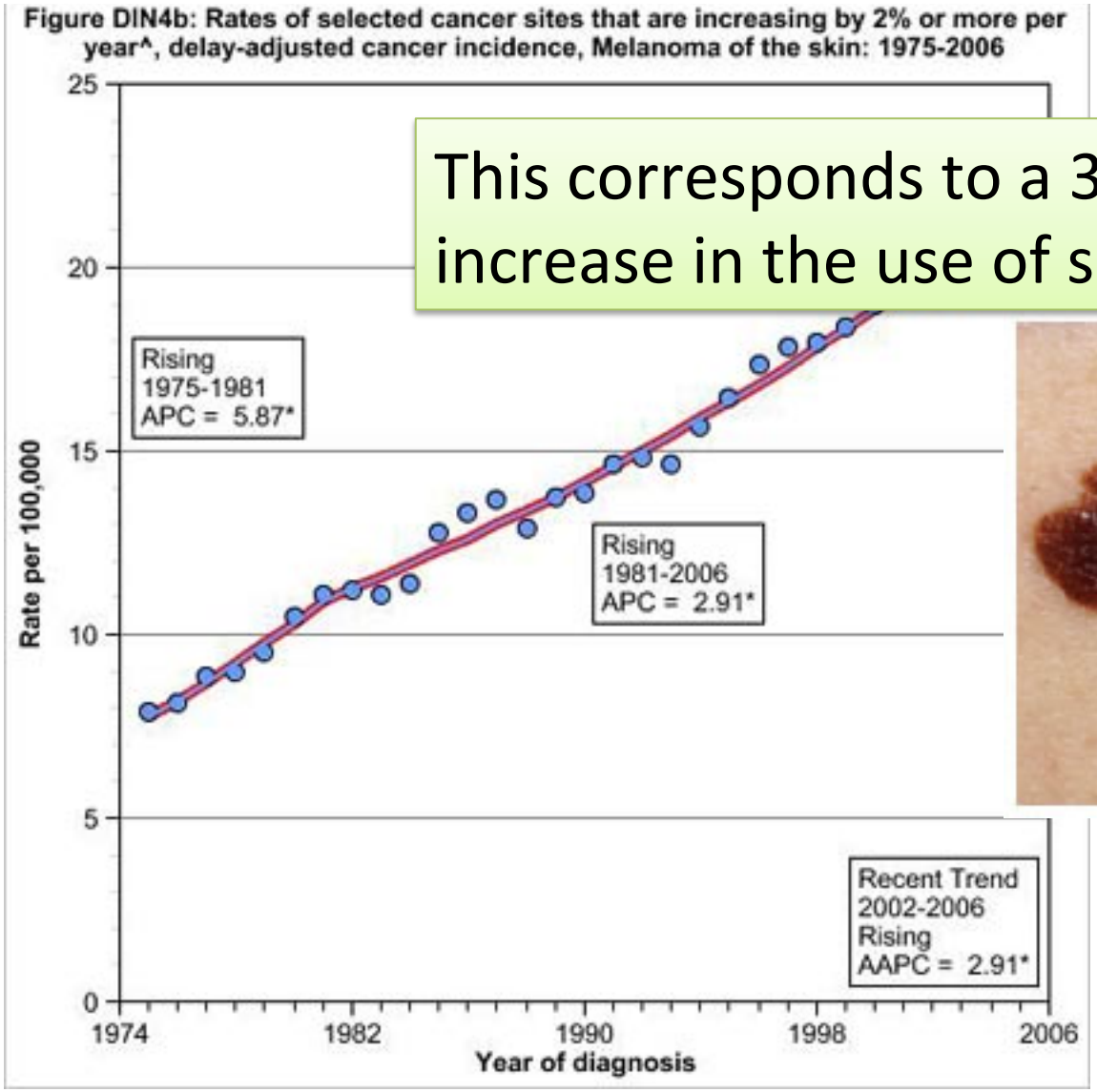
* P.P. Manish et al., Arteriosclerosis, Thrombosis and Vascular Biology, Epub, 2012 http://www.medpagetoday.com/Cardiology/Dyslipidemia/34561.

# Ultraviolet Exposure and Mortality among Women in Sweden*

- 38,472 Women selected in 1991-1992, aged 30-49
  - monitored for 15 years
- Questionnaire asked about frequency of sunbathing vacations and sunburn
  - *Increased* sunburn frequency associated with *reduced* all-cause mortality
  - Sunbathing vacations more than once a year *reduced* risk to cardiovascular disease and mortality

* Yang et al., Cancer Epidemiol Biomarkers Prev. 20(4):683-690, 2011

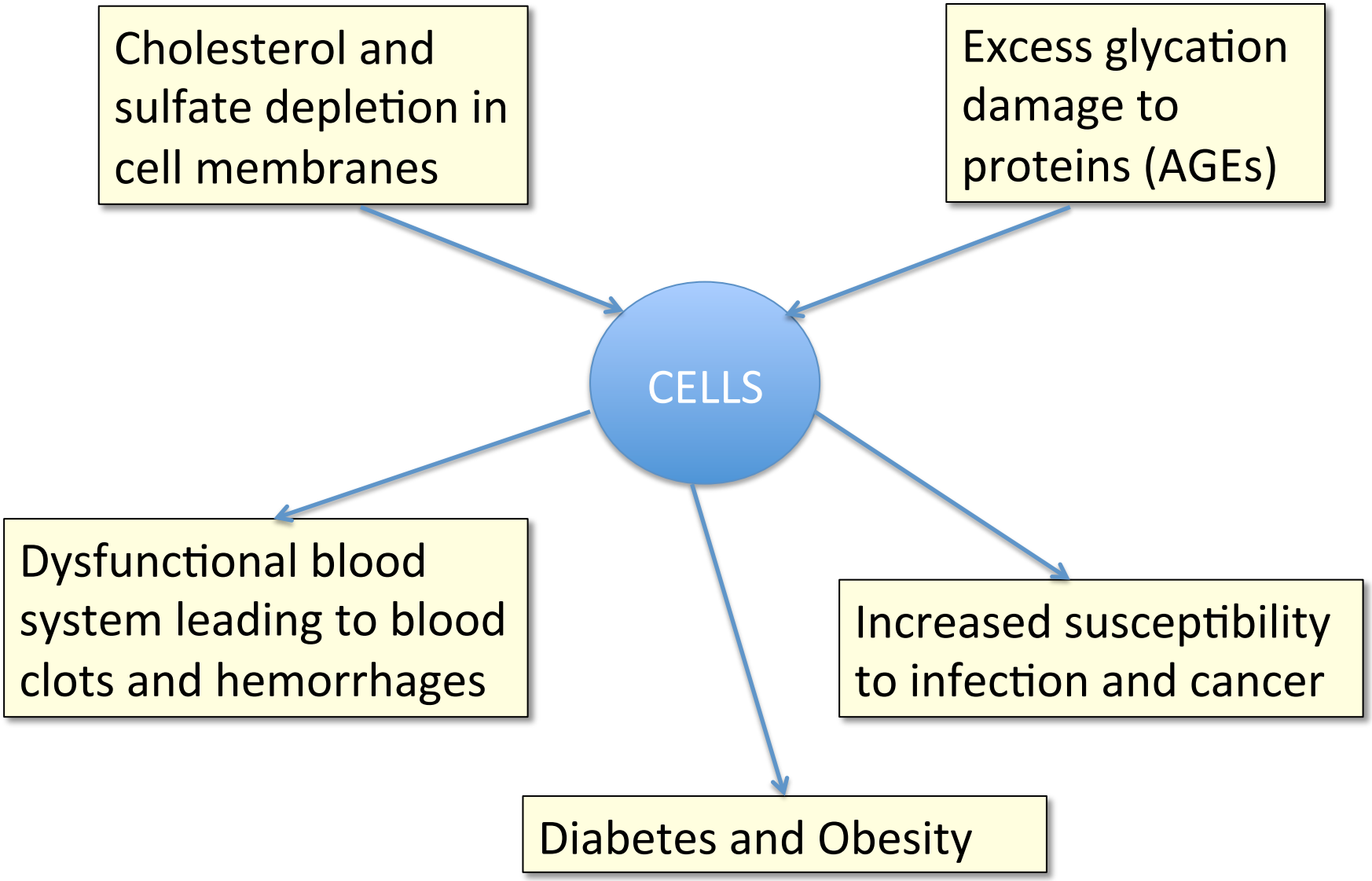
# Skin Melanoma Increasing 2%/Yr since 1974*



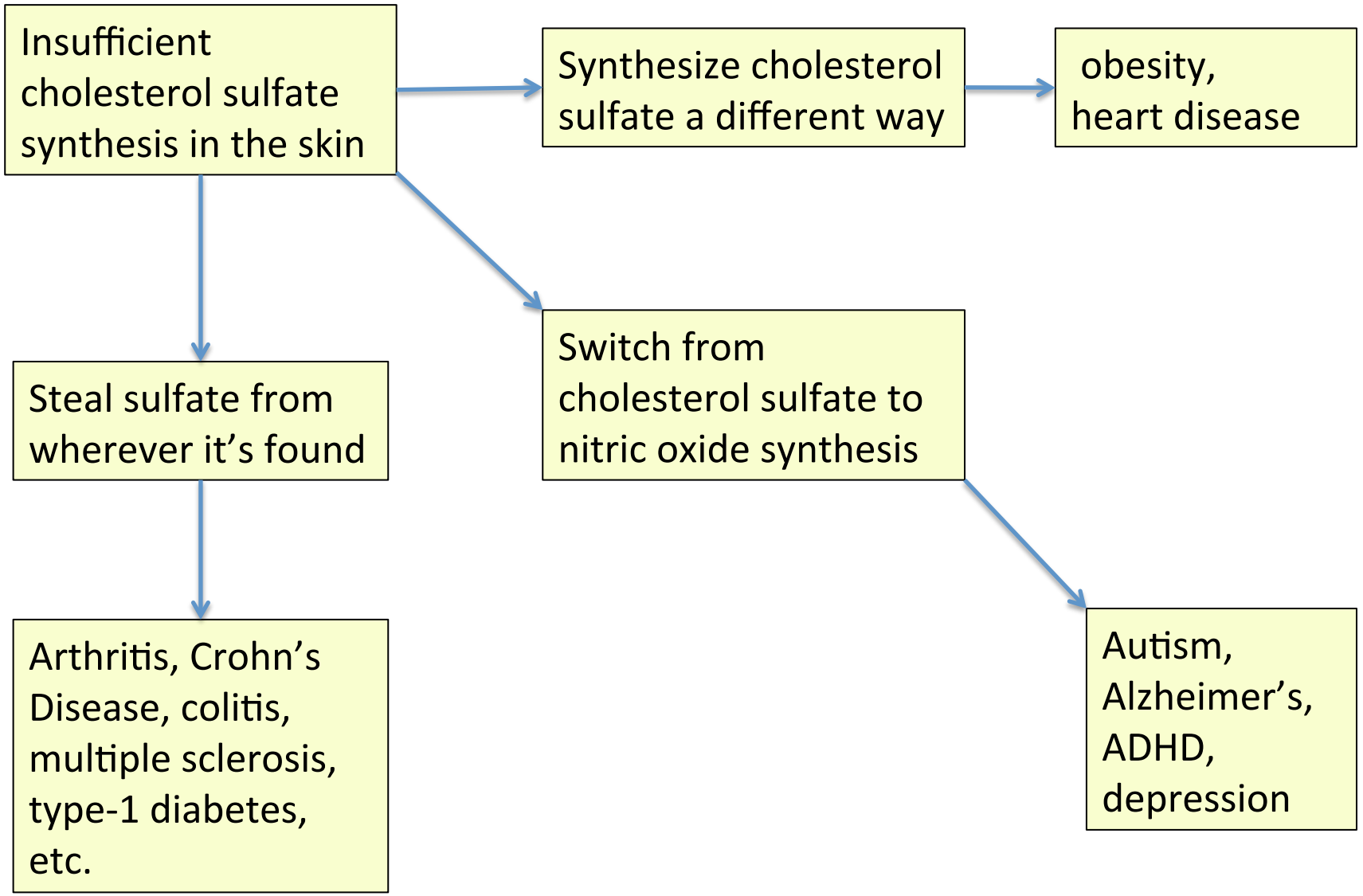

This corresponds to a 30-fold increase in the use of sunscreen

* Andrew Schneider, Aol News, May 24, 2010
aolnews.com/2010/05/24/study-many-sunscreens-may-be-accelerating-cancer

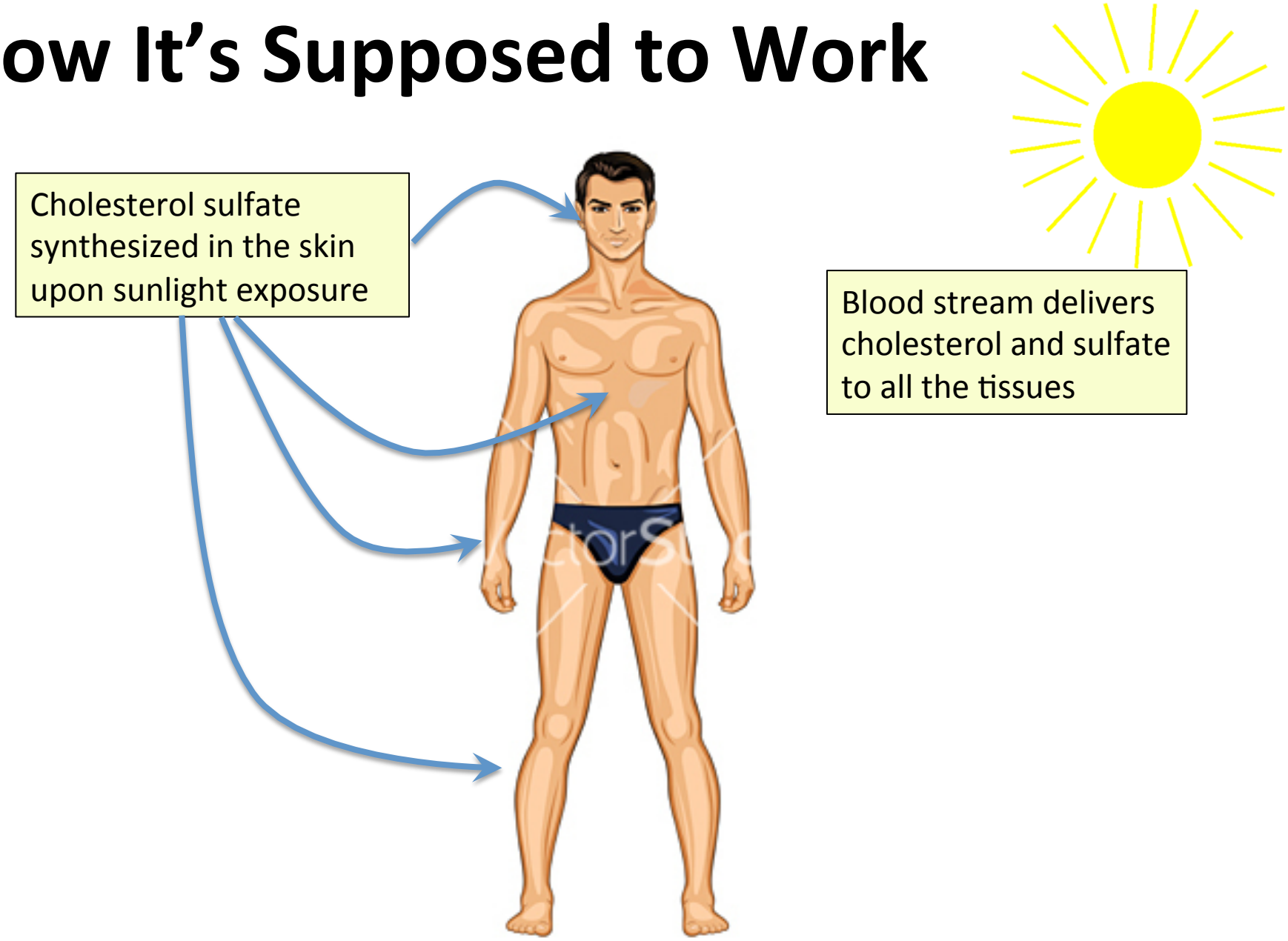
# A Biological Definition of Aging



# Several Roads to Hell



# How It's Supposed to Work



# How It Works Instead

Aggressive sun protection prevents skin from producing cholesterol sulfate


Cholesterol sulfate is synthesized in the atherosclerotic plaque instead (from homocysteine and LDL)

# And/Or …..

Sulfate is stolen from whatever tissue is willing to give it up!

From the brain
Alzheimer's, Multiple Sclerosis


From the gut
(Crohn's disease, colitis)

From the pancreas
(diabetes, pancreatic cancer)

From the joints (arthritis)

# And/Or …..

The body gives up on sulfate supply and confronts blood instabilities


Blood clots

hemorrhaging

Sudden cardiac arrest

Nitric oxide poisoning

# Cholesterol Transport

HDL is “good” cholesterol and LDL is “bad” cholesterol – true or false?

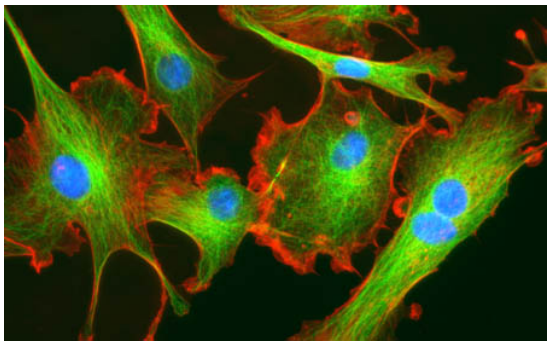
FALSE!

Neither HDL nor LDL is “cholesterol”!

Both HDL and LDL are particles that transport cholesterol, fat, fat-soluble vitamins and antioxidants to all tissues

# Some Facts and Speculation

- Fact: Fibroblasts in the skin are major suppliers of cholesterol to HDL
  - Speculation: they depend upon sulfation for export of cholesterol to HDL

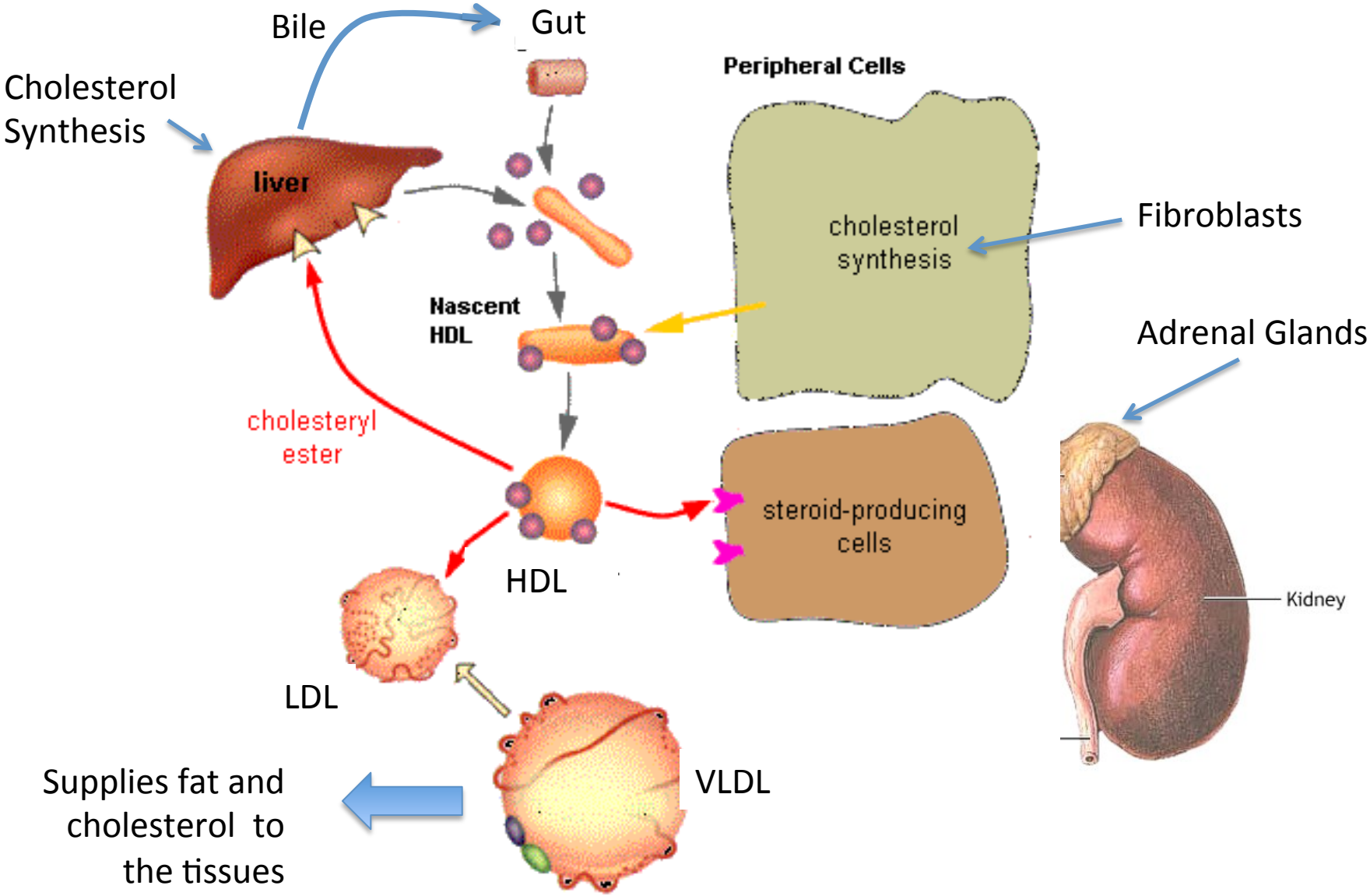


- Fact: Cholesterol sulfate is synthesized in large quantities in the skin upon sunlight exposure
  - Speculation: this is a major supplier of cholesterol and sulfate to the tissues
  - Reduces need for serum LDL

# HDL, VLDL, LDL, Chylomicron

- HDL: Liver produces “nascent” particle; cholesterol-synthesizing cells (fibroblasts) fill it up
- VLDL: Liver produces large particles; carry fat, cholesterol, fat-soluble vitamins and antioxidants
- LDL: “remnant” after VLDL has delivered its goods
- Chylomicron: HUGE particle produced in gut to deliver ingested fats and cholesterol to the tissues (mainly heart, liver and muscles)

# Cholesterol Transport



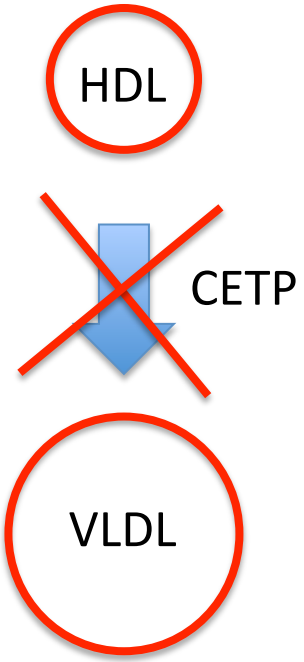

VLDL

HDL is like a rowboat that can go in to shore to pick up goods and deliver them to the ocean liner, which is the VLDL particle

HDL

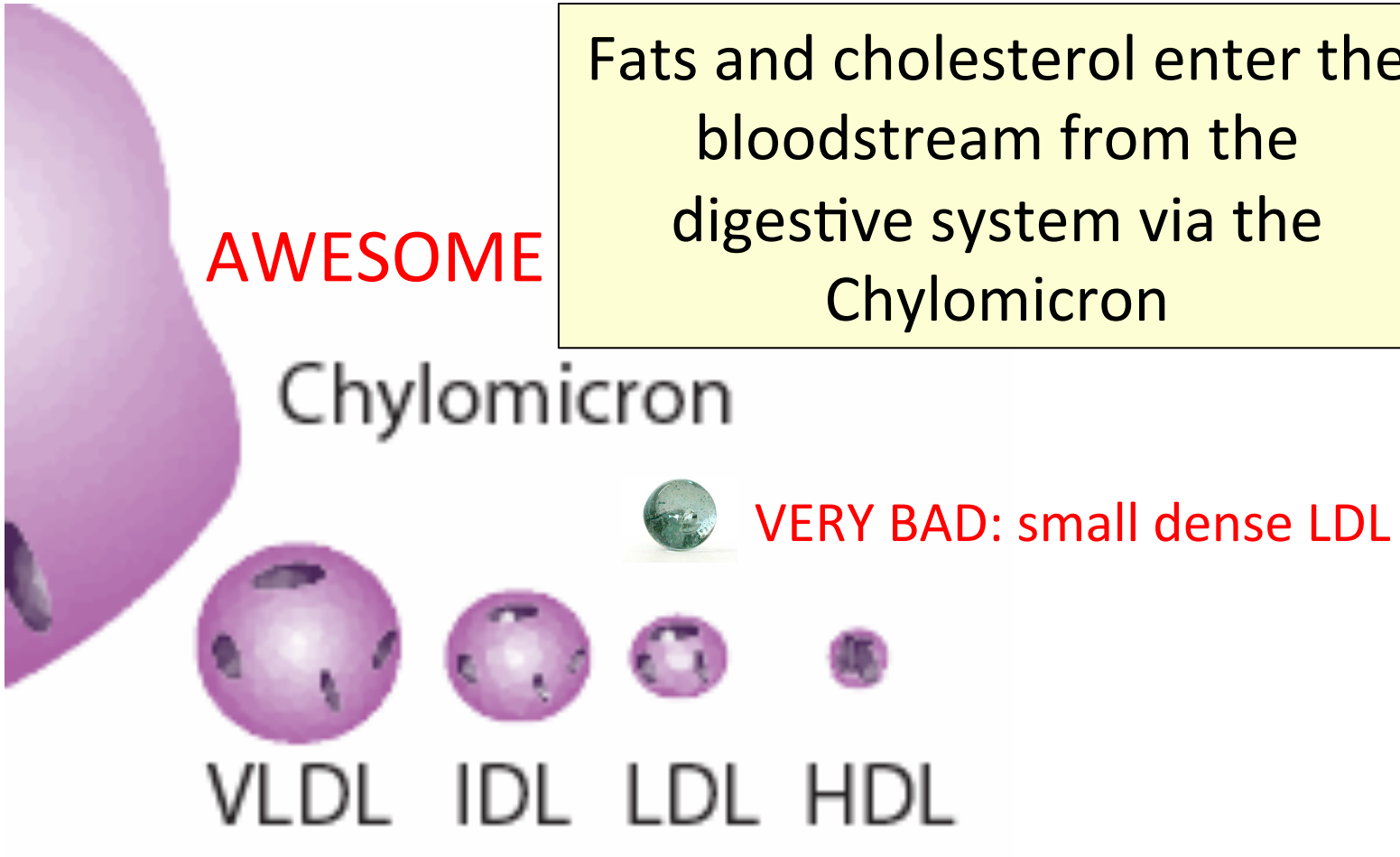
# Cholesterol Ester Transport (CEPTP) Inhibitors*

- This is a new class of drugs that raises HDL dramatically
  - It does so by trapping cholesterol in the HDL particle
- These drugs have failed miserably in trials
  - Problem is that they prevent HDL from doing its job: delivering cholesterol from the cells in the skin that make it to the VLDL particles

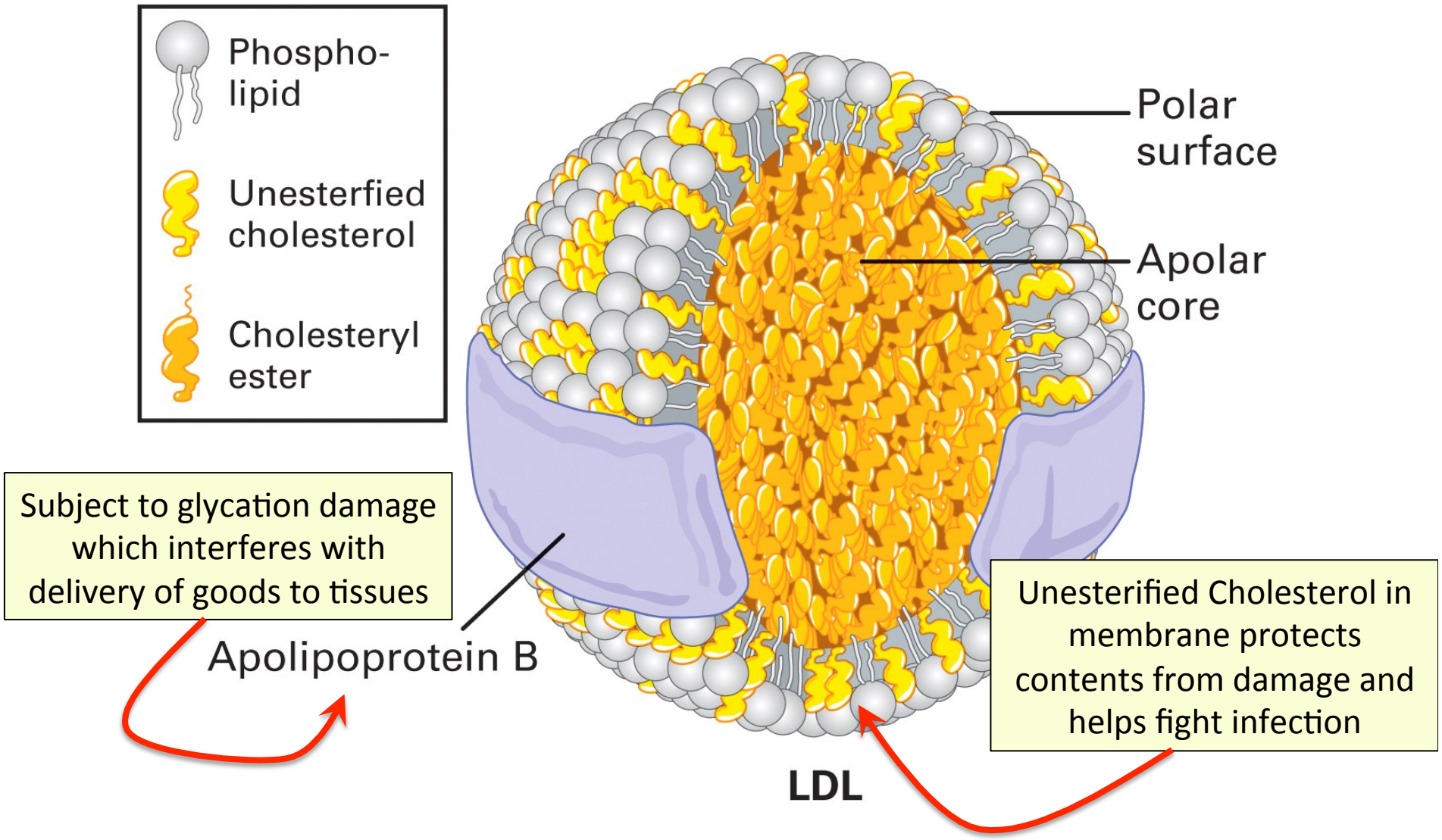



* Barkowski and Frishman, Cardiol Rev. 2008 16(3):154-62.

# Sizes of Lipoprotein Particles in Blood Serum

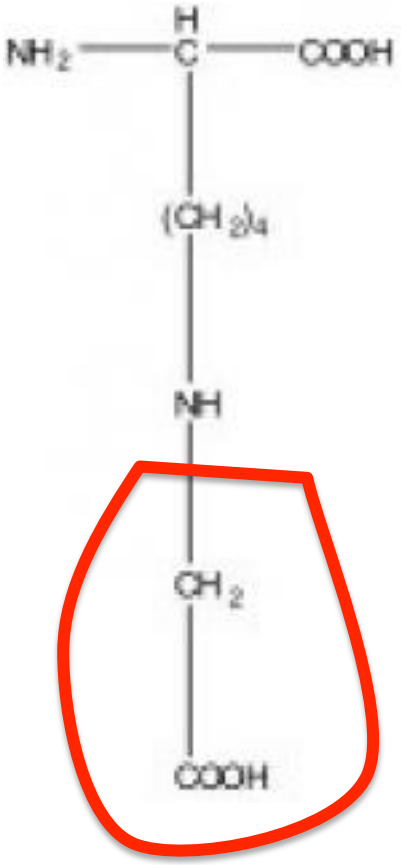


# Structure of LDL

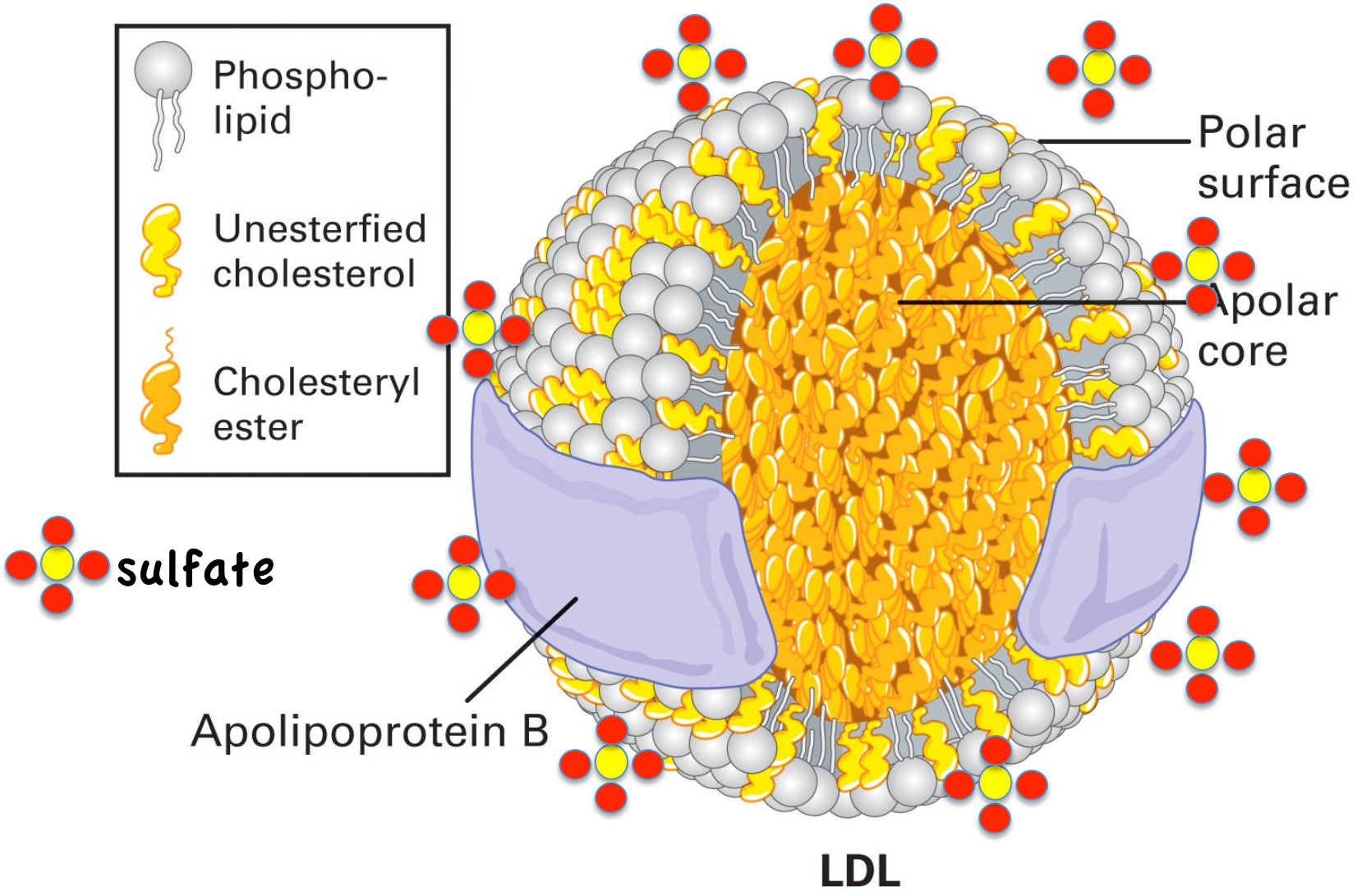


# Glycation Damage

- Due to excess sugars in the blood stream
  - Especially high fructose corn syrup
- Messes up cholesterol transport
  - Glycated LDL is less efficient at delivering cholesterol to tissues
  - Glycated LDL can't be recycled through receptors in liver (LDLR)
  - Builds up in blood serum as "small dense particles" (The bad kind)
- Cells become deficient in cholesterol

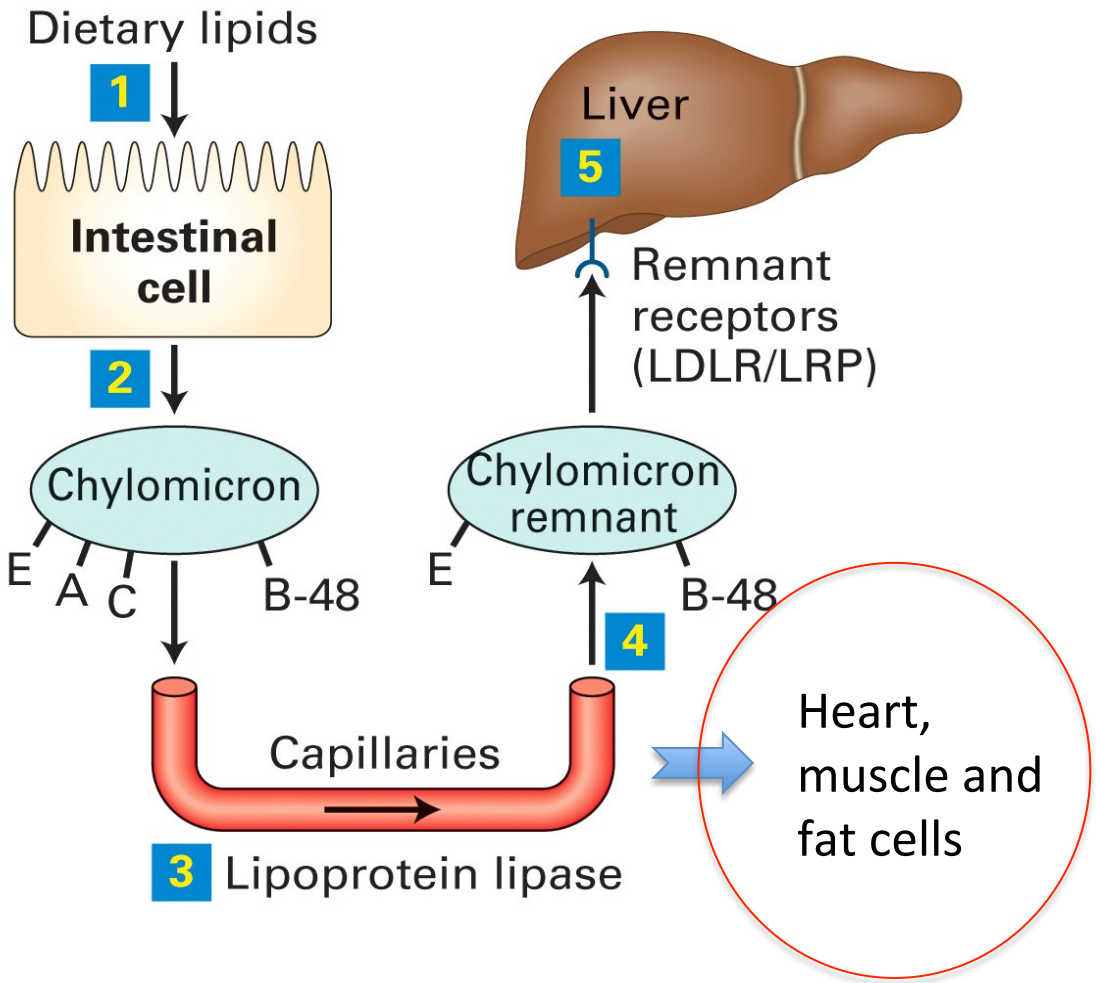


# Cholesterol Sulfate Protects LDL*




* E.H. Epstein et al., Science, 214(4521, 659-660, Nov. 6 1981

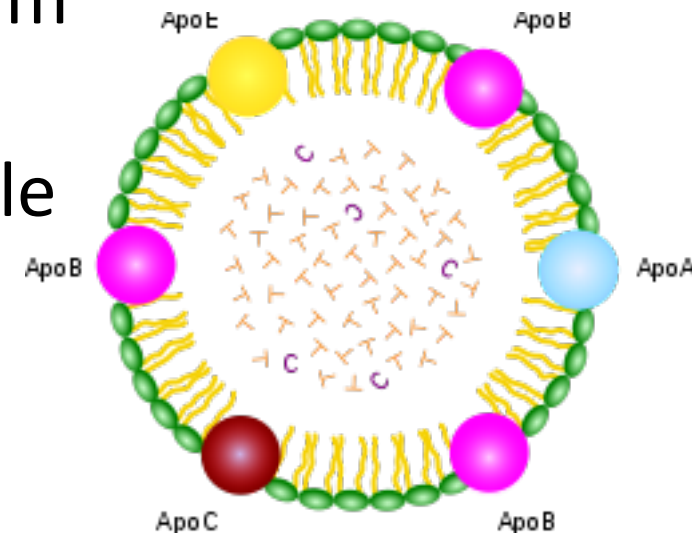
# The Life Cycle of the Chylomicron



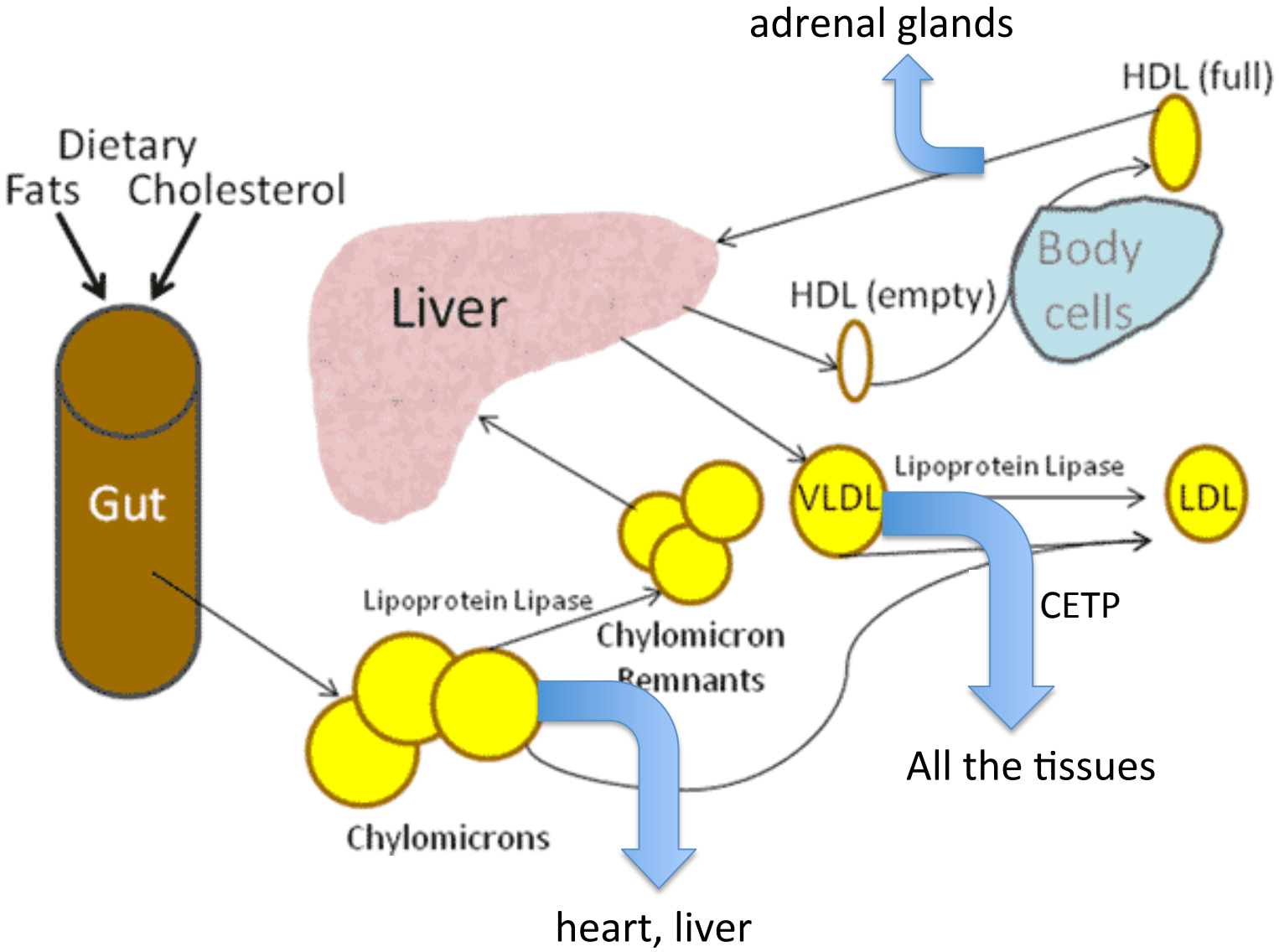

- Triglyceride composition closely resembles dietary intake

# Dietary Cholesterol

- Dietary fat and cholesterol enter the lymph system directly from the gut and exit into the blood stream at the subclavian vein
- They travel in a large lipid particle called a chylomicron
- It's a short path from this major vein to the heart
- Conclusion: the heart gets first dibs on dietary cholesterol and fat



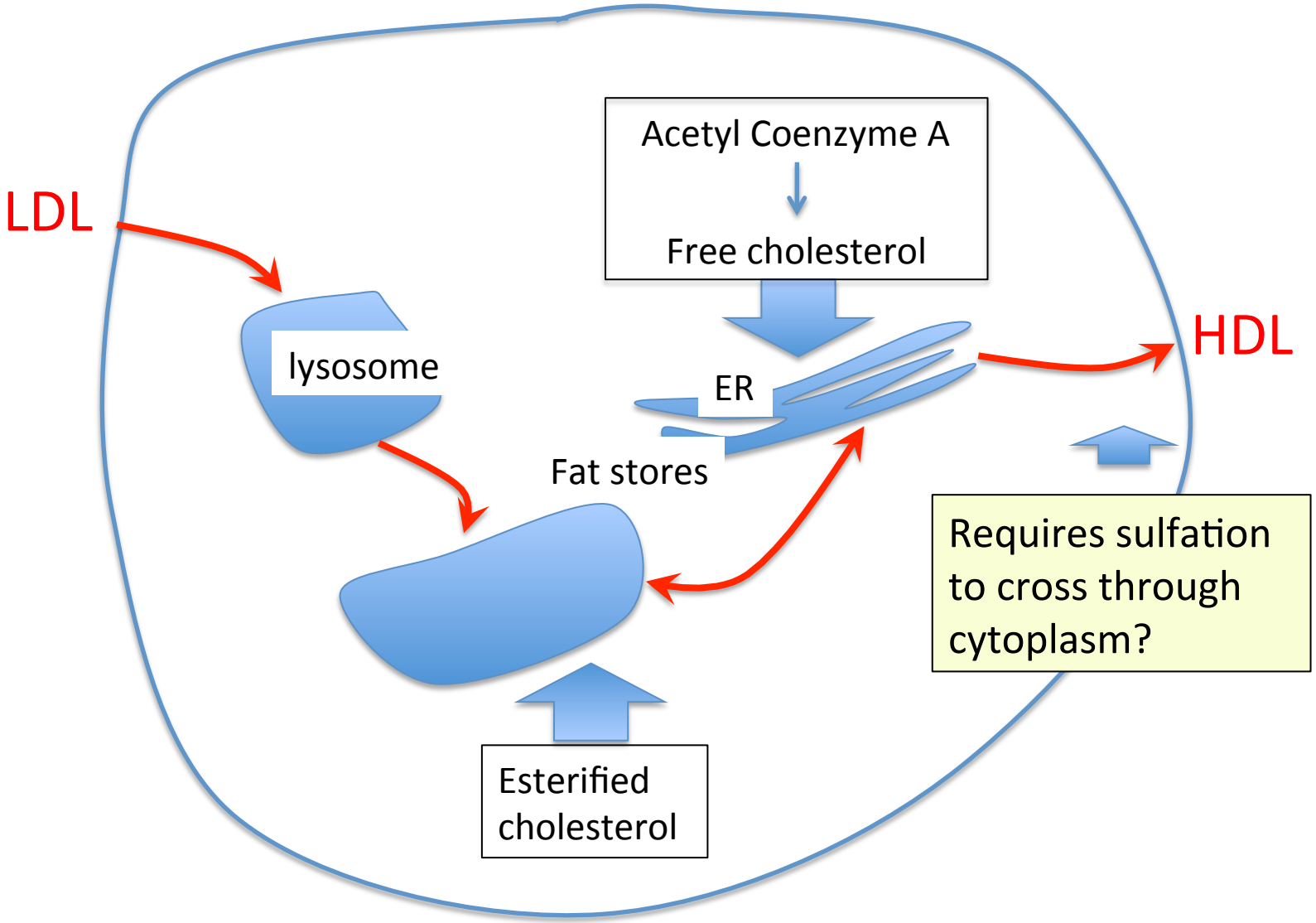
# Cholesterol Metabolism



# Cholesterol Transport to HDL

- Cells in the skin are major producers of cholesterol sulfate and major suppliers of cholesterol to HDL-A1
- Cells synthesize cholesterol in Endoplasmic Reticulum (ER)
- Transport from ER to cell membrane
  - Is not vesicular
  - Ergo, requires migration through cytoplasm
  - Proposal: sulfation of cholesterol necessary step in transport (renders molecule water-soluble)

# Cholesterol Management in the Cell*

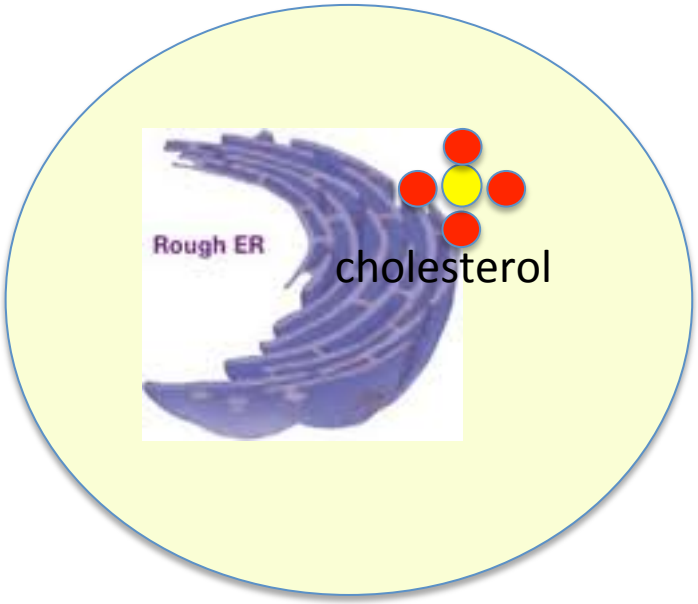



* E.M.L. Bastiaanse, Cardiovascular Research 33, 272–283, 1997

# Sulfate and Cholesterol Transport

- Researchers are puzzled as to how cholesterol is transported from the ER, where it is synthesized, to the cell membrane
  - Cholesterol's insolubility in water is problematic
- I propose that the solution is to add sulfate to make the molecule water-soluble
  - This allows cholesterol (as cholesterol sulfate) to move freely through the cytoplasm
  - This is how synthesized cholesterol makes it to the cell membrane AND how it gets from the membrane to HDL, the particle that transports it in the blood stream

# Sulfate Gives Cholesterol Free Passage!




HDL

Low cholesterol content in HDL is the strongest lipid predictor of heart disease

# Cholesterol Sulfate Deficiency Explains Autistic Correlates

- Autism is associated with several health issues:
  - Eczema, asthma, digestive problems (leaky gut)
  - Increased susceptibility to infection
- Cholesterol sulfate in skin stimulates filaggrin synthesis*
  - Filaggrin deficiency leads to eczema and asthma
- Sulfate protects cells in blood from bacteria
- Sulfate deficiency leads to leaky gut syndrome **

* Nakae et al. The FASEB Journal 22:782.2, 2008

** Waring and Klovrza, J Nutr & Environ Medicine 10, 25-32, 2000.

# Recent Papers

"Might cholesterol sulfate deficiency contribute to the development of autistic spectrum disorder?"

Stephanie Seneff, Robert Davidson, Luca Mascitelli

*Medical Hypotheses* 2012, 78, 213–217

"Impaired Sulfate Metabolism and Epigenetics: Is There a Link in Autism?"

Samantha Hartzell and Stephanie Seneff
*Entropy* 2012, *14*, 1953-1977

# Recapitulation

- HDL, LDL, and chylomicron provide cholesterol and fat to all the tissues
  - Chylomicron supplies dietary cholesterol preferentially to the heart
- High serum LDL is an indication of insufficient sulfate and excess glycation damage
- Sulfate is essential for export of cholesterol to HDL particles
- Cholesterol sulfate deficiency can explain autism

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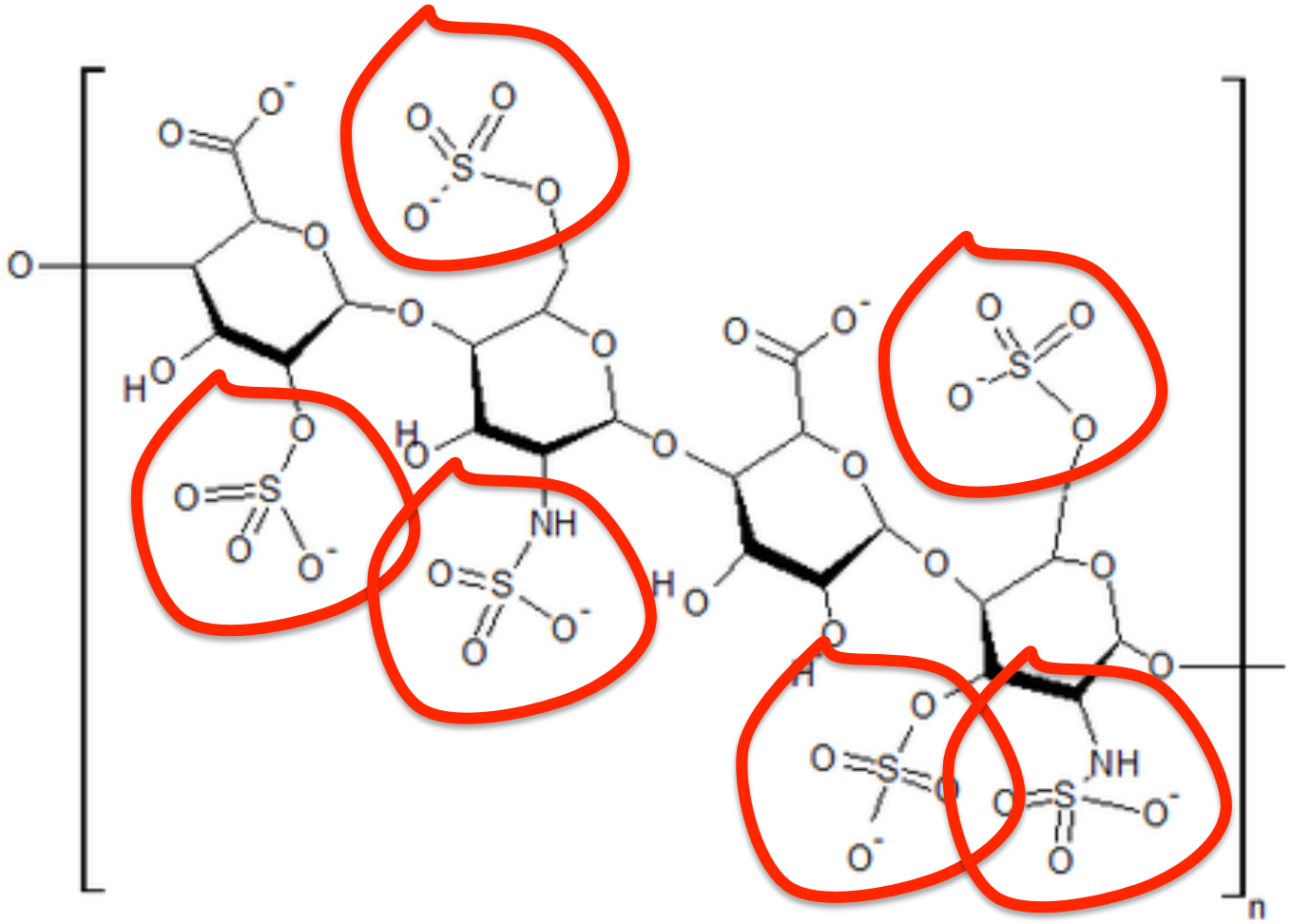
# Sulfate Deficiency

The body depends upon heparan sulfate proteoglycans (HSPGs) as a temporary storage bin for glucose

Impaired sulfate synthesis in the skin disrupts this process and leads to diabetes

# Heparan Sulfate Proteoglycans (HSPGs)

# Heparan Sulfate: Wonder Worker



Polymers of  sugars with attached nitrogen and sulfates: safe glucose storage

# A Provocative Hypothesis

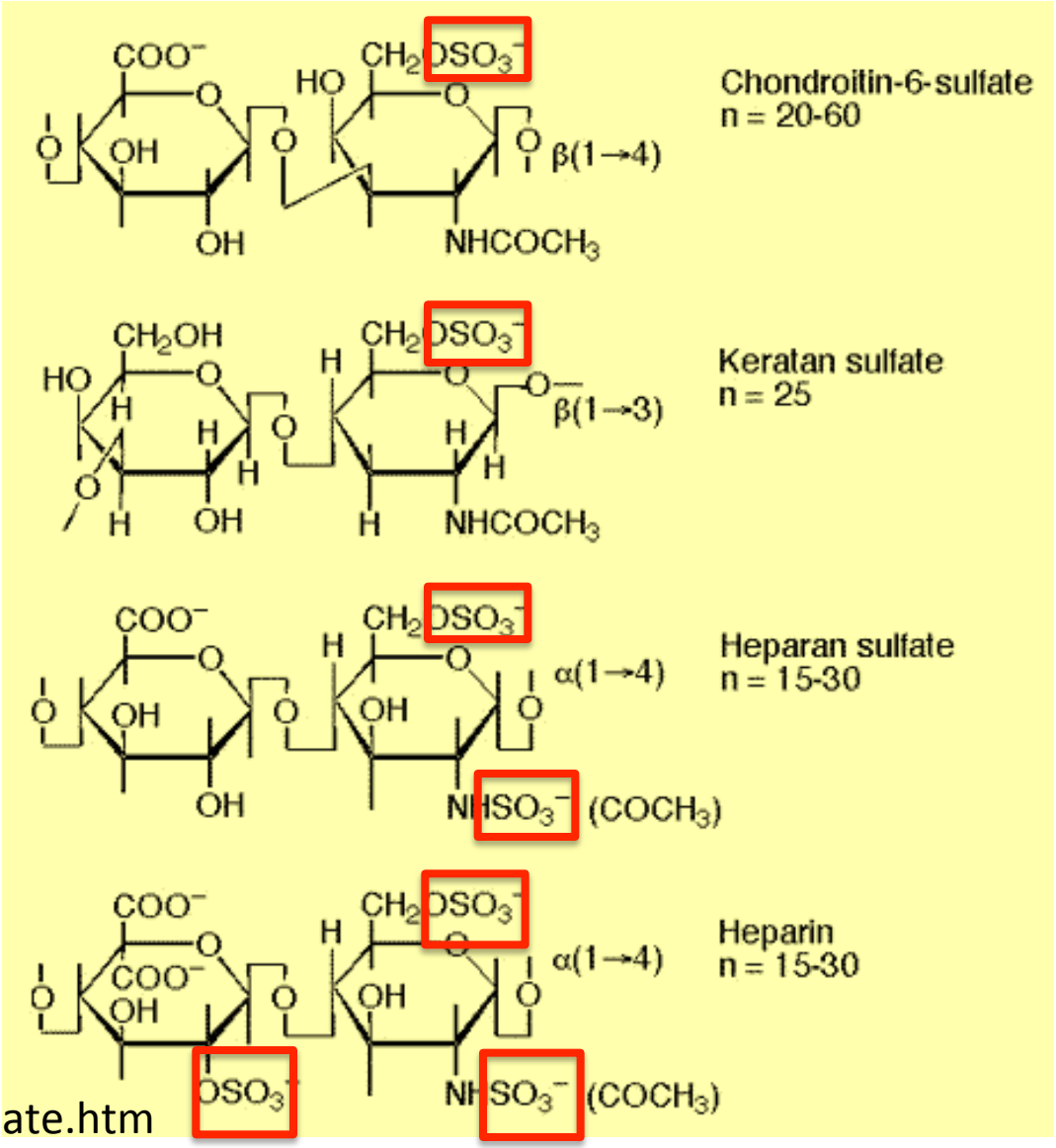
When cholesterol and sulfate are in short supply, storage of sugars in HSPGs becomes impaired

Cells store glucose temporarily in HSPGs, protected by sulfate

This is the source of insulin resistance and diabetes!

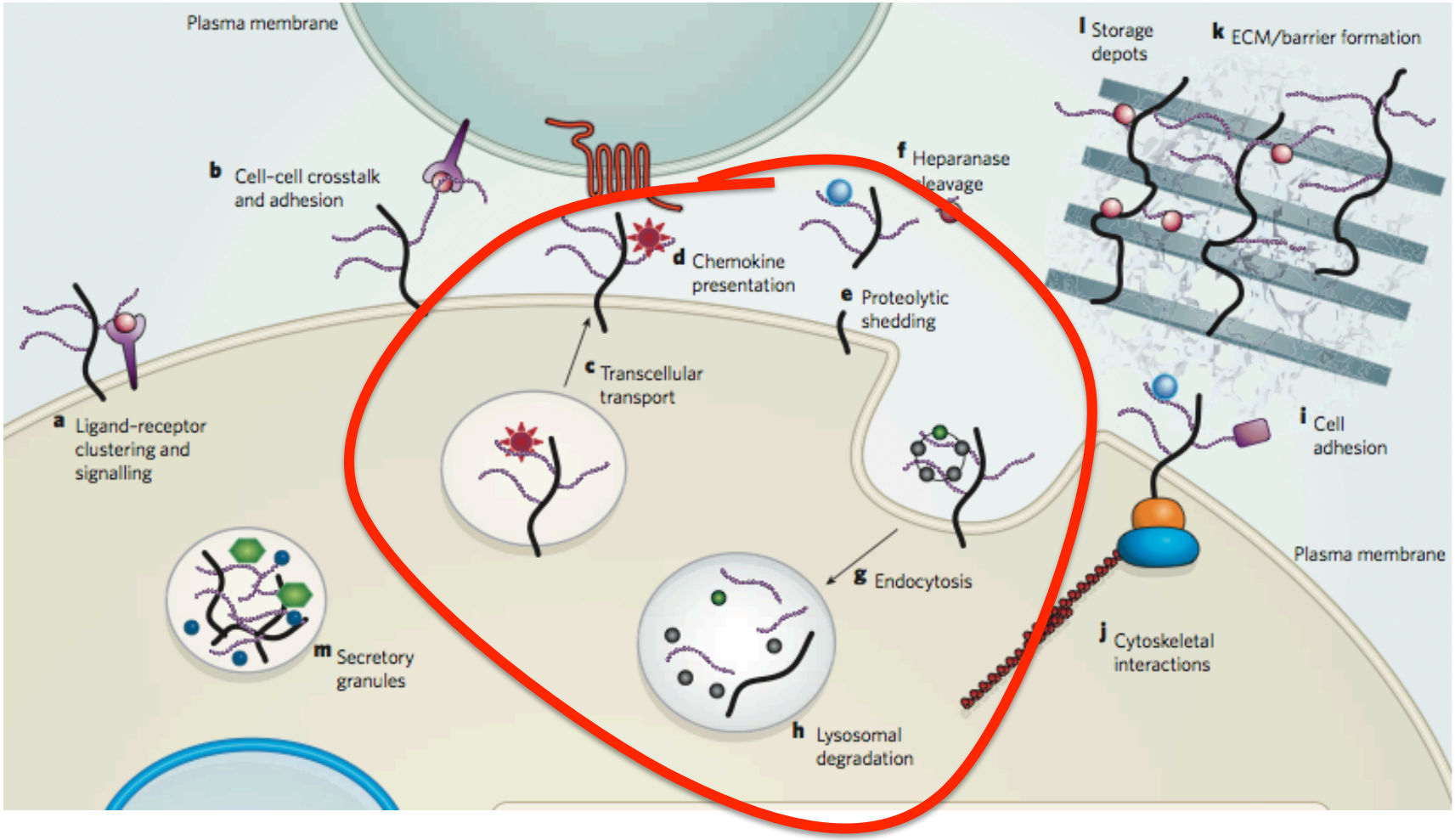
# Sulfated Glycosaminoglycans (GAGs)

- Prominent in extracellular matrix of all cells
- Amount of sulfate depends on availability
- Crucial for maintaining negative charge and protecting from infection




http://www.science-autism.org/sulphate.htm

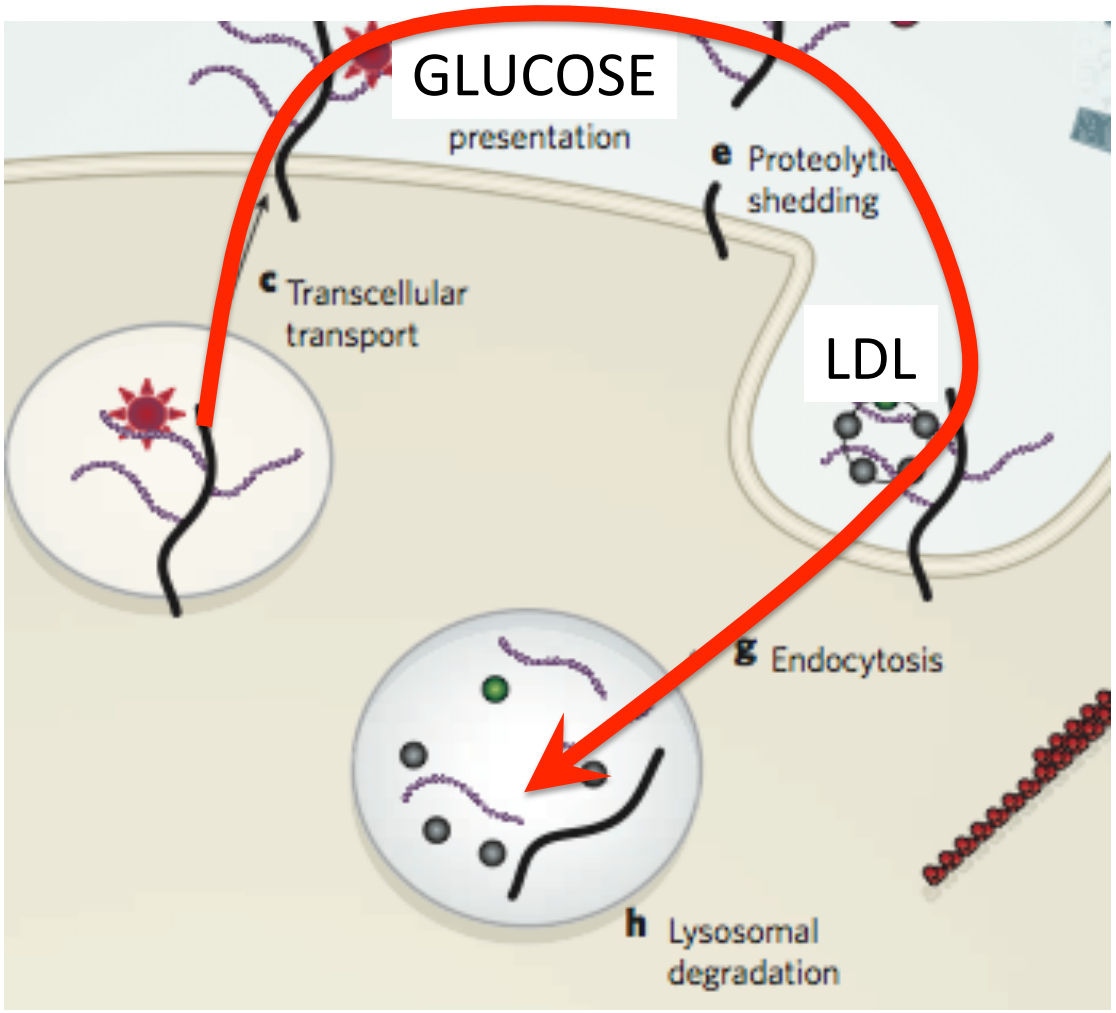
# Amazing Number of Roles for Heparan Sulfate in Cells*




* J.R. Bishop et al, Nature 446, 1030-1037, Apr. 2007

# Recycling HSPGs w/ LDL*

- Hypothesis: Sugars are temporarily housed in HSPGs
- Hypothesis: sulfate in HSPGs is critically needed to recycle LDL




* J.R. Bishop et al, Nature 446, 1030-1037, Apr. 2007

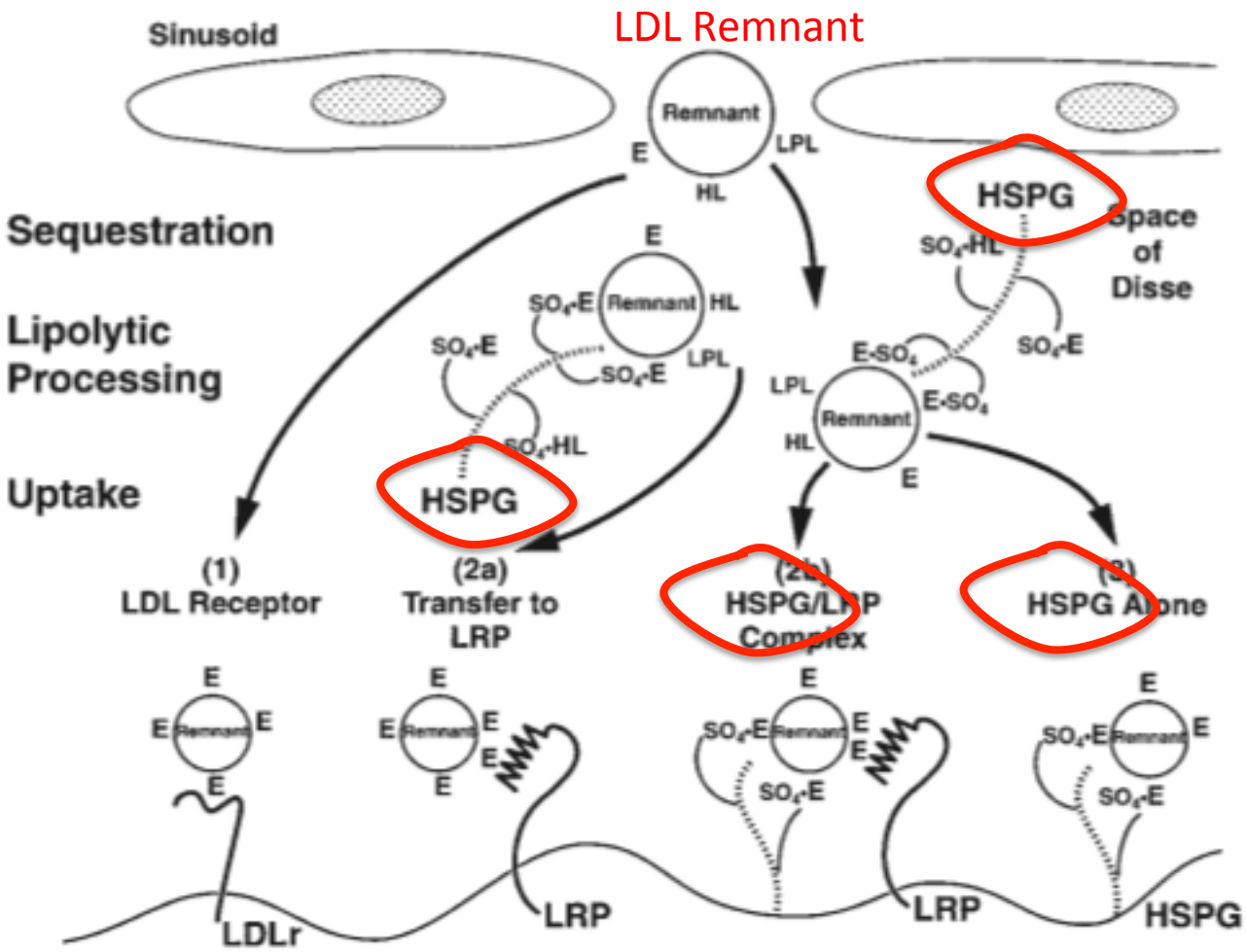
# LDL Uptake by Liver depends on Sulfate in Heparan Sulfate Proteoglycans (HSPGs)*

- LDL recycled mainly through uptake by liver
- This uptake depends critically on sulfate in HSPGs
- Insufficient HSPGs leads to elevated serum LDL

"Our studies have shown that HSPG participate not only in the initial sequestration step, but also in the uptake step, either in association with the LRP or acting alone as a receptor."

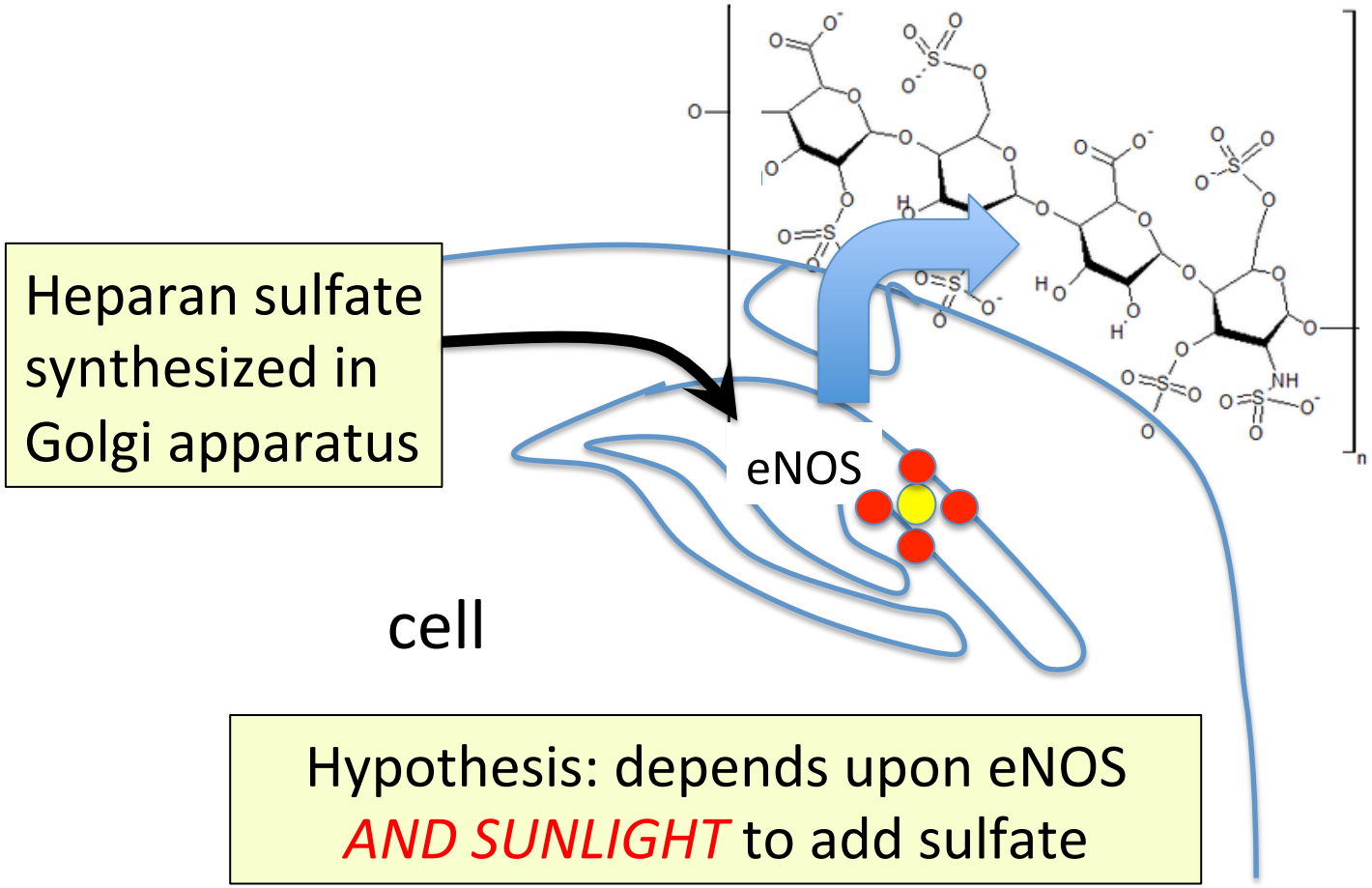
* Mahli and Ji, J. Lipid Research 40, 1999, 1-16.

# LDL Clearance Depends on HSPGs*




* Figure 1, in Mahli and Ji, J. Lipid Research 40, 1999, 1-16.

# Proposed Role of eNOS in Heparan Sulfate synthesis




Hypothesis: depends upon eNOS *AND SUNLIGHT* to add sulfate

# Recapitulation

- Heparan sulfate proteoglycans (HSPGs) are everywhere in the body
  - They play a crucial role in ion transport, nutrient uptake, and cell signalling
  - They are central in LDL reuptake and recycling
- I propose that they also provide a temporary storage depot for glucose
  - Deficient sulfate leads to diabetes
- I propose that eNOS provides the sulfate, catalyzed by sun exposure

# Outline

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  - Sulfate Deficiency
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  - SiNiC
- Consequences
  - Blood Clots and Hemorrhages
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  - Infection
  - Impaired Autophagy
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  - Environmental Toxins
  - Polyphenols
- Summary

# Obesity

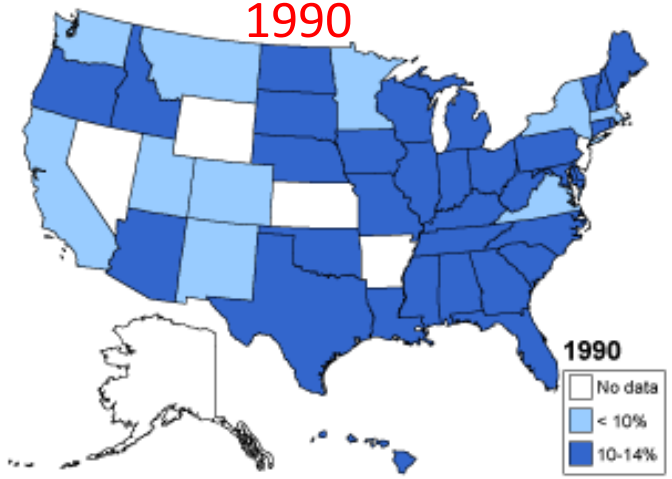
Insufficient HSPGs impairs muscles' ability to utilize glucose as a fuel

Fat cells insinuate themselves into the loop by transforming glucose to fat for later release to supply safe usable fuel to muscles

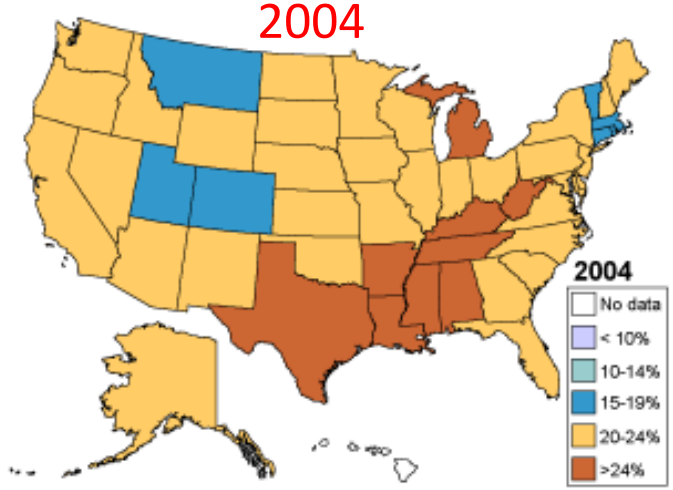
# Fat Cells to the Rescue

# The U.S. Obesity Epidemic*

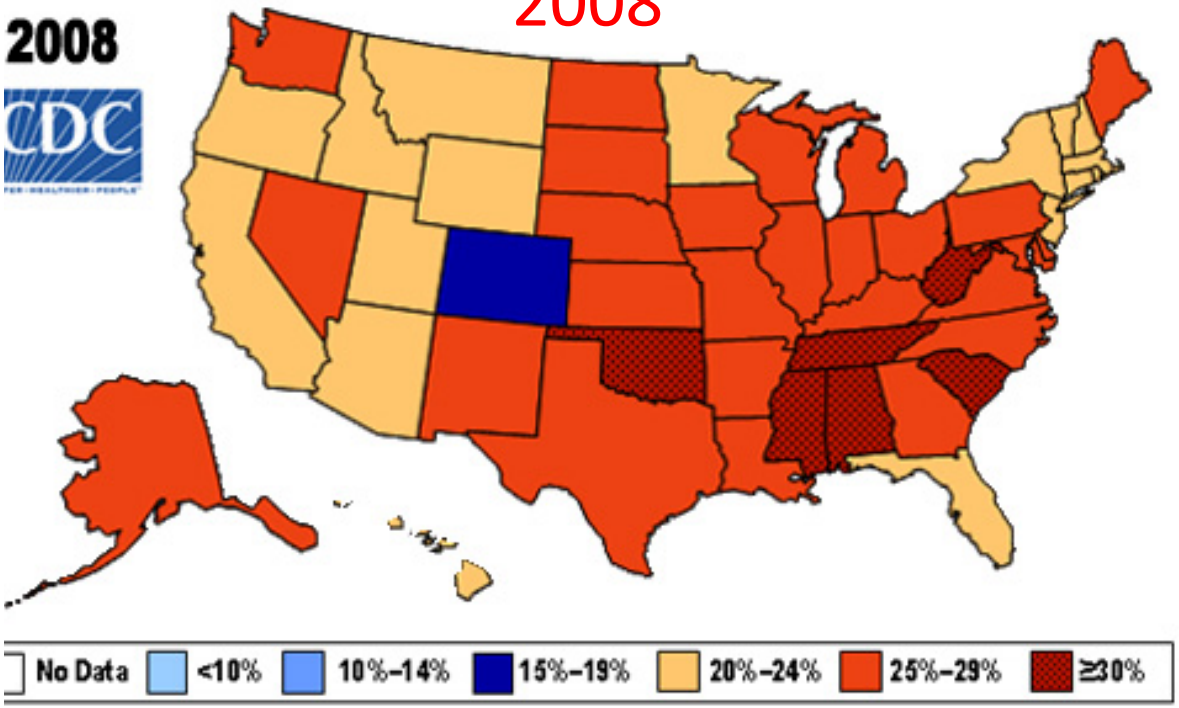
1990




2004




2008

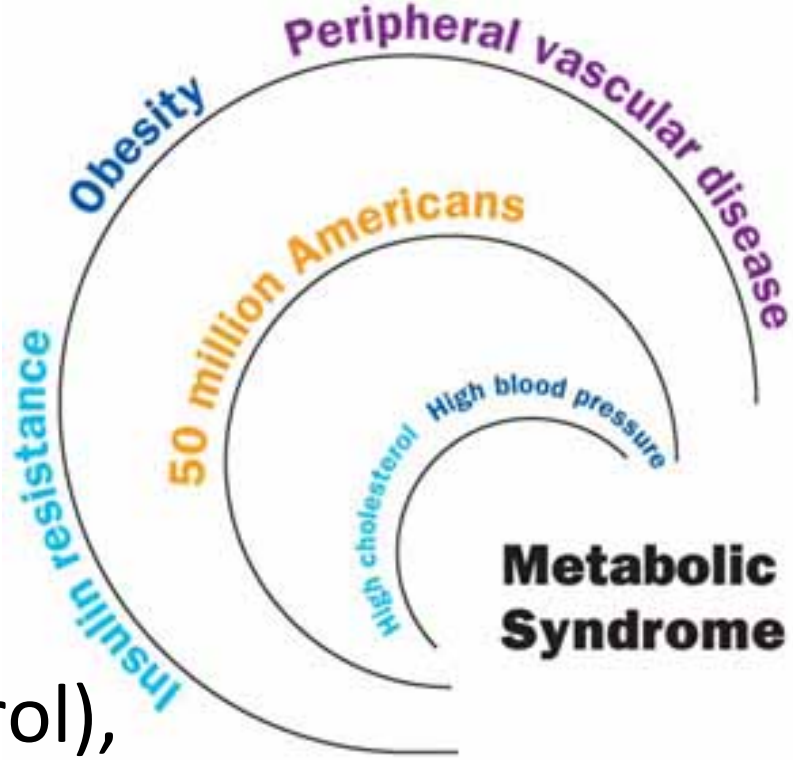



**WHY ARE WE GETTING FATTER AND FATTER?**

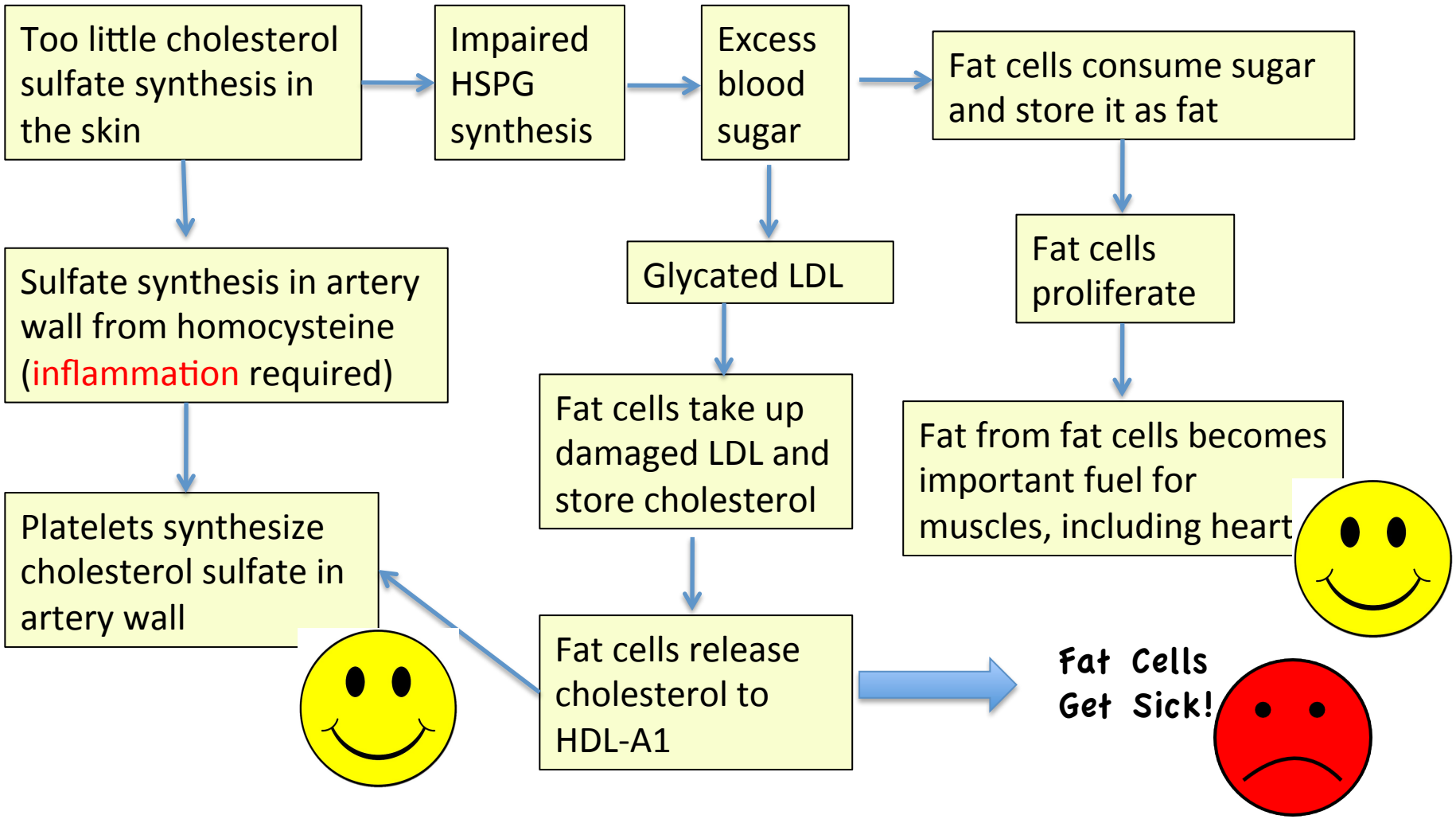
* Source: CDC Behavioral Risk Factor Surveillance System

# The Metabolic Syndrome

- Abdominal obesity
- Insulin resistance
- High blood pressure
- High serum triglycerides
- High LDL ("bad" cholesterol), especially “small dense LDL”
- Low HDL ("good" cholesterol)
- Increased risk to heart disease

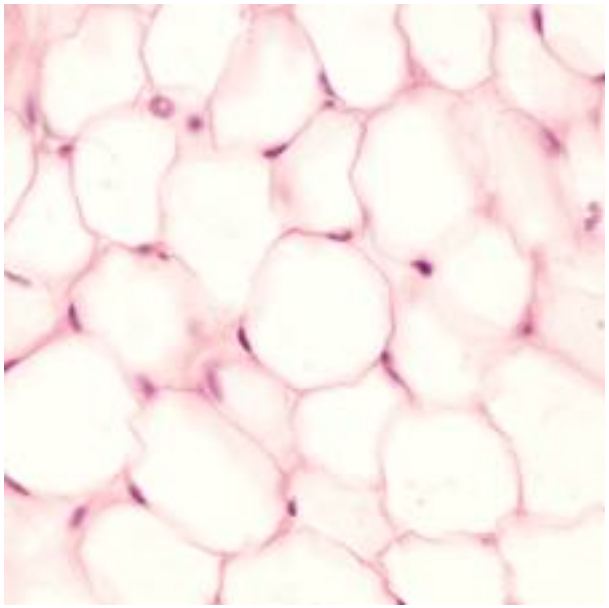


# The Path to Obesity and Heart Disease



# Fat Cells Get Sick!

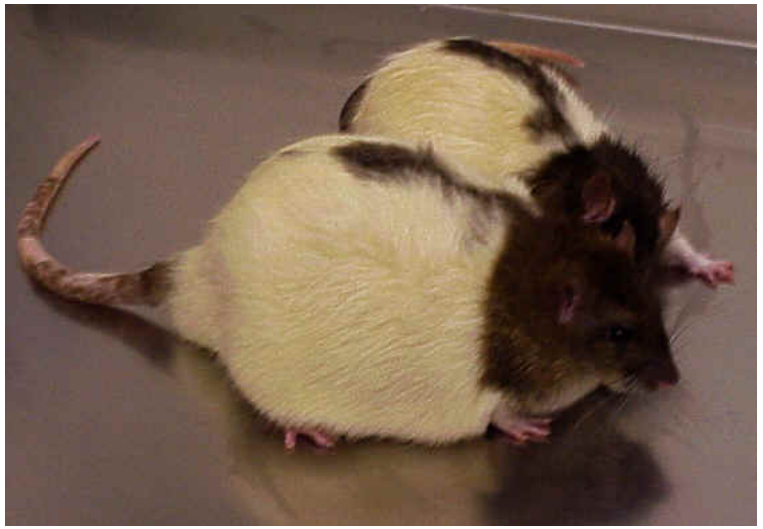
- Cholesterol depletion in membrane induces cholesterol synthesis and storage
- Fat cells need sulfate to release fat stores
- Too much cholesterol stuck inside the cell becomes problematic
- Endoplasmic reticulum has trouble folding proteins
- Fat cell calls in macrophages to orchestrate cell death and clean up the mess



# Experiment on Cholesterol Depletion in Fat Cells*

Normal fat cells from rats were exposed in vitro to MβCD

- Depleted cholesterol from cell walls
- Caused activation of cholesterol-acquiring proteins
- Caused insulin resistance and increased synthesis of TNF-α, angiotensinogen, and IL-6
  - Related to hypertension, inflammation, and atherosclerosis



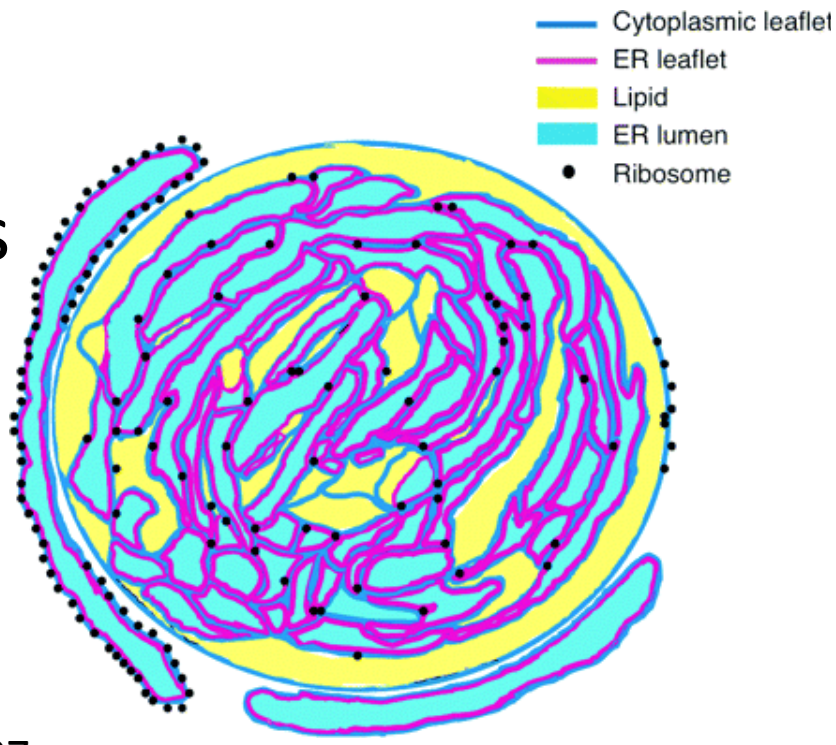
Conclusion:

fat cell membrane cholesterol depletion is key manifestation of metabolic syndrome

* Soazig Le Lay et al., J. Biol. Chem., 2001

# Endoplasmic Reticulum Becomes Sick

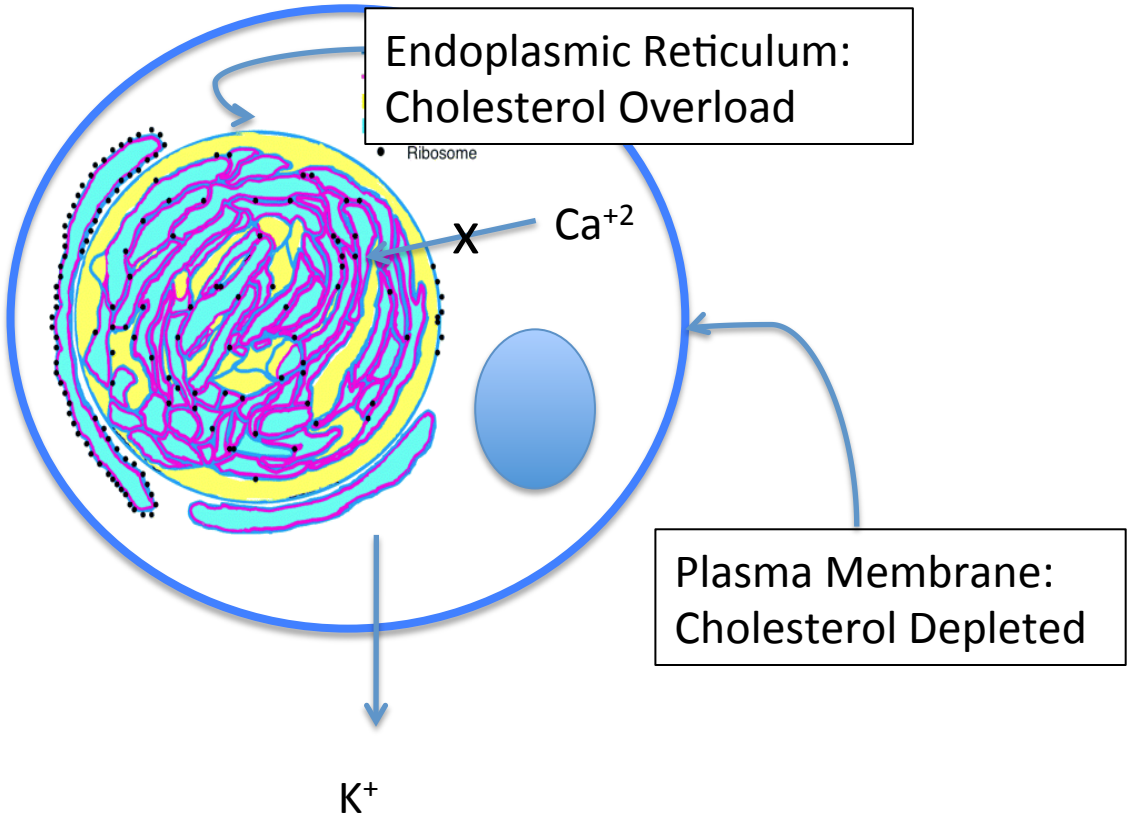
- Endoplasmic reticulum essential for protein folding and activation
- Absence of sulfate leads to internal cholesterol accumulation
- Excess cholesterol destroys cell's ability to fold proteins
- This causes major dysfunction and eventually leads to cell disintegration




Picture from Wan et Al., The FASEB Journal, 2007

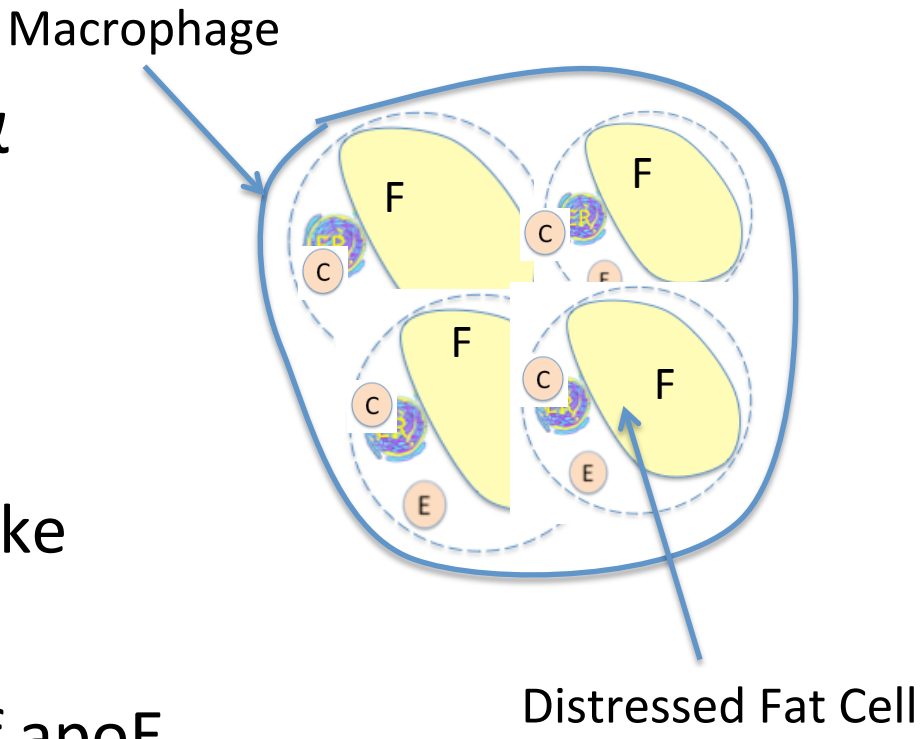
# Fat Cells Become Dysfunctional

- Endoplasmic Reticulum has too *much* cholesterol which inhibits calcium transport
- Plasma membrane has too *little* cholesterol, which causes potassium leaks



# Fat Cells Launch Distress Cascade

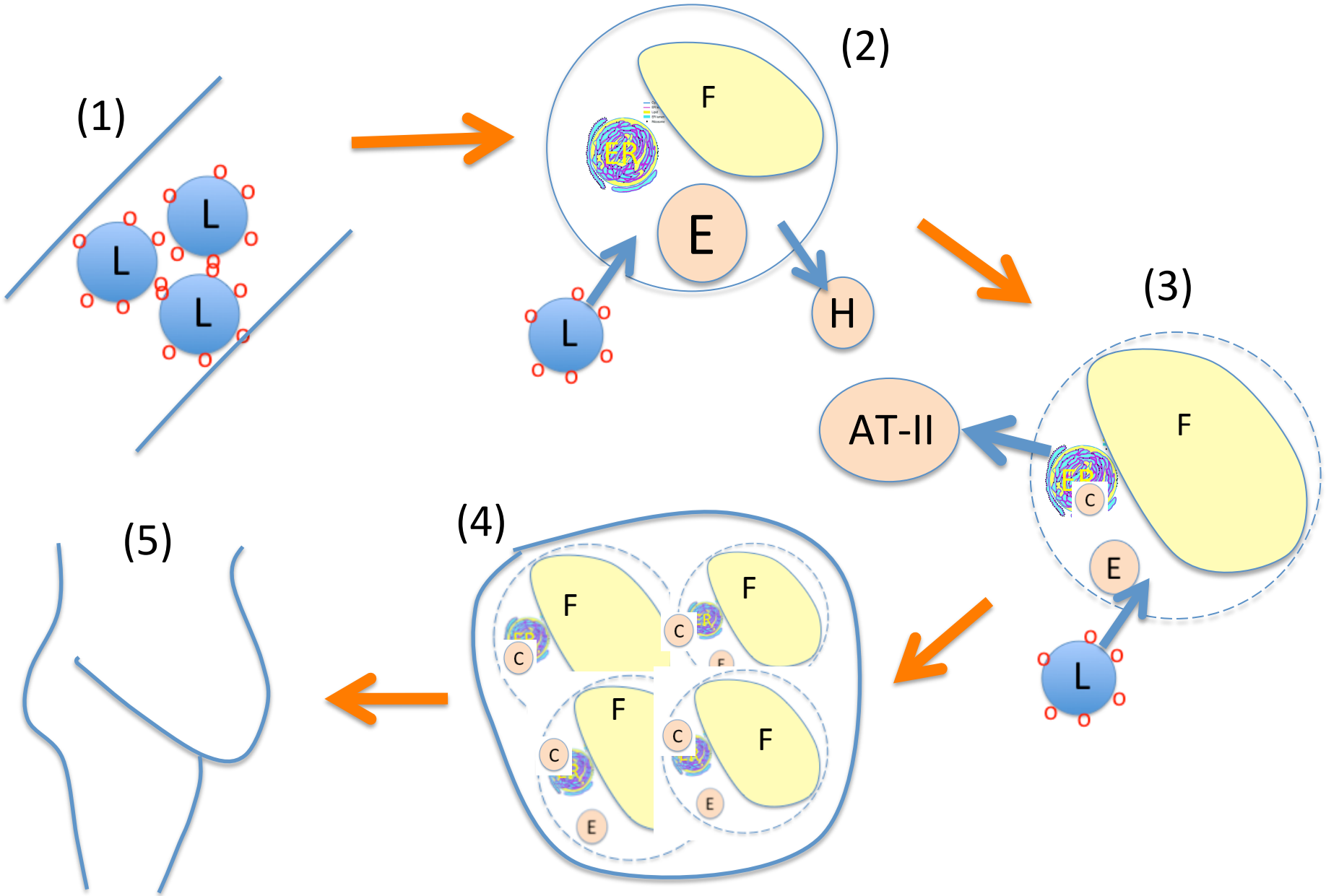
- Release IL-6 and TNF-α
  - Calls in macrophages
  - Induces inflammation
- Release leptin
  - Decreases glucose uptake
- Release angiotensin-II
  - Suppresses synthesis of apoE
  - Increases thirst due to sodium leaks
  - Promotes uptake of cholesterol from HDL for plasma membrane



# Visceral Fat

- Damaged VLDL remnants leads to widespread cholesterol deficiency
- Major organs are especially susceptible (e.g., pancreas, liver, adrenal glands, reproductive organs, etc.)
- Hypothesis: Visceral fat deposits store reserve cholesterol supply for critical organs

# Putting it All Together



# "Reverse Epidemiology"*

- Obesity, hypertension, and high cholesterol are all protective in:
  - Heart failure
  - Rheumatoid arthritis
  - AIDS
  - Post myocardial infarction
  - Cancer
  - The elderly
- This is estimated to apply to 30 million Americans

* George T. Griffing, MD, Medscape Today, Aug 19, 2009. http://www.medscape.com/viewarticle/707334?src=mp&spon=22

While these measures are risk factors for heart disease, they play a protective role in preventing other consequences that are arguably worse than heart disease

# 17 Year Study on Elderly*


- Begun in 1990: all subjects were at least 70 years old
- Measured serum cholesterol, ability to synthesize cholesterol, and ability to absorb cholesterol through the intestines
- Low values of all three parameters were associated with accelerated mental decline and increased physical frailty
- Subjects with low values on all three had 4 ½ years decreased life span

* Tilvis et al., Annals of Medicine, Early Online, 2011

# AIDS Increases Risk to Heart Disease*

"Patients with HIV with chronic infection have significant vascular inflammation, and thus added CVD [cardiovascular disease] risk, beyond that estimated by traditional risk factors."

I propose that AIDS is a cholesterol sulfate deficiency disease

* S. Subramanian, et al., Arterial Inflammation in Patients With HIV. JAMA, July 25, 201, 308( 4379), p. 386

# High Fat Diet Protects from Heart Failure*

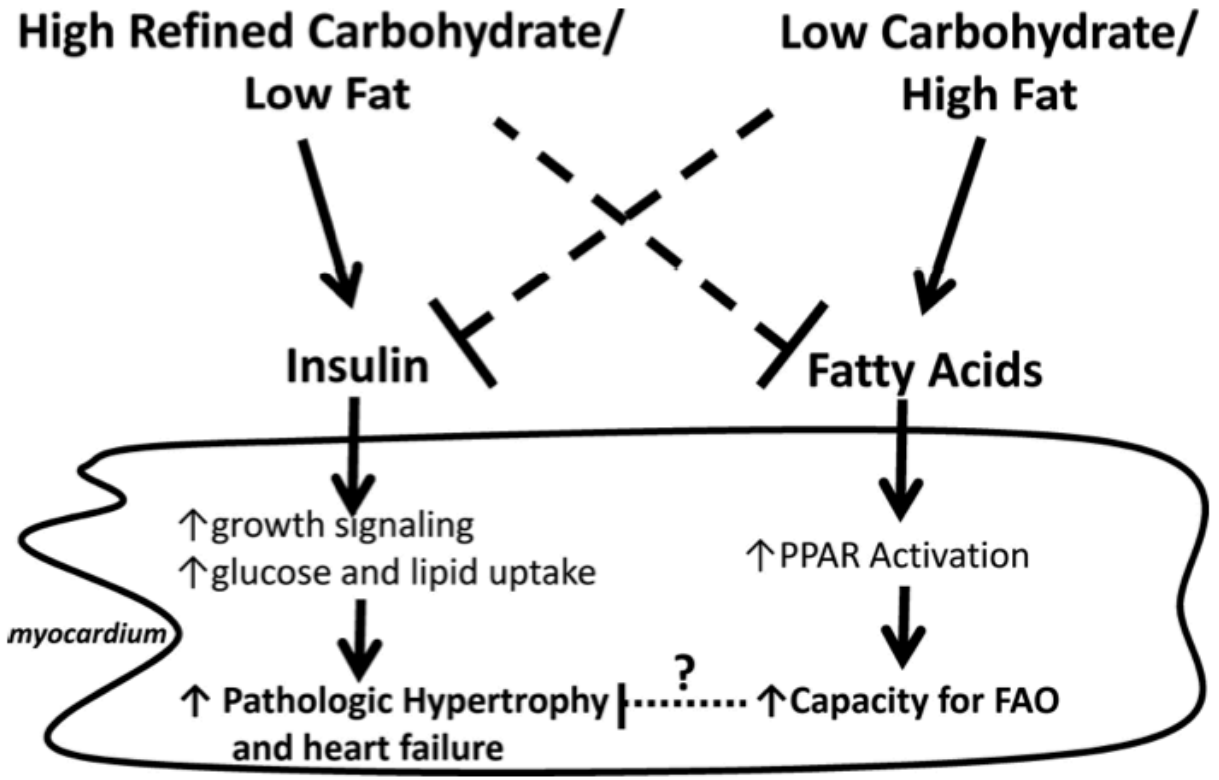



**Figure 3. Schematic depiction of the effect of dietary fat and CHO on cardiomyocytes.**

*From W.C. Stanley *et al.*, Dietary Fat and Heart Failure: Moving From Lipotoxicity to Lipoprotection, *Circulation Research* 2012, 110:764-776

# Recapitulation

- Fat cells play an important service in cleaning up busted LDL and supplying cholesterol and fats to insulin-resistant muscle cells
- This is a dirty job, and fat cells get sick
  - Too little membrane cholesterol, too much internal cholesterol
- Macrophages are called in to clean up dying cells
  - This leads to inflammatory response and widespread tissue damage
- Metabolic syndrome is protective in several modern diseases and conditions
- High fat diet protects from these diseases

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# eNOS

eNOS (endothelial Nitric Oxide Synthase) is a "moonlighting" enzyme: it makes sulfate upon sunlight stimulation, and switches to nitric oxide (nitrate) under stress

# Endothelial nitric oxide synthase (eNOS)

# eNOS!!

- Endothelial nitric oxide synthase (eNOS) is a very interesting molecule
- It's known for its role in synthesizing nitric oxide (NO) from L-arginine
- But I think it has a much more important role as well, which is its primary role:
  - To synthesize sulfate from sulfur in the presence of sunlight

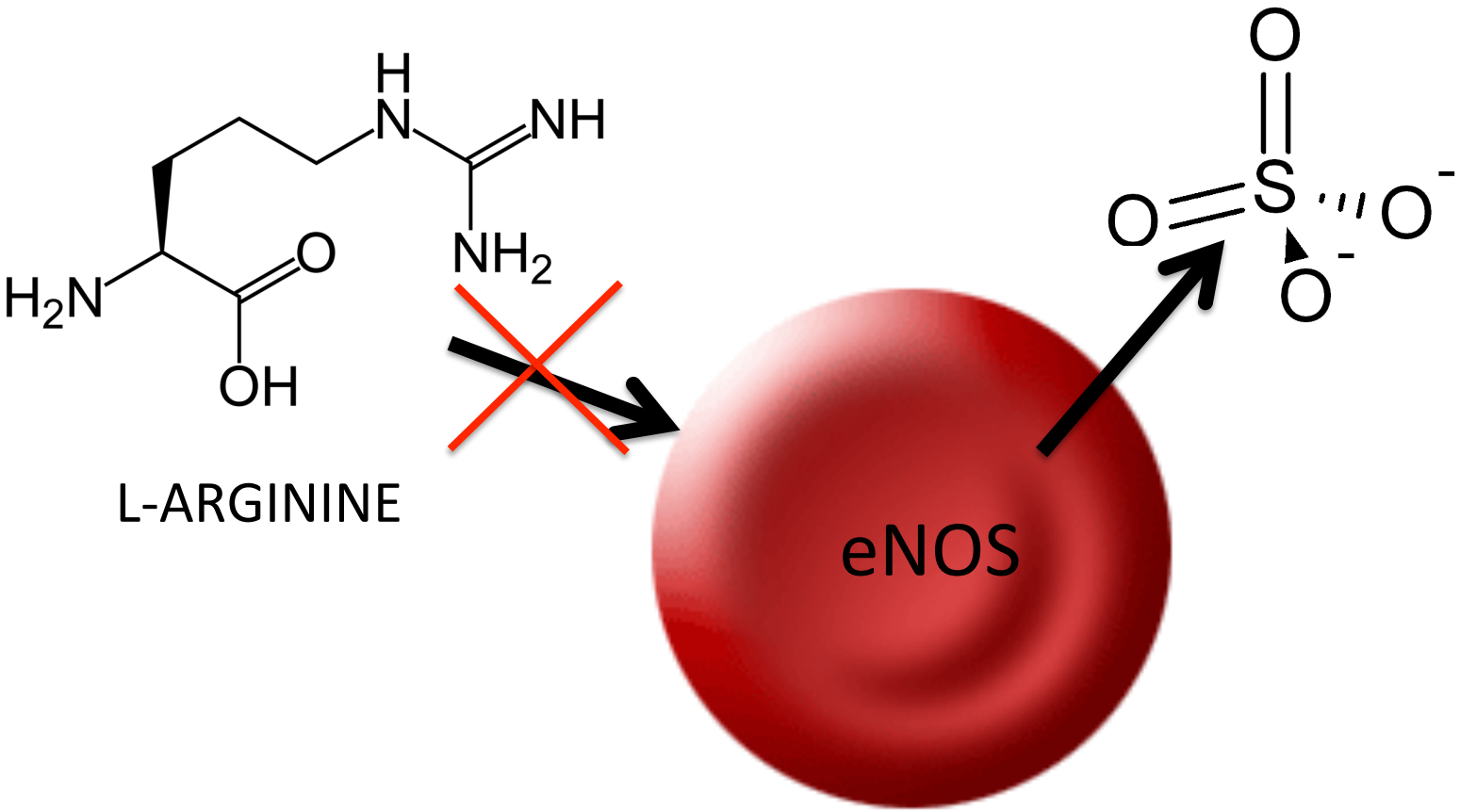
$$N \rightarrow NO \rightarrow NO_3^{-2}$$
$$S \rightarrow SO_2 \rightarrow SO_4^{-2}$$

# Where is eNOS Found? What does it do?

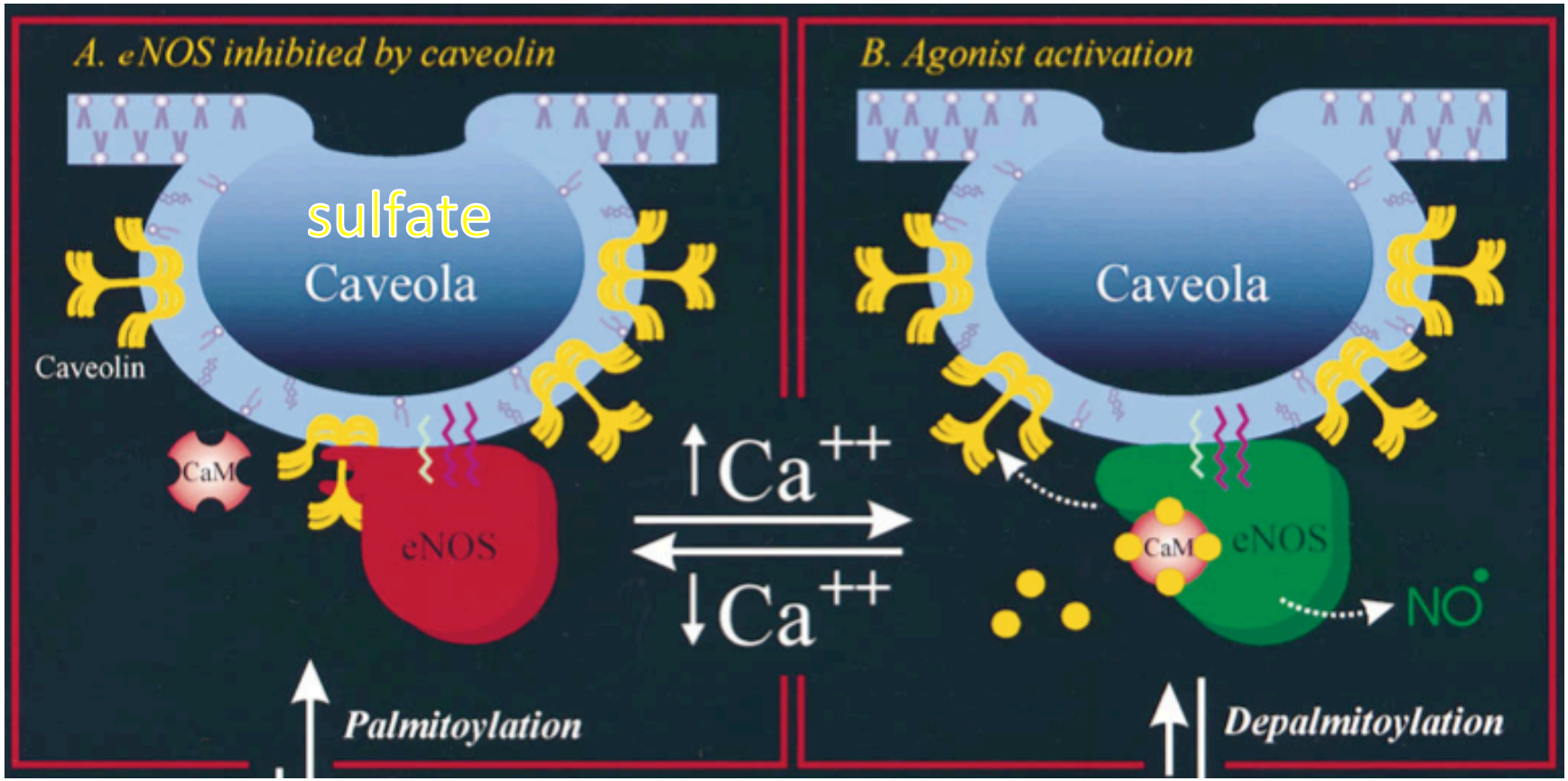
- In keratinocytes in the skin
- In the endothelial cells lining artery walls
- In several cell types in the blood:
  - red blood cells, platelets, mast cells
- eNOS synthesizes nitric oxide from L-arginine

It makes no sense for a red blood cell to synthesize nitric oxide!!

The red blood cell keeps the substrate for nitric oxide synthesis out:
I hypothesize that it uses eNOS to make sulfate instead !

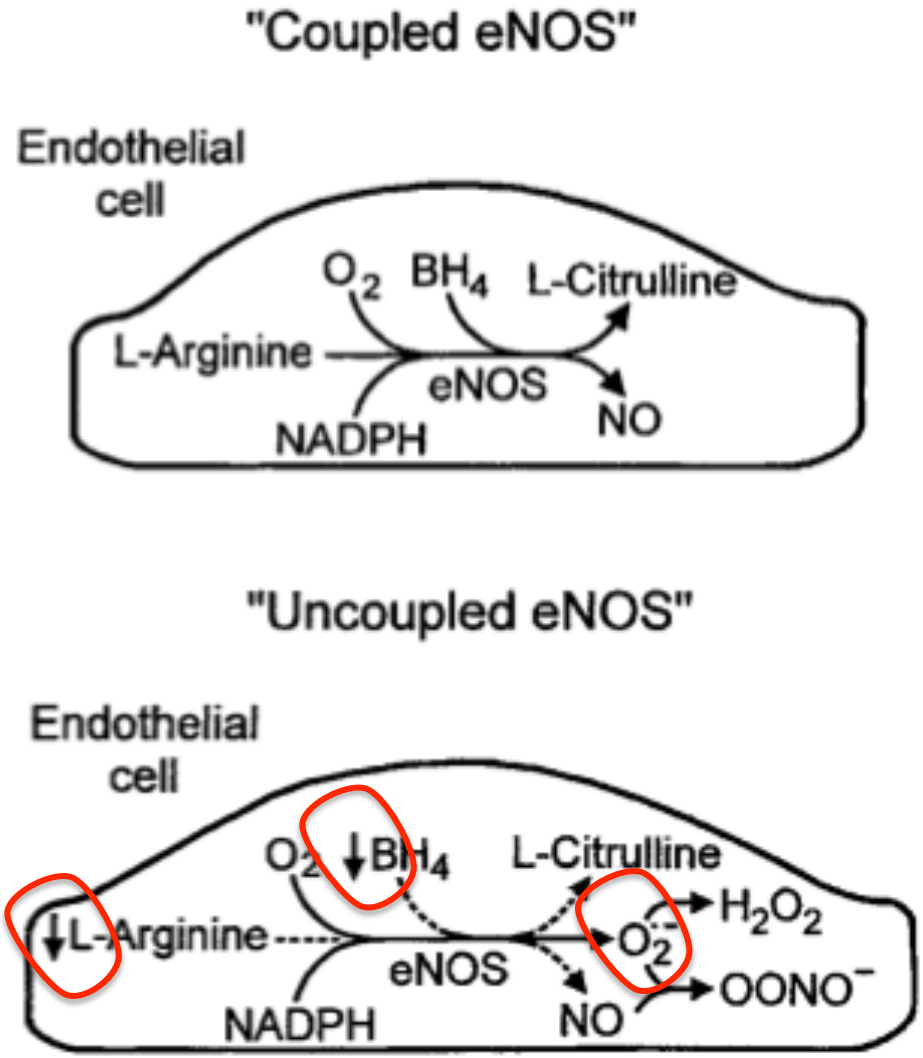


# eNOS and Caveolae




Michel and Feron, J. Clin. Invest. 100(9) 2146-2152, 1997

# eNOS with and without L-Arginine



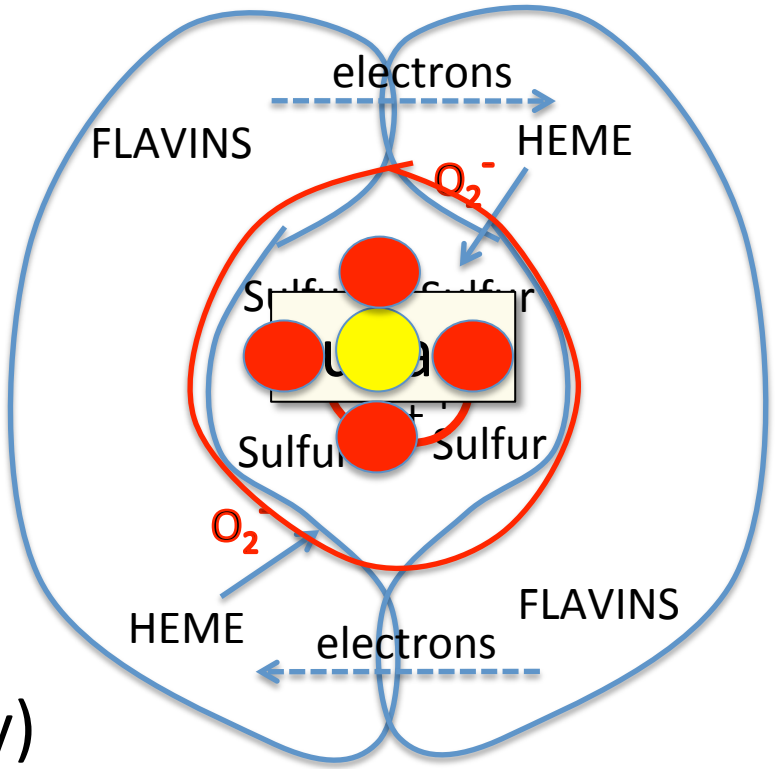

If either L-arginine substrate or $BH_4$ cofactor is reduced, eNOS synthesizes $O_2^-$ (superoxide) instead of NO

$O_2^-$ and NO combine to make $OONO^-$, a potent oxidizing agent

**Katusic, *Am J Physiol Heart Circ Physiol 281:H981-H986, 2001.***

# eNOS Dimer Synthesizes Sulfate: A proposal

- Dimer (two eNOS molecules) creates cavity
- Zinc atom in cavity creates positive charge field
- Sulfurs in cysteine peptides attracted to zinc
- Flavins respond to sunlight by emitting electrons
- Electrons create charged oxygen dimers ($O_2^-$)
- Oxygen combines with sulfur to form sulfate (capture energy)



# Recapitulation

- eNOS is a moonlighting enzyme whose day job is cholesterol sulfate synthesis
  - Depends on sunlight to catalyze reaction
  - Switches to nitric oxide (nitrate) synthesis under pathological conditions
- Red blood cells, platelets, endothelial cells, keratinocytes and other cells use eNOS to make sulfate
  - Mechanism involves zinc atom in cavity in eNOS dimer
  - These cells need sulfate to produce cholesterol sulfate and/or heparan sulfate

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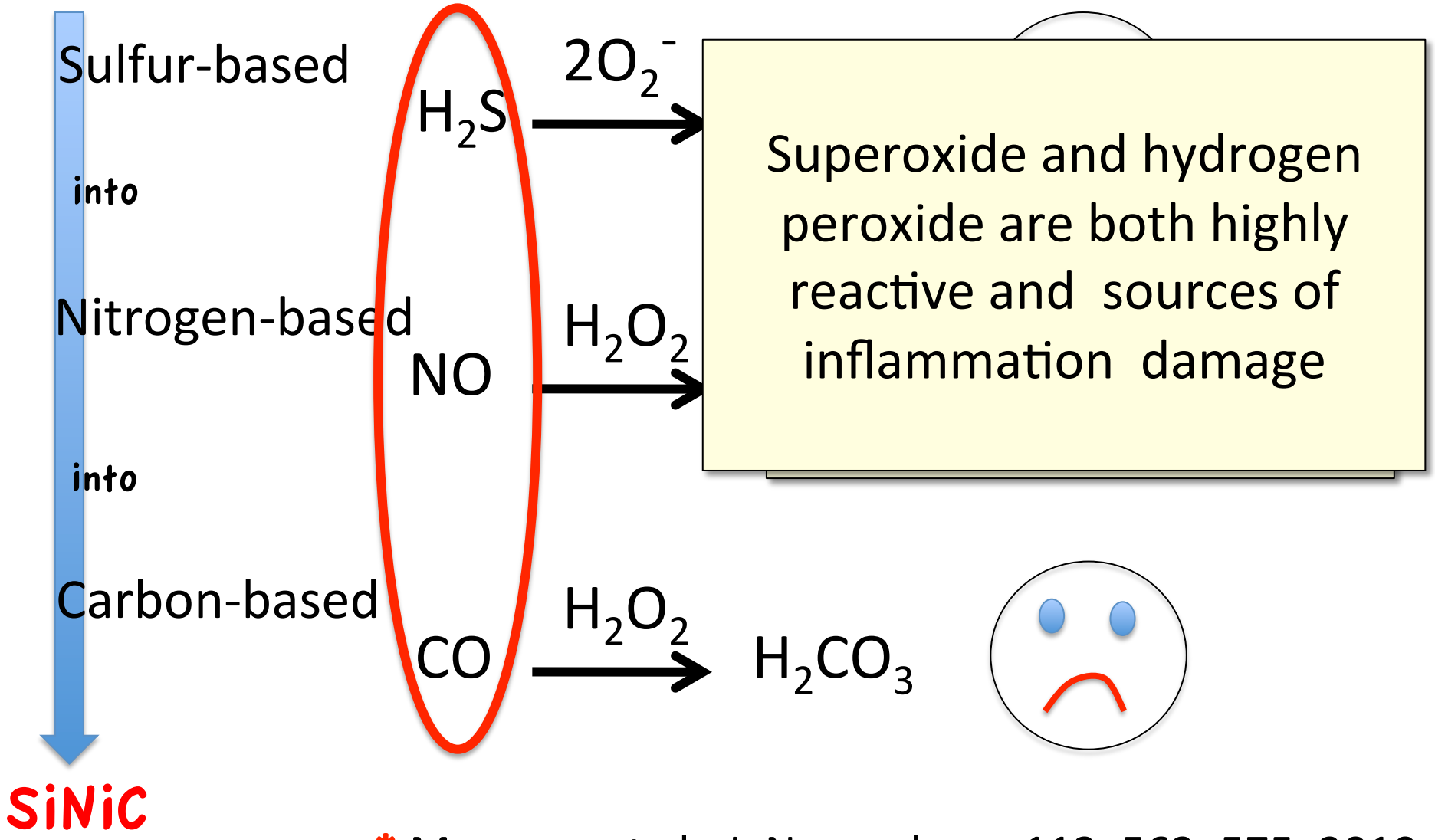
# SiNiC

The slide from Sulfur into Nitrogen into Carbon
as we age

Oxygen transport in the blood depends upon
these three atomic elements (S, N, C)
which can react with oxygen and form anions
that stabilize the blood and safely carry oxygen

Sulfur is the healthiest choice;
nitrogen is associated with many pathologies;
carbon acting alone will lead to
acidosis and cancer

# Signaling Gas Molecules and Oxygen*

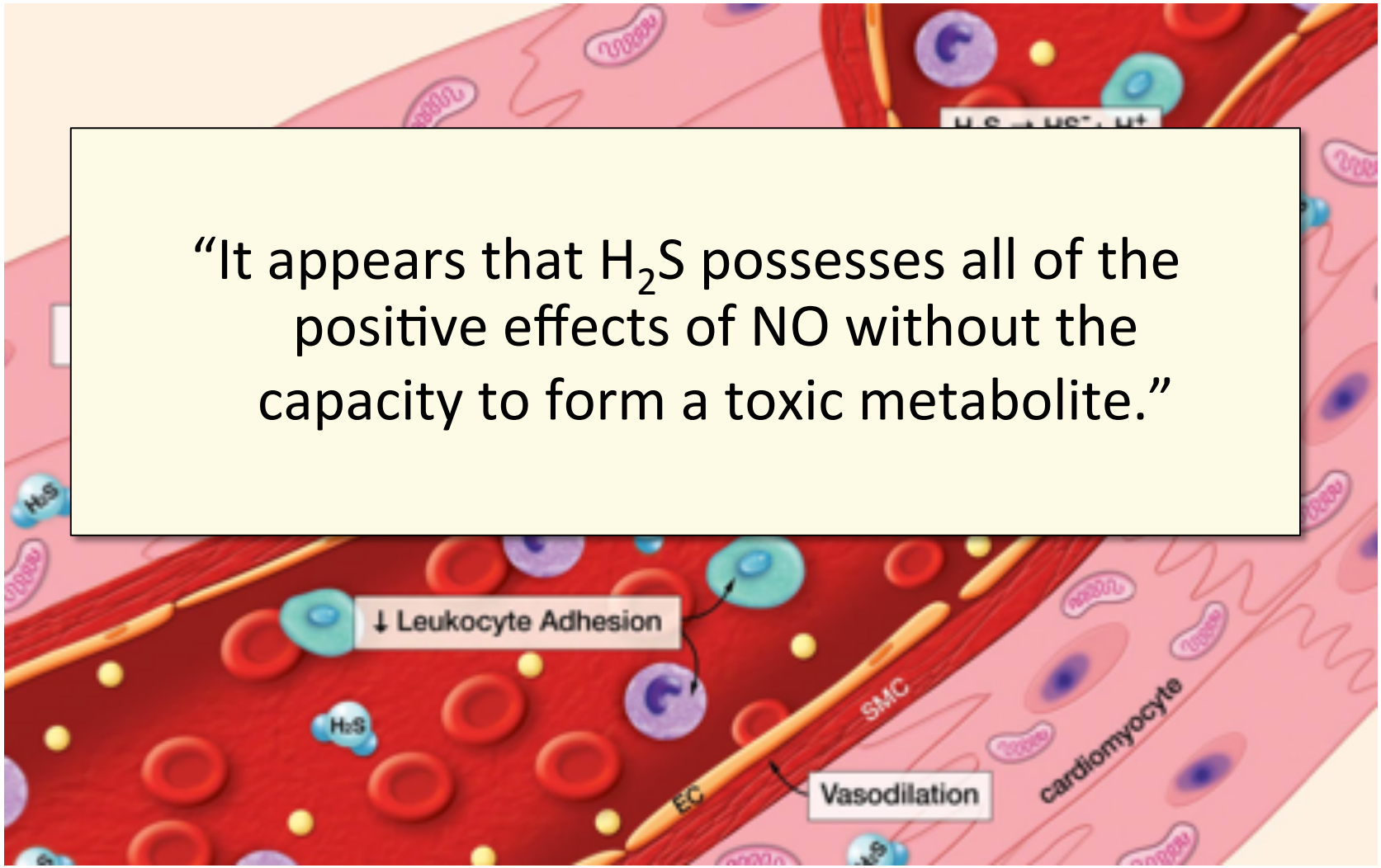



* Mancuso et al., J. Neurochem. 113, 563–575, 2010

# Signaling Gases

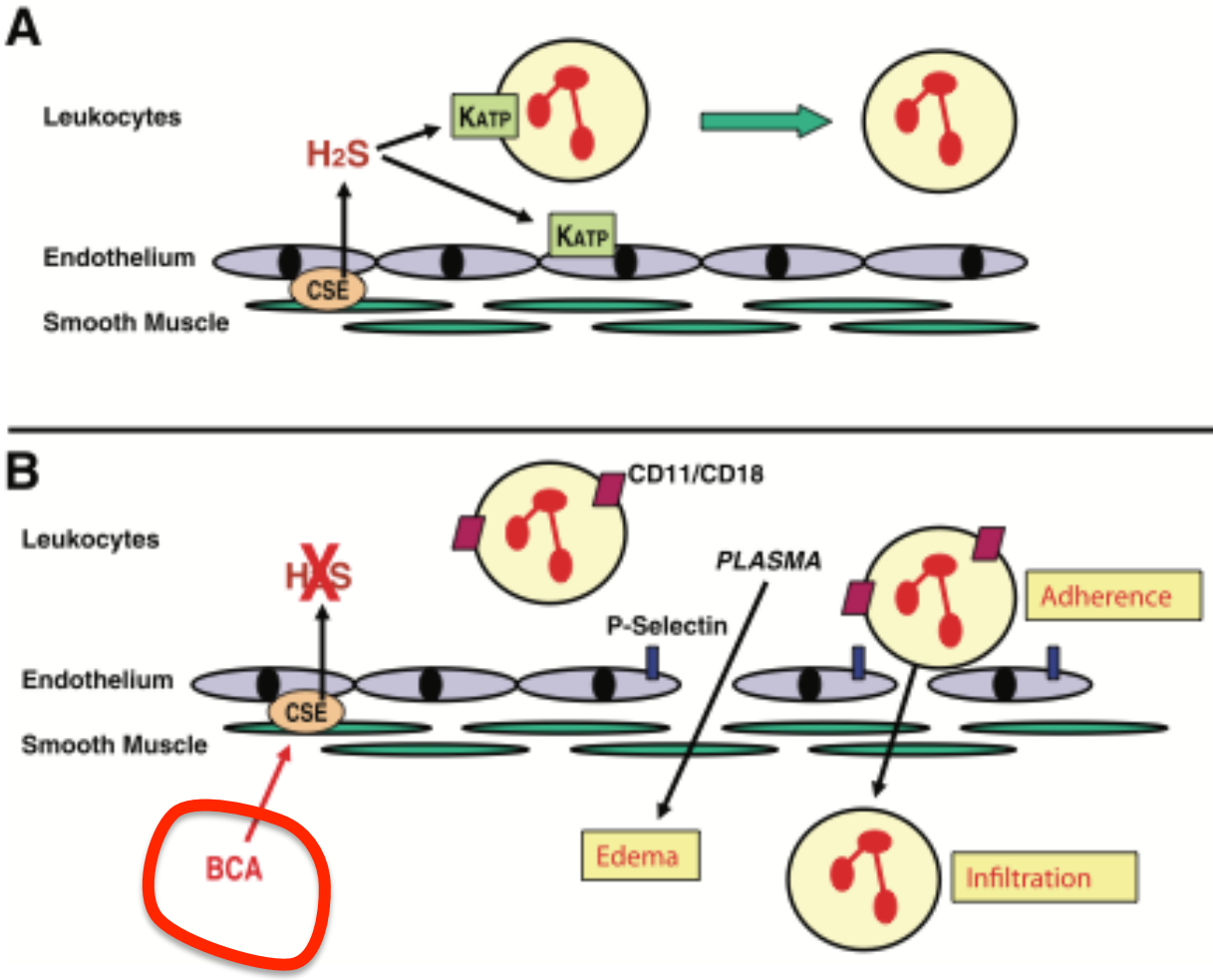
- Hydrogen sulfide ($H_2S$), nitric oxide (NO), and carbon monoxide (CO) are all released into the blood stream in small amounts
- All of these gases have the effect of relaxing the artery wall and increasing oxygen supply to the tissues

# Hydrogen Sulfide as a Signaling Molecule*




* D. Lefer, PNAS 104(46), 17907–17908, 2007

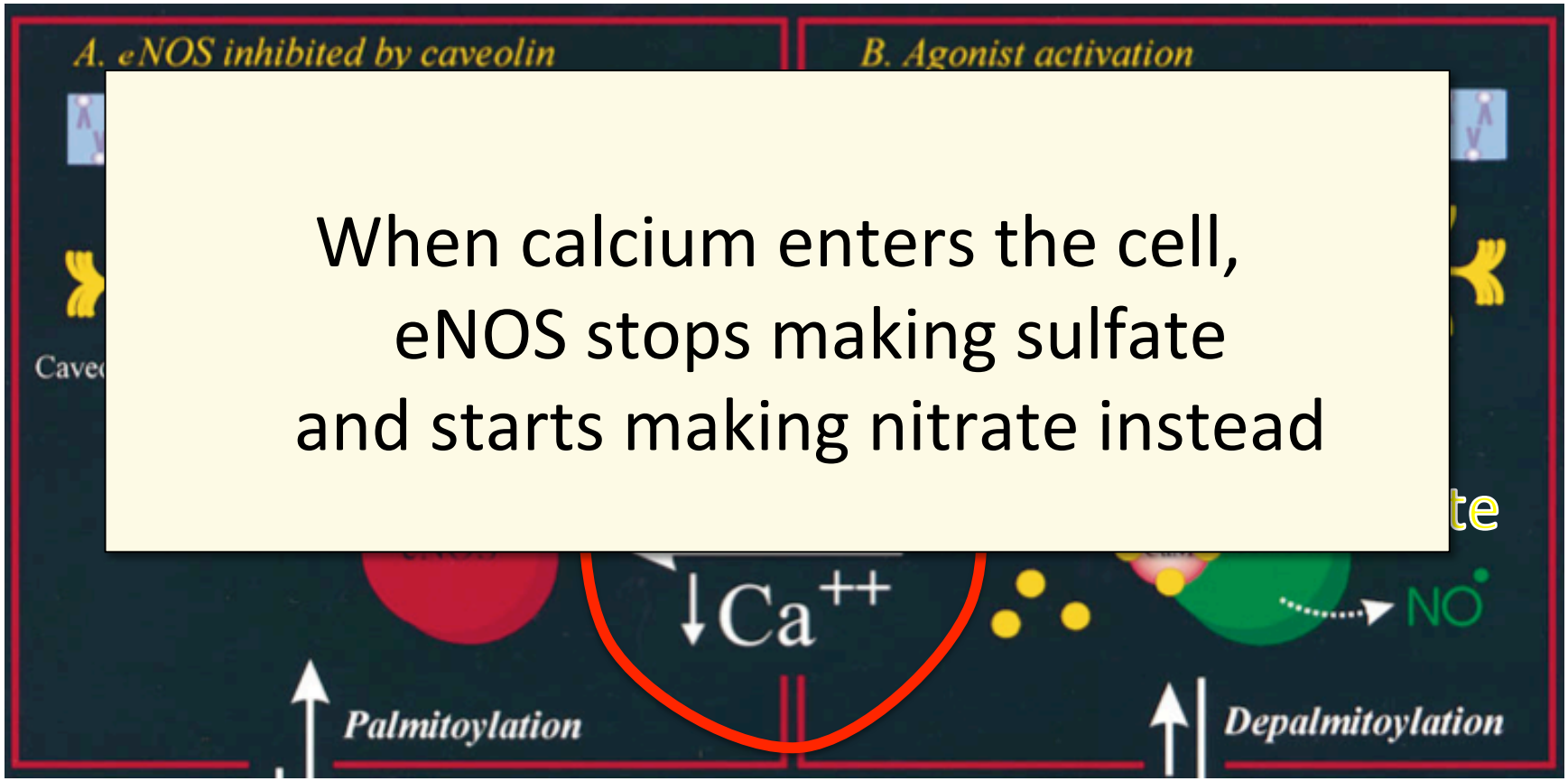
# Hydrogen Sulfide Protects from Cardiovascular Disease*




- BCA blocks $H_2S$ Synthesis
- This causes white blood cell adherence to the artery wall and infiltration beyond it, leading to atherosclerosis

* Renada et al., The Faseb Journal, 20, E1411-E1418, 2006.

# eNOS and Caveolae*

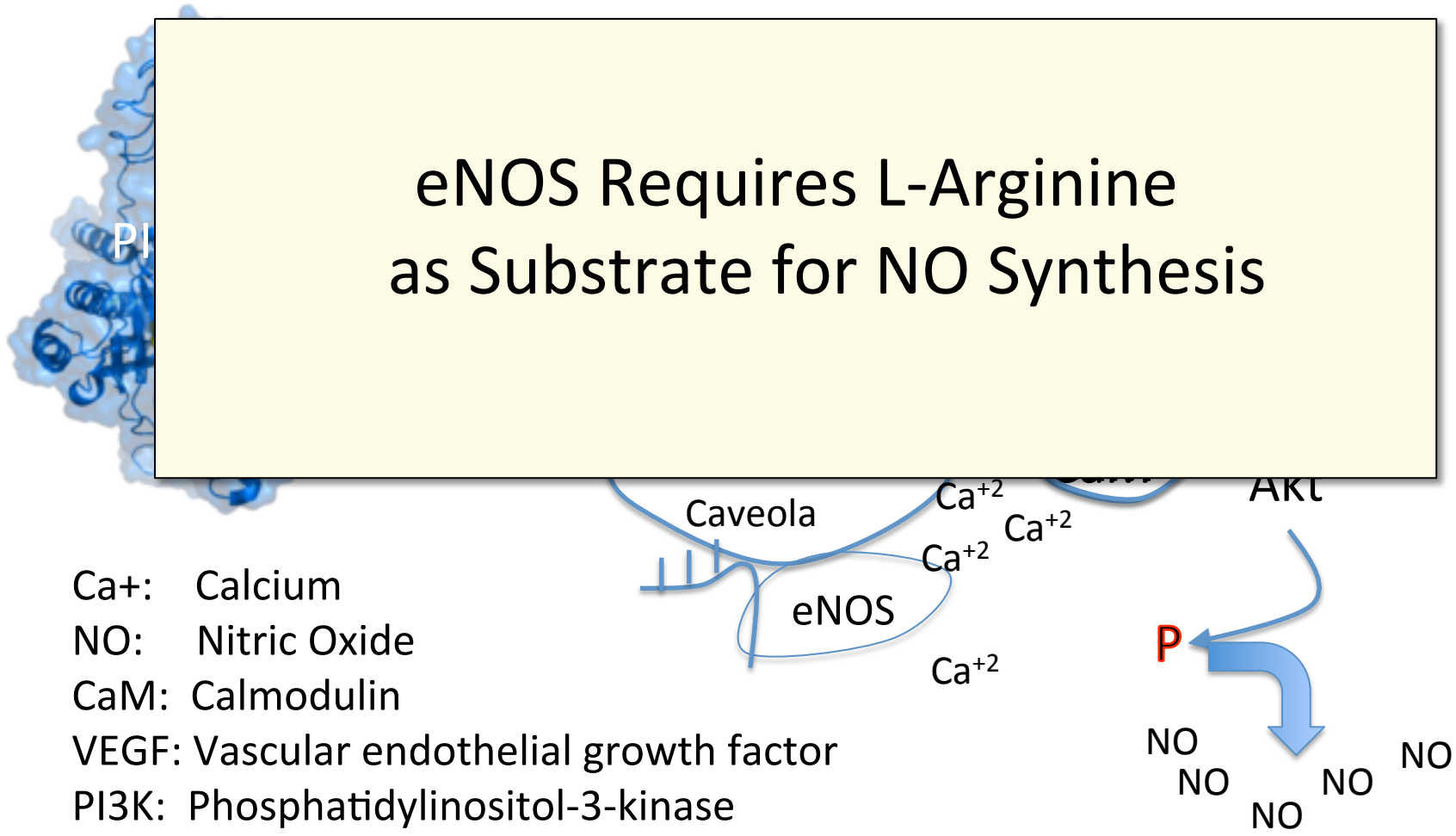



When calcium enters the cell,
eNOS stops making sulfate
and starts making nitrate instead

* Michel and Feron, J. Clin. Invest. 100(9) 2146-2152, 1997

# Transition to Nitrogen-based Oxygen Transport*

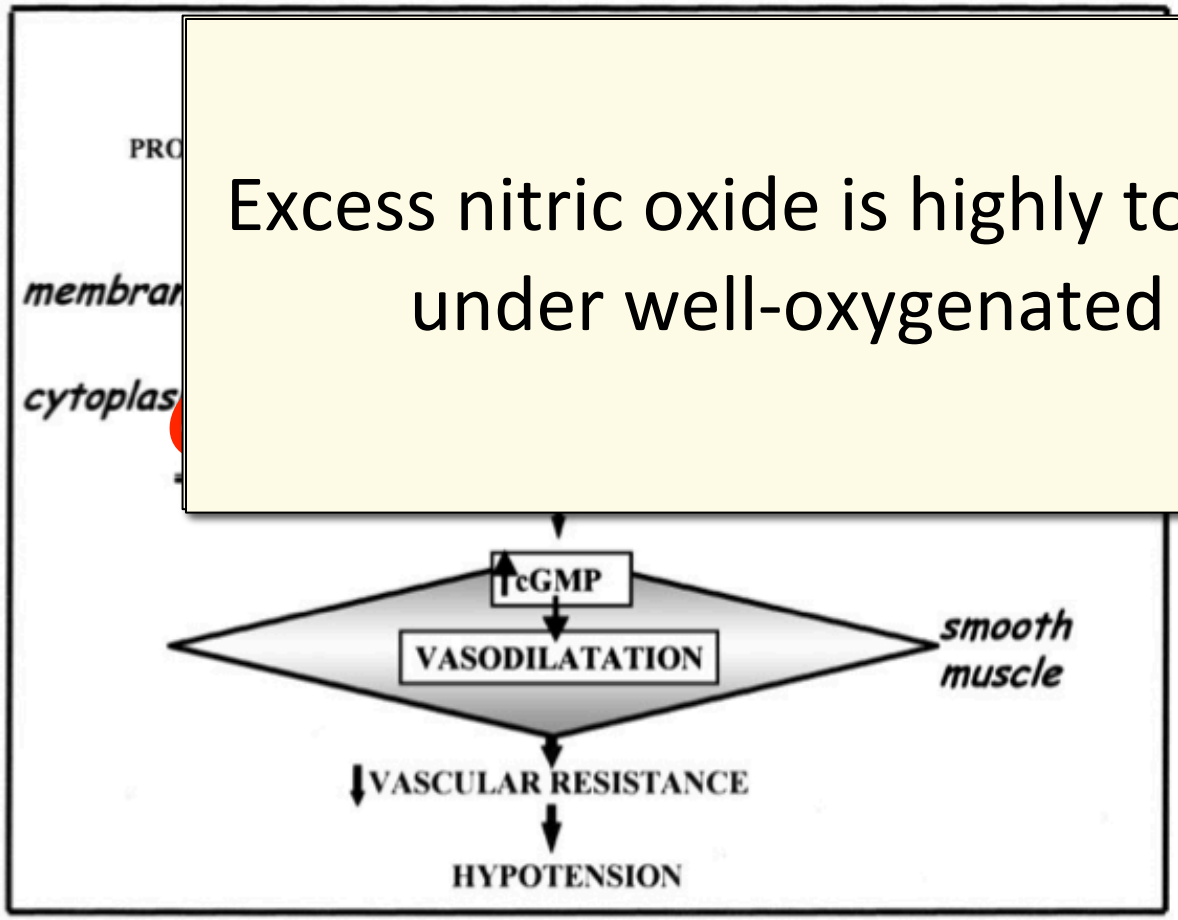
eNOS Requires L-Arginine
as Substrate for NO Synthesis




Ca+: Calcium
NO: Nitric Oxide
CaM: Calmodulin
VEGF: Vascular endothelial growth factor
PI3K: Phosphatidylinositol-3-kinase
eNOS: endothelial nitric oxide synthase

* Navarro et al., The Faseb Journal 18, 2004

# Protamine Treatment*




Excess nitric oxide is highly toxic, especially under well-oxygenated conditions

cationic
hly
ginine
tment
eme
ension,
pulmonary hypertension, anaphylactic shock and cardiac failure

* Viaro et al., *Chest 122;1061-1066, 2002*

# The Relationship with Pathogens

Keep the pathogens out

$H_2S \xrightarrow{2O_2} H_2SO_4$

Fight the pathogens

$NO \xrightarrow{H_2O_2} H_2NO_3$

Let the pathogens win

$CO \xrightarrow{H_2O_2} H_2CO_3$

# How it Works

- Keep the pathogens out (sulfur)
  - Both cholesterol sulfate in the skin and sulfate ions surrounding individual cells protect from bacterial invasion
- Fight the pathogens (nitrogen)
  - Once the pathogens get in, nitric oxide is a good toxin to kill them
- Let the pathogens win (carbon)
  - When cells themselves are too vulnerable to damage, it's better to just learn to live with the pathogens –> autoimmune disease

# Interleukins Control Strategy

IL-1, TNF-α; superoxide

$H_2S \xrightarrow{2O_2^-} H_2SO_4$

IL-6; muscle loss, anemia

$NO \xrightarrow{H_2O_2} H_2NO_3$

IL-10; autoimmunity

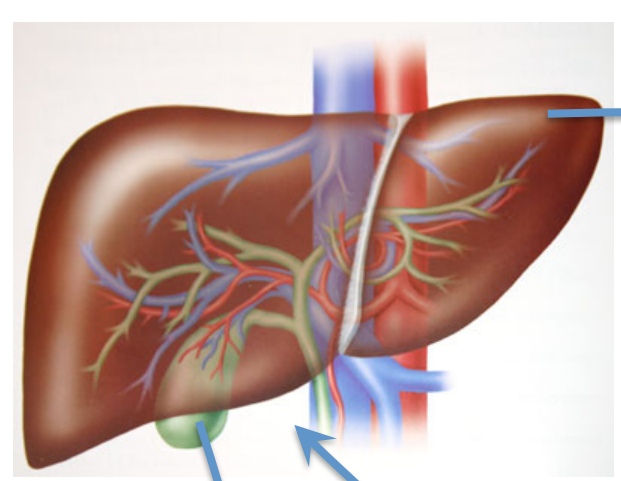
$CO \xrightarrow{H_2O_2} H_2CO_3$

# How Insufficient Sulfate Leads to Anemia*

- Bacterial infections rage in blood stream
  - Triggers IL-6 synthesis by macrophages
- Induces mechanisms to deplete blood of iron
  - Iron feeds the bacteria!
- Iron is drawn into macrophages, to lure bacteria to their death through nitric oxide poisoning
- Serum iron is depleted → anemia

* Paradkar et al. Blood, 112:3, 866-874, 2008

# How Bacterial Infection Leads to Anemia through IL-6*



bacteria

hepcidin

hepcidin

IRON

IRON

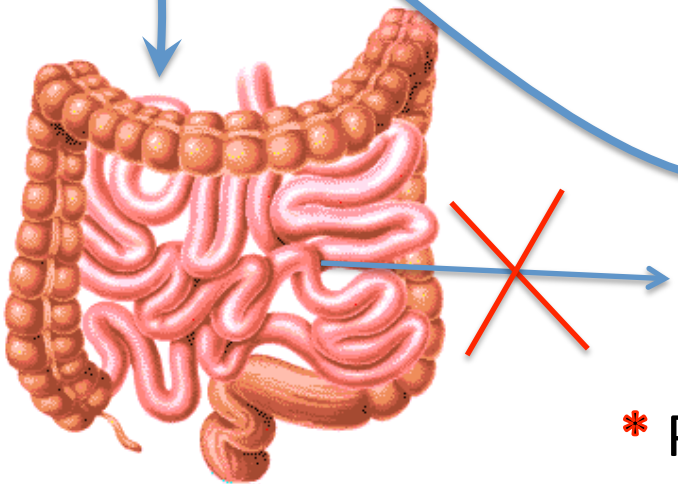
IL-6

IRON

IRON

IRON

IRON



* Paradkar et al. Blood, 112:3, 866-874, 2008

# Conditions Associated with Deficiencies

Obesity, heart disease, arthritis

$$H_2S \xrightarrow{2O_2} H_2SO_4$$

Anorexia, cachexia, ALS

$$NO \xrightarrow{H_2O_2} H_2NO_3$$

Lupus, AIDS, cancer

$$CO \xrightarrow{H_2O_2} H_2CO_3$$

# Recapitulation

- Three signaling gases relate to three systems of oxygen/acid management: S, N, and C
- Adequate sunlight exposure to skin allows S system to thrive
- When S is deficient, bacteria invade
- N based system fights bacteria
- C based system gives up →

  autoimmune disease

BREAK!

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# Blood Dysfunction

The stability of the blood colloidal system depends upon adequate cholesterol sulfate.

When sulfate is depleted, suspended cells and particles become deficient in negative charge, and start to stick together.

This leads to blood clots and hemorrhaging

# Blood Clots and Hemorrhages

# Blood Clots and Disease

"How and when our blood clots is one of those incredibly complex and important processes in our body that we rarely think about. If your blood doesn't clot and you cut yourself, you could bleed to death, if your blood clots too much, you could be in line for a heart attack or stroke. "*

* http://medicalxpress.com/news/2012-03-mystery-blood-clotting.html

# "Platelet Up-and-Comers"*

"The quest for ever-more effective drugs to decrease platelet aggregation and reduce the risk of thrombus formation, without unduly increasing the risk of bleeding, will always be a holy grail of cardiovascular disease research. With each new contender comes fresh excitement as well as more questions."

* http://www.theheart.org/collection/Antiplatelet-up-and-comers.do

# Hemostasis

- Hemostasis keeps the blood in circulation and prevents
- Maintain ng clotting factors
- Complex cium, thrombo l serum proteins
  - Extrins
  - Intrins
  - Common pathway
- Final product is insoluble fibrin (blood clot)

**Hypothesis: insufficient sulfate supply to blood leads to fragile state of constant tension between hemorrhage and blood clots**

# The Glycocalyx

- Negatively charged gel-like mesh lining the walls of all arteries, veins and capillaries
- Depends crucially on sulfated polysaccharides
  - Particularly heparan sulfate proteoglycans (HSPGs)
- Sulfate creates exclusion zones
  - Helps protect cells in wall from ion leaks and contact with enzymes suspended in blood
  - Greatly reduces amount of flowing blood (decreases resistance, lowers blood pressure)

# The Glycocalyx

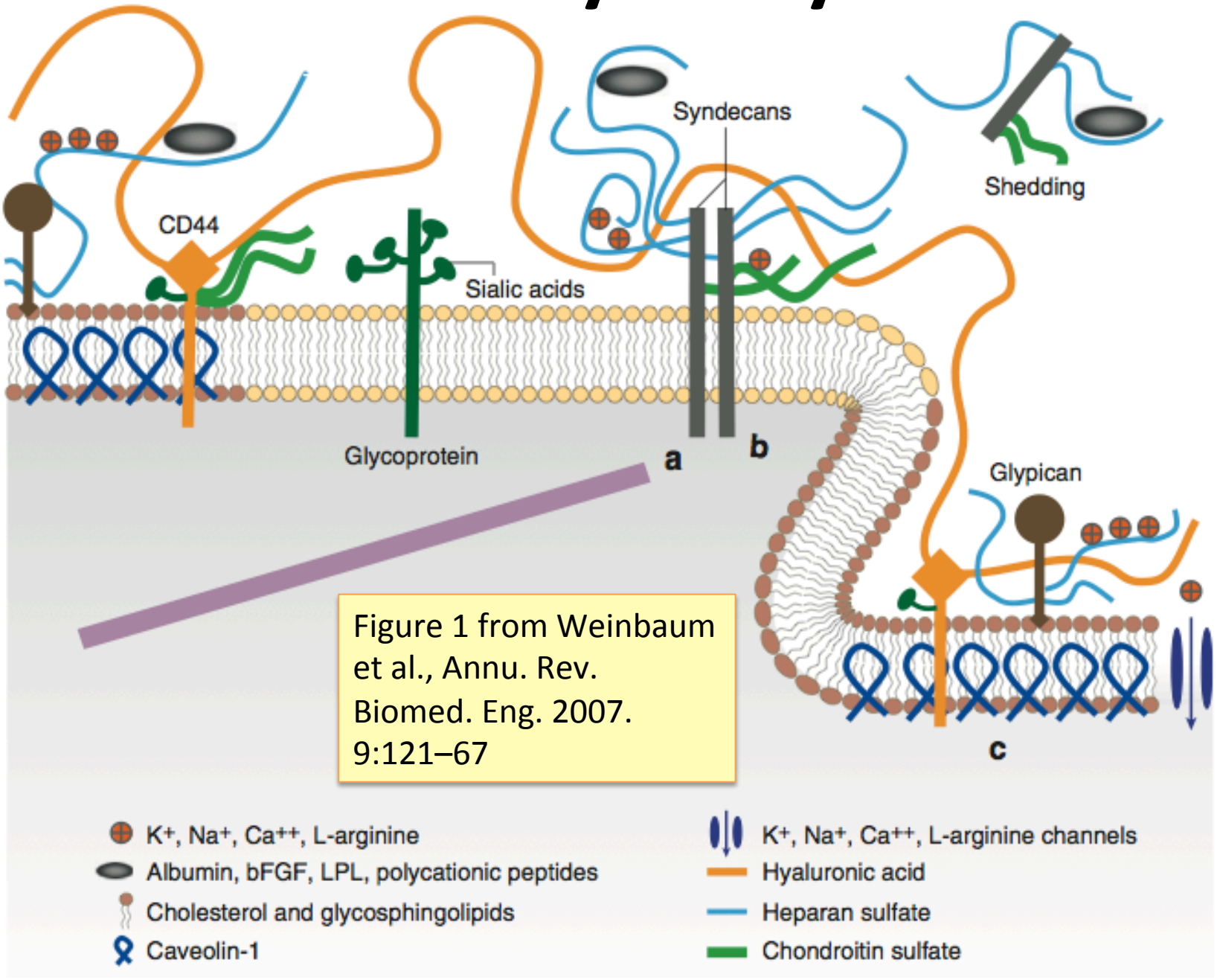



Figure 1 from Weinbaum et al., Annu. Rev. Biomed. Eng. 2007. 9:121–67

# The Glycocalyx

Sulfate maintains negative charge and creates exclusion zones

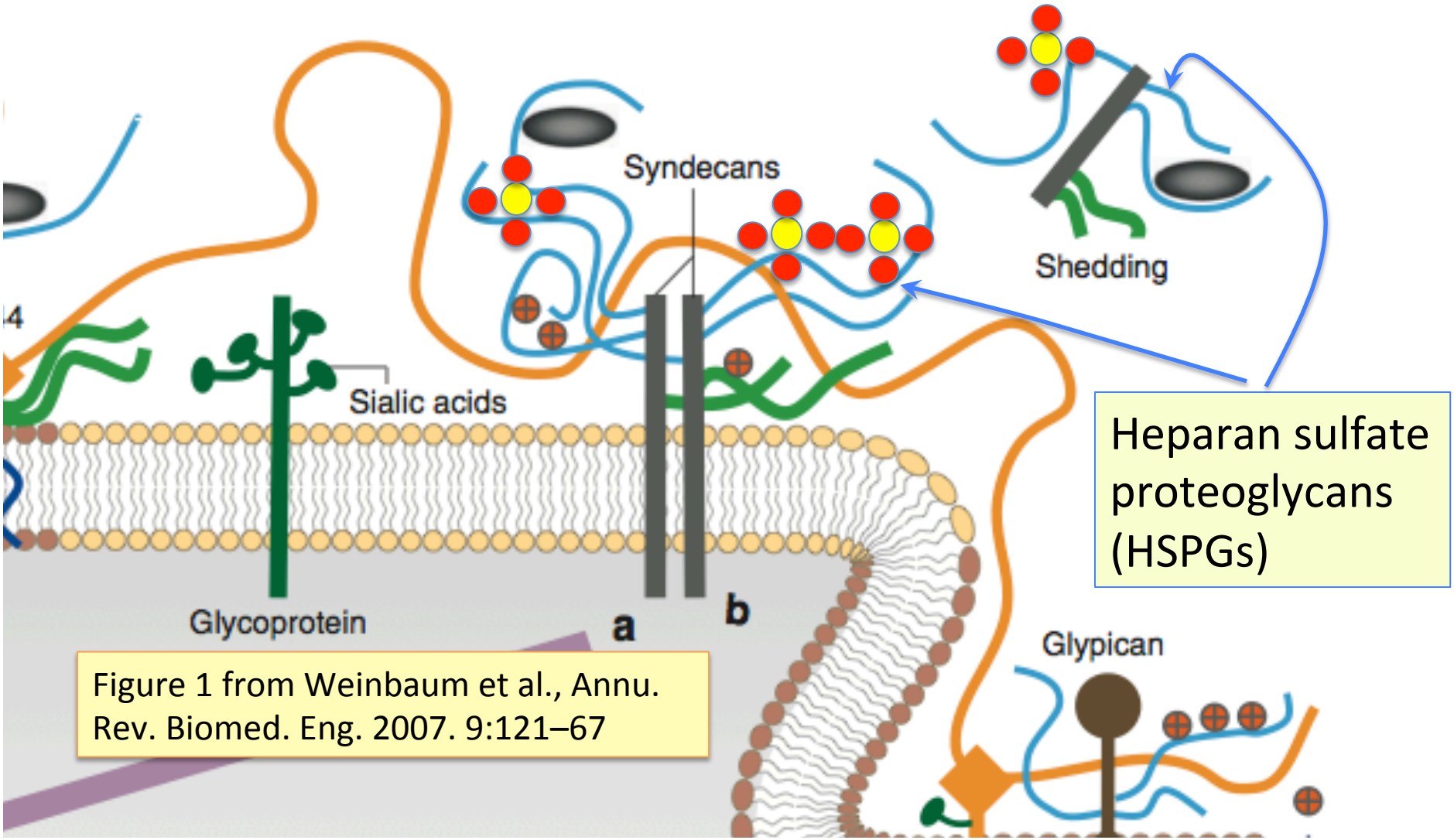
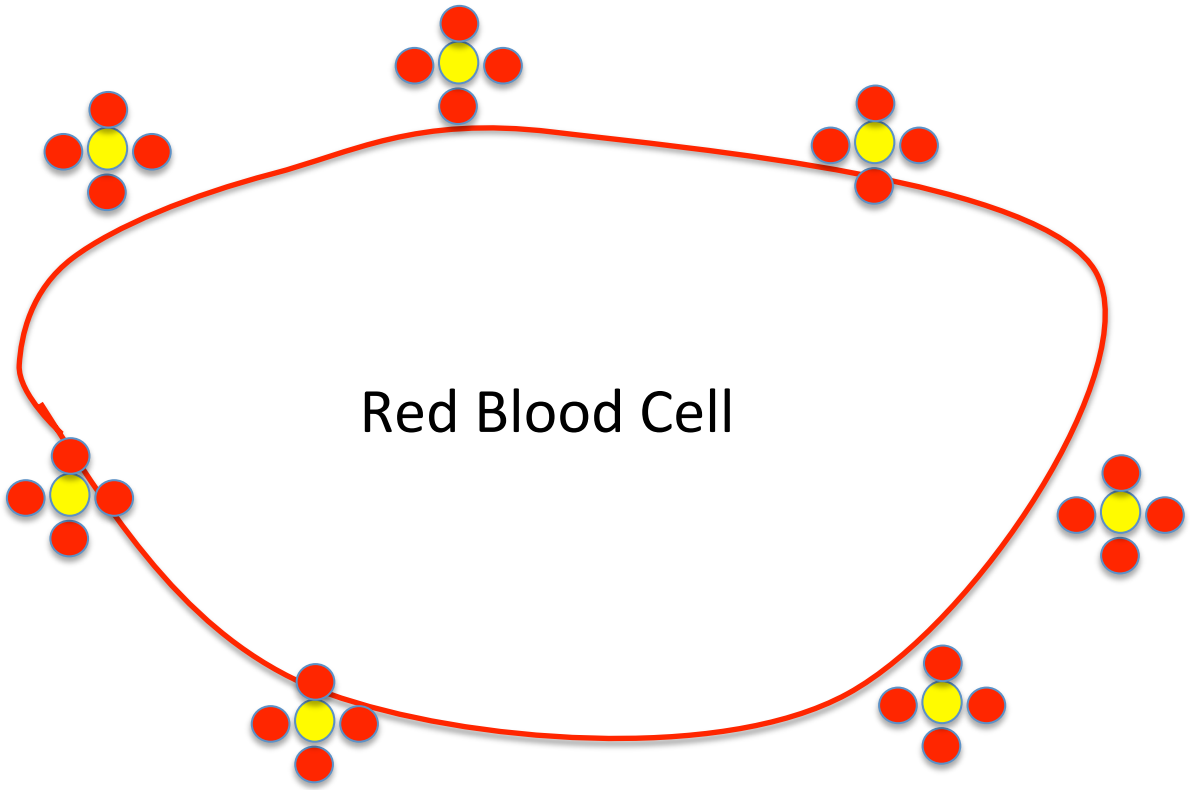



Figure 1 from Weinbaum et al., Annu. Rev. Biomed. Eng. 2007. 9:121–67

# Key Role for Sulfates

- Sulfates decorate exterior of cell, e.g., as heparan sulfate proteoglycans (HSPGs) or as cholesterol sulfate (Ch-S)
- Sulfates carry negative charge and keep cells from sticking together
- Sulfates, as kosmotropes, create exclusion zone – gel-like environment to protect cell



# Zeta Potential

- Zeta potential indicates degree of repulsion between similarly charged particles in a dispersion (e.g., the blood)
- High zeta potential confers stability
  - RBCs and platelets resist aggregation

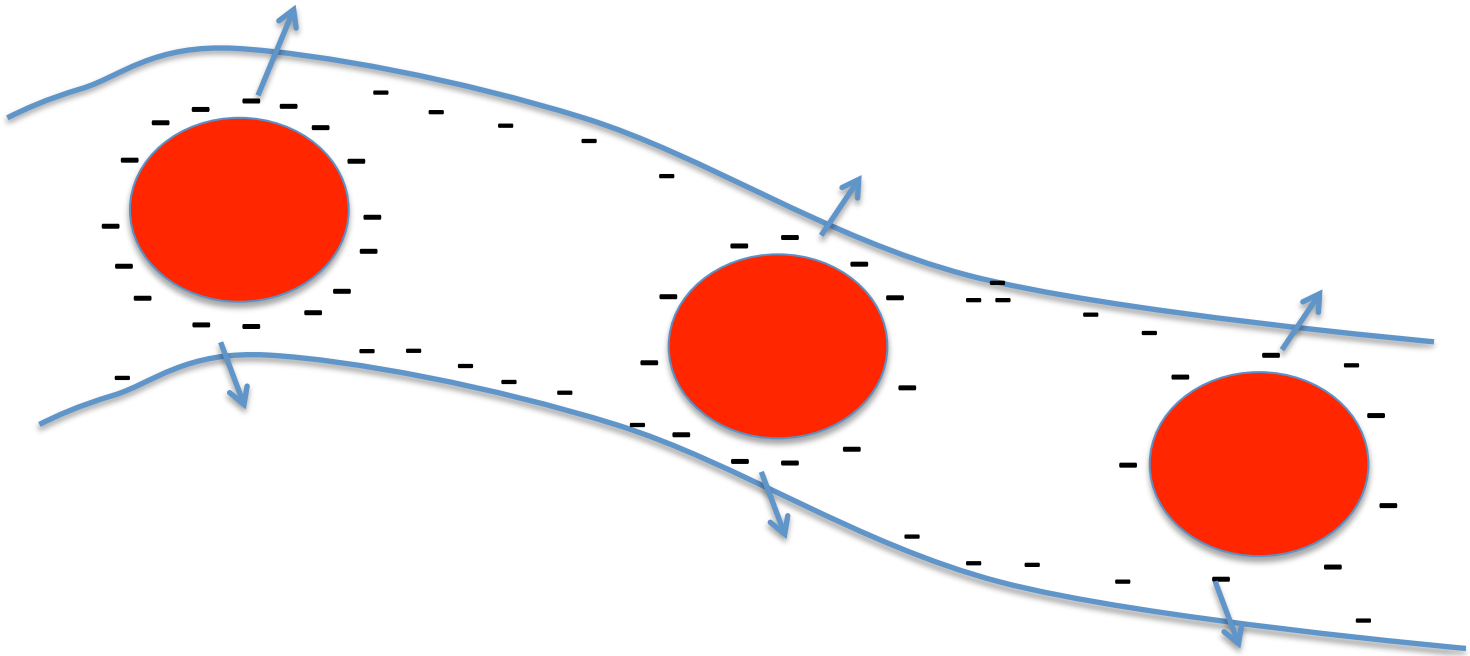

- Low zeta potential causes flocculation and coagulation (blood clot)
- Proposal: a steady drop in zeta potential in the blood as we age  is the source of many modern diseases

# Grounding the Human Body Reduces Blood Viscosity*

- Blood viscosity is strongly influenced by the surface charge on the suspended particles (like RBCs)
- A higher repulsive surface charge increases spacing between RBCs, reduces clumping, lowers viscosity, and lowers peripheral resistance to flow (blood pressure)
- Grounding transfers electrons from soil to the body
  - Increases zeta potential, lowers blood viscosity

*G. Chevalier et al., J Alternative and Complementary Medicine, 1–9, 2012

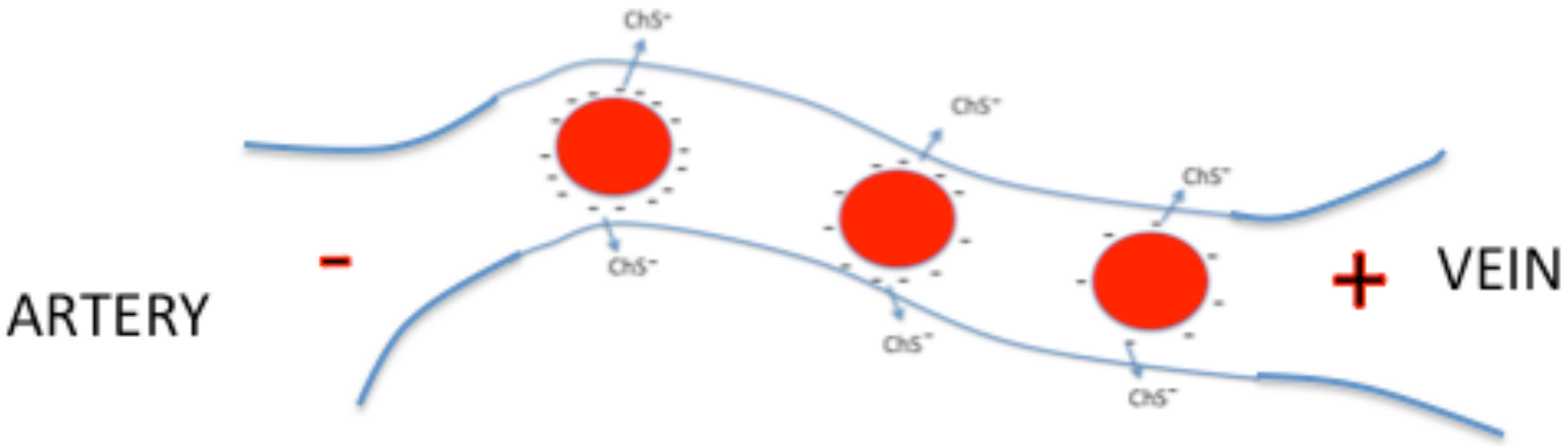
# Negative Charge Builds on Artery Wall*



Red blood cells export cholesterol sulfate to the artery wall, supplying it with cholesterol, sulfate, and negative charge

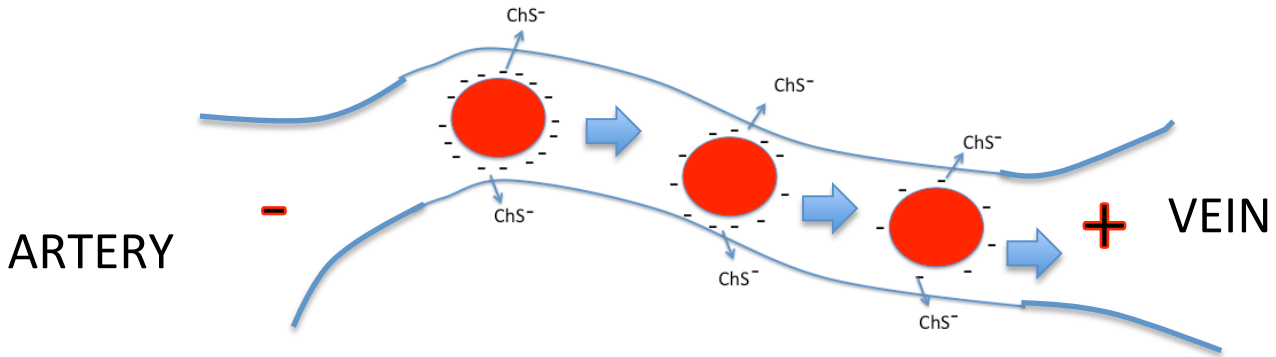
* Davidson and Seneff, Entropy 14, 1399-1442, 2012.

# A Battery!




* Davidson and Seneff, Entropy 14, 1399-1442, 2012.

# Battery Poles Between Artery and Vein




- RBC's lose charge as they travel through the capillary
- This sets up voltage gradient between vein and artery
- Negatively charged RBC's are propelled towards positive pole of "battery"

This creates force field that promotes blood flow

* Davidson and Seneff, Entropy 14, 1399-1442, 2012.

Zeta potential measures rate at which negatively charged particles travel in an electric force field

# Hypothesis

- When blood becomes deficient in negative charge (sulfates), glycocalyx becomes unhealthy
- Endothelial cells develop gaps that allow blood to seep into tissues
- Blood clots are needed to plug the holes
- Mistakes can lead to dangerous blockage
  - Deep vein thrombosis
  - Pulmonary thrombosis

# Recapitulation

- Hemostasis is the regulatory system that maintains blood stability
  - Tension between clotting and hemhorraging
  - Sulfates in the glycocalyx are protective
- Grounding can improve blood stability by providing negative charge through currents from the ground
- Red blood cells discharge negative charge by releasing cholesterol sulfate in capillaries
  - This helps maintain pH difference between arteries and veins to drive circulation

# Outline

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  - Sulfate Deficiency
  - Obesity
  - Endothelial Nitric Oxide Synthase (eNOS)
  - SiNiC
- Consequences
  - Blood Clots and Hemorrhages
  - Cardiovascular Disease
  - Impaired Gut Bacteria
  - Infection
  - Impaired Autophagy
- The Environment
  - Environmental Toxins
  - Polyphenols
- Summary

# Heart Disease

Cardiovascular plaque develops as an alternative mechanism to produce cholesterol sulfate from damaged LDL and homocysteine

# What Happens when Cholesterol Sulfate Synthesis is Impaired??

**Cardiovascular disease!!!**

Activities in Plaque Produce
Cholesterol Sulfate to Supply the Heart

# They Knew a Long Time Ago*

- Article published in 1960
- Fed  cholesterol to monkeys
  - induced atherosclerosis
- If sulfur-containing nutrients are added, atherosclerosis is prevented
- These nutrients provide source of sulfate to enable cholesterol transport

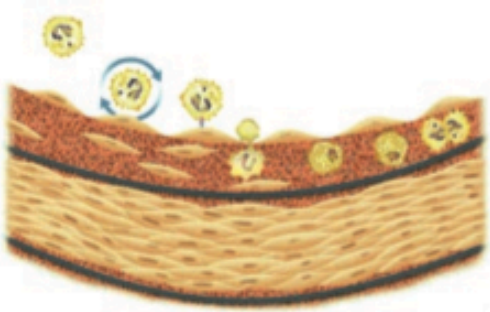
* G.V. Mann et al., Am. J. Clin. Nutr. 8, 491-497, 1960.

# Steps in Atherosclerosis*

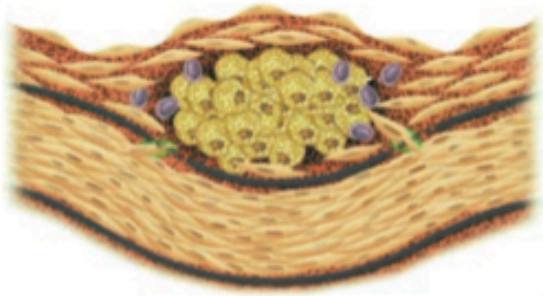
1. Inflamed endothelium provides adhesion molecules to trap and hold macrophages
2. Macrophages through scavenger process take up oxidized LDL and become foam cells
3. Interleukins and growth factors promote proliferation of smooth muscle cells (artery thickening)
4. Extracellular matrix proteins are degraded
5. Vulnerable plaque eruption: thrombosis

* Libby et al., Circulation 105:1135-1143, 2002

# Steps in Atherosclerosis



Entrapment of macrophages



Build up of fatty deposits

Rupture and thrombosis

Adapted from Libby, et al., Circulation 105:1135-1143, 2002

# Many Good Reasons for ROS

- ROS (reactive oxygen species) are a key component of inflammation in the artery
- ROS are needed to produce sulfate *
- Oxidation of glycated LDL makes it accessible to macrophages for breakdown**
- Peroxynitrite (product of reaction between superoxide and nitric oxide) is toxic to pathogens***

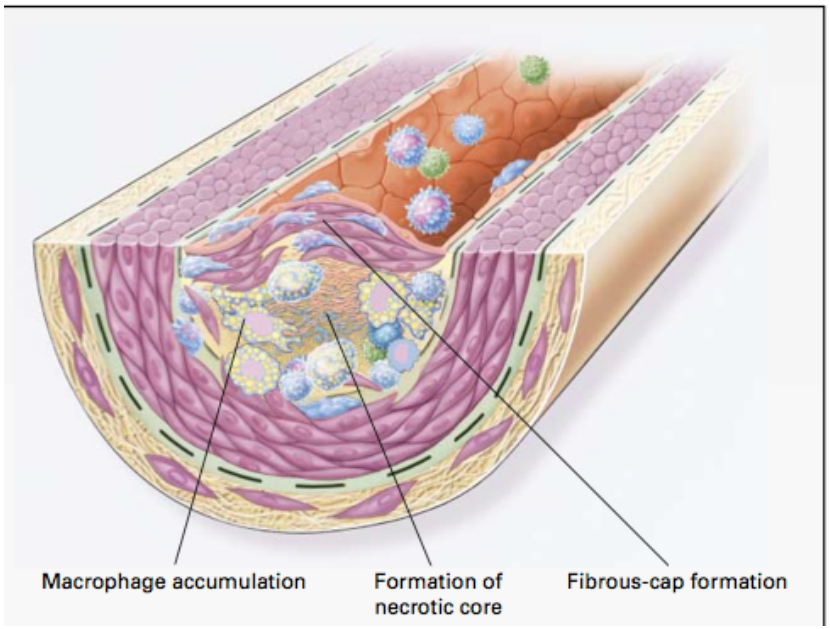

* Mitsuhashi et al. Shock 24(6) 529-34, 2005.

** Kaplan and Aviram, Arterioscler Thromb Vasc Biol 21(3) 386-93 2001.

*** Alvarez et al., J. Biol. Chem. 286, 6627-6640, 2011.

# Macrophages and Cholesterol*

- Macrophages in artery wall take up oxidized LDL and export extracted cholesterol to HDL-A1
- Unsaturated fatty acids interfere with export process
- Macrophages eventually become damaged by exposure to oxidizing and glycating agents → necrotic core



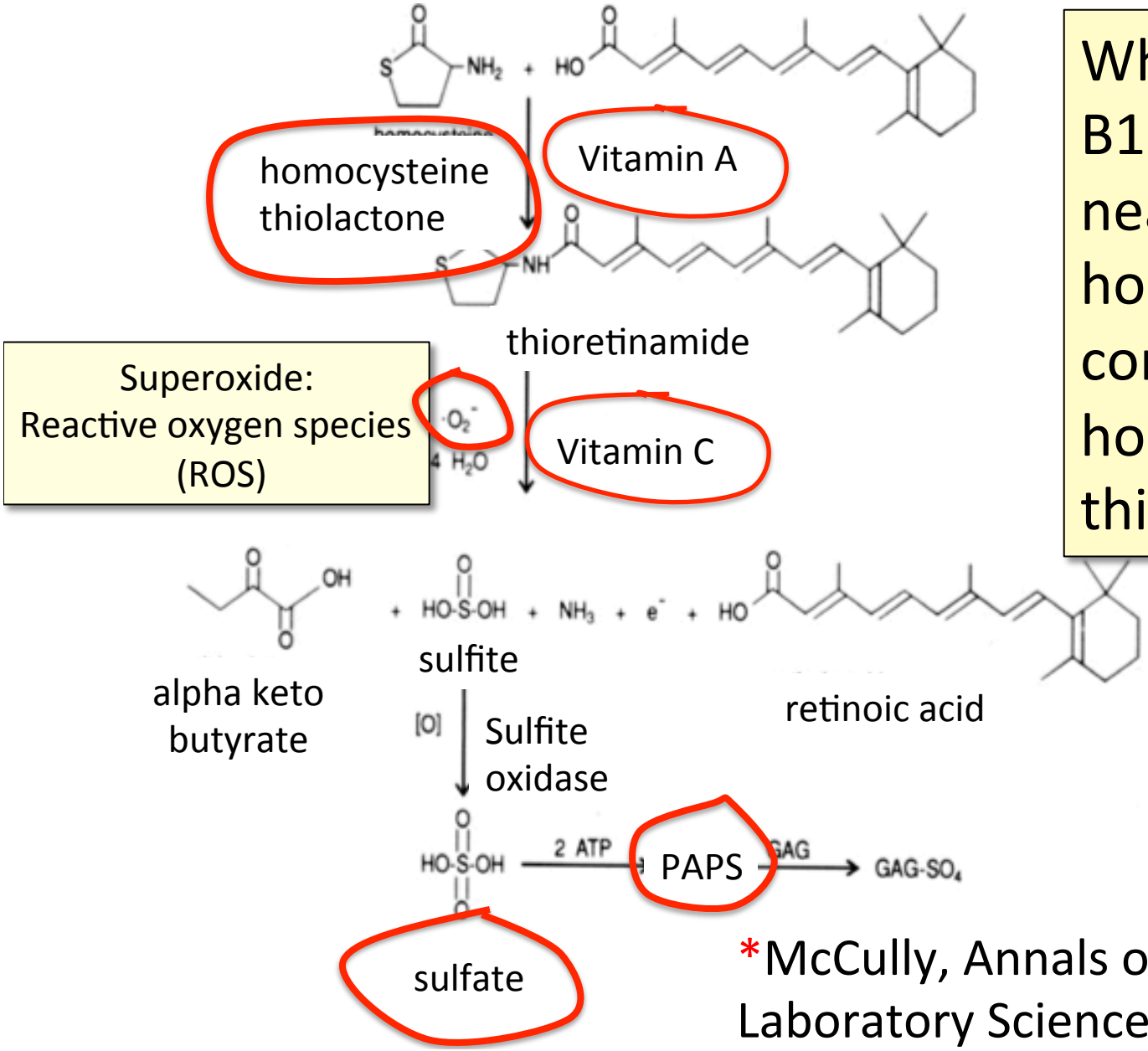

* Wang and Oram, J. Biol. Chem. 277 (7) , 5692–5697, 2002

# Quote from the Abstract*

"These findings raise the possibility that an increased supply of *unsaturated* fatty acids in the artery wall promotes atherogenesis by impairing the ABCA1 cholesterol secretory pathway in macrophages."

* Wang and Oram, J. Biol. Chem. 277 (7) , 5692–5697, 2002

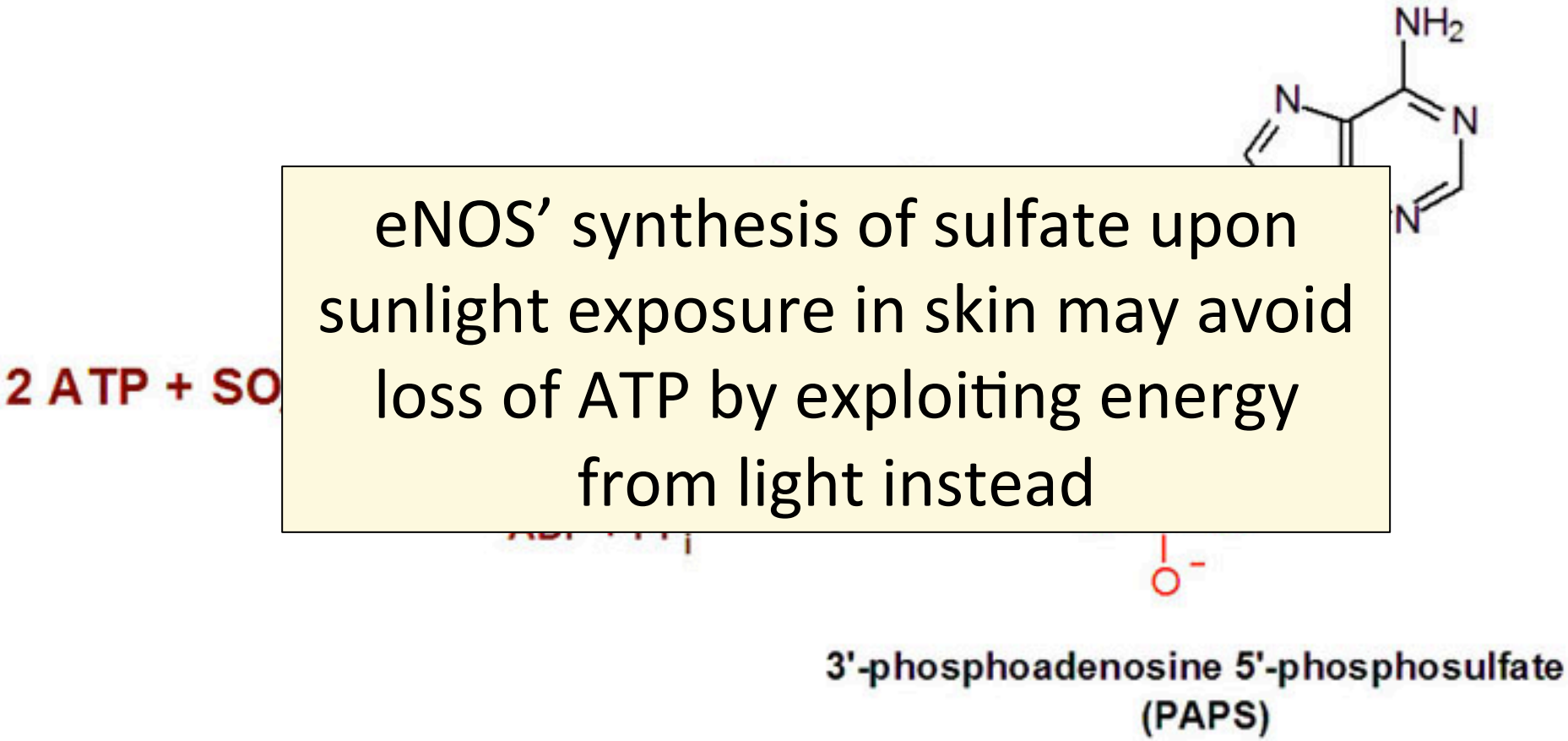
# Pathway from Homocysteine to Sulfate*




When folate and B12 are deficient, nearly all homocysteine is converted to homocysteine thiolactone

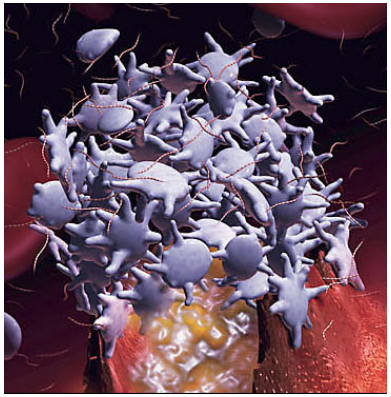
*McCully, Annals of Clinical and Laboratory Science 41:4, 300-313, 2011

# Synthesis of PAPS consumes ATP




eNOS' synthesis of sulfate upon sunlight exposure in skin may avoid loss of ATP by exploiting energy from light instead

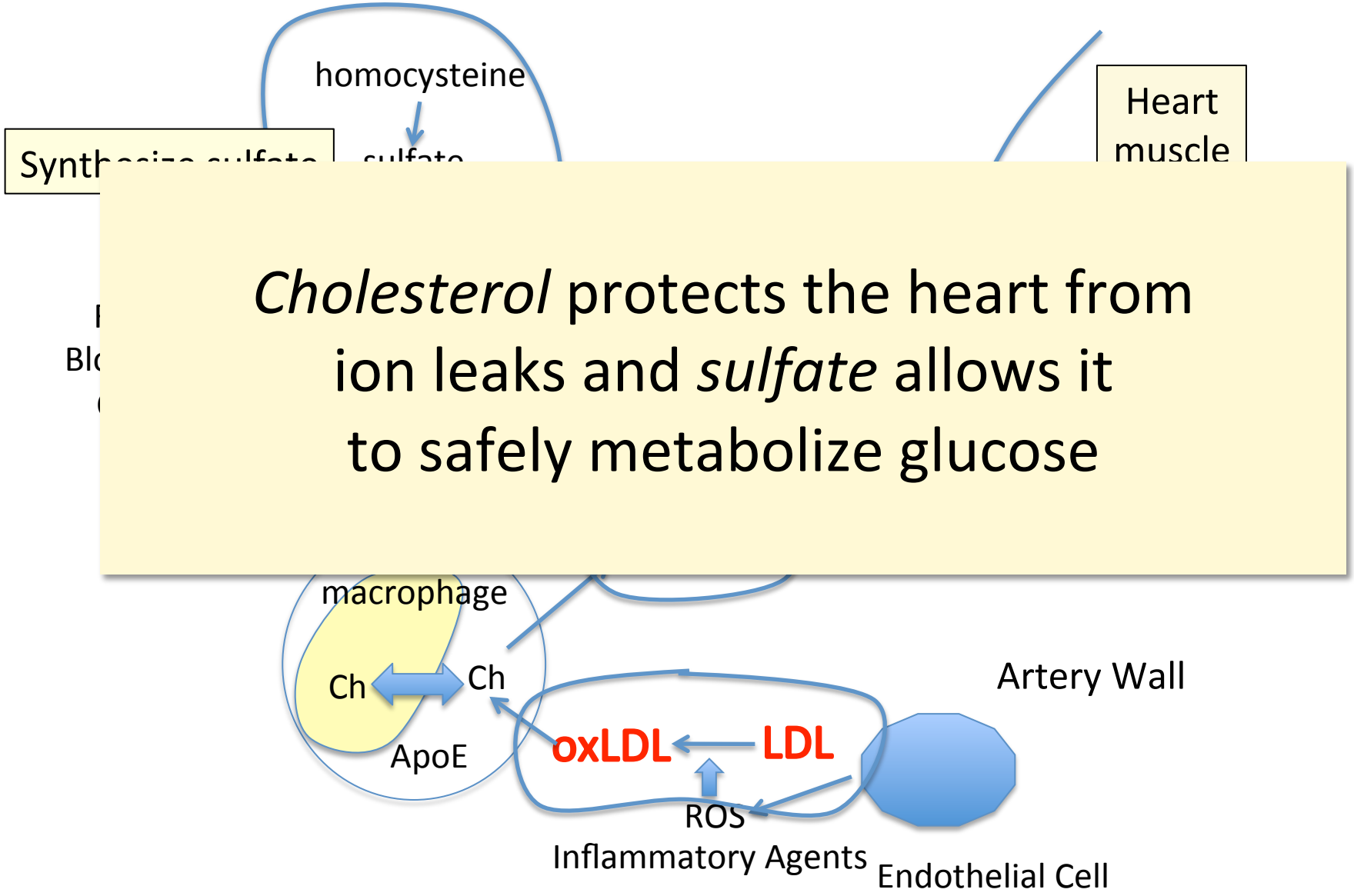
# Platelets and Cholesterol Sulfate*



- Platelets and RBCs both synthesize cholesterol sulfate (Ch-S)
  - Ch-S is present in the atherosclerotic lesions in the aorta
  - Platelets will accept cholesterol only from HDL-A1
  - Platelet synthesis rate increases 300-fold when PAPS is available.
  - PAPS is formed from ATP and sulfate
- Platelet aggregation leads to thrombosis
  - HDL suppresses aggregation; LDL promotes it

* Yanai et al, Circulation 109, 92-96, 2004

# Putting it All Together




*Cholesterol* protects the heart from ion leaks and *sulfate* allows it to safely metabolize glucose

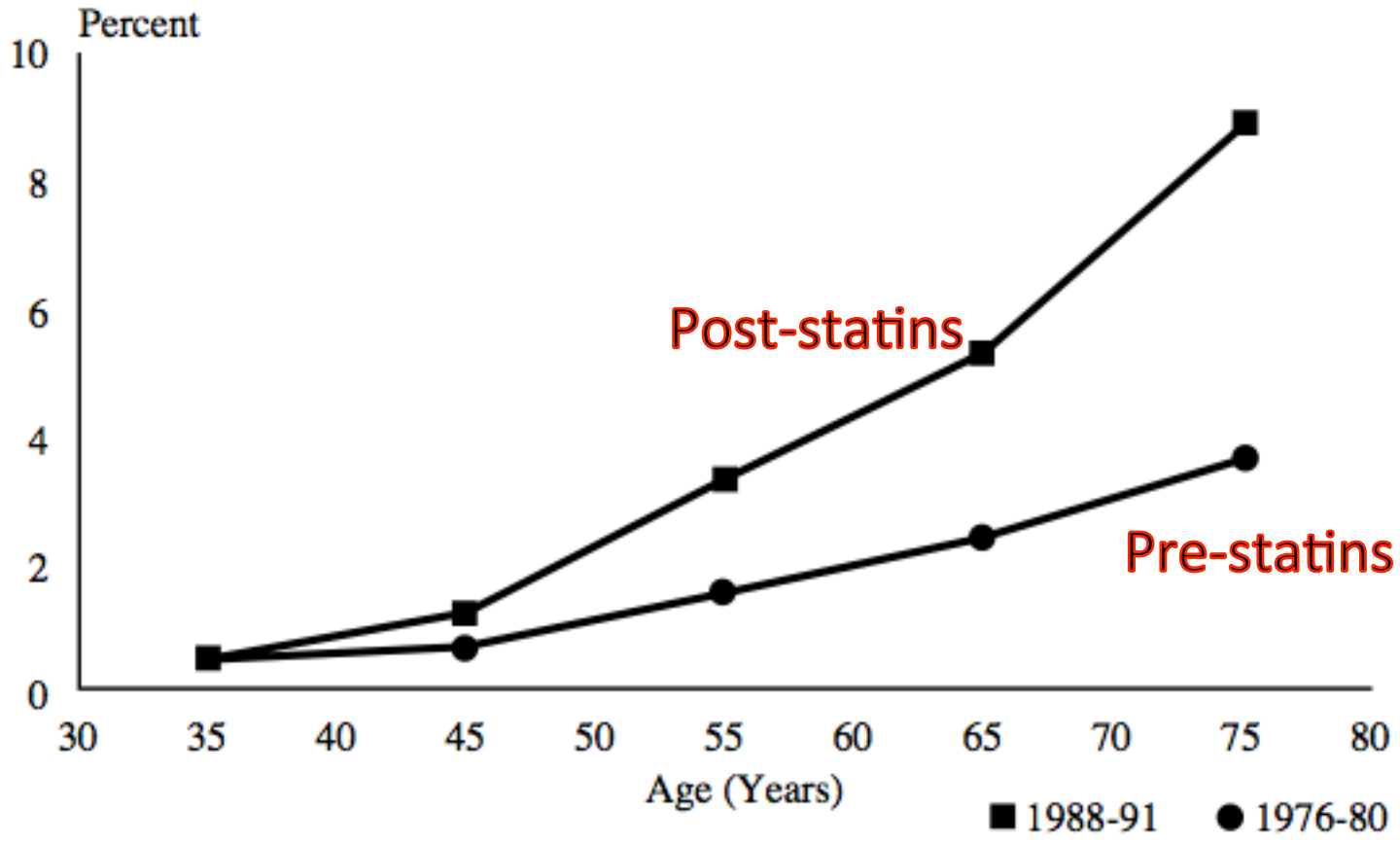
# Steps in Atherosclerosis: Reinterpreted

1. Endothelial cells lining artery walls feeding the heart release inflammatory agents
2. Macrophages infiltrate artery wall
3. Macrophages extract cholesterol from oxidized LDL and deliver it to HDL-A1.
4. Platelets extract cholesterol from HLD-A1 and convert it to cholesterol sulfate, with help from PAPS
5. Macrophages die and build up necrotic core

I hypothesize that treatments aimed at reducing the supply of cholesterol to the plaque will eventually lead to severe deficiencies in cholesterol and sulfate supply to the heart, resulting in heart failure.

**Figure 5**

**Prevalence of CHF, by Age, 1976-80 and 1988-91**




*Source: National Health and Nutrition Examination Survey (1976-80 and 1988-91), National Center for Health Statistics.*

**National Heart, Lung, and Blood Institute National Institutes of Health Data Fact Sheet**

# Recapitulation

- I believe that cardiovascular disease can be best characterized as a factory to supply *cholesterol* and *sulfate* to the heart
- *Unsaturated* fats, but not saturated fats, interfere with the supply chain
- Statin drugs, through their ability to deplete the supply of cholesterol to the plaque, can lead to heart *failure* down the road

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# Impaired Gut Bacteria

# Gut Bacteria and Health*

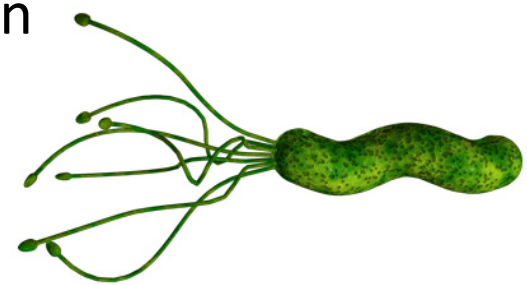
- We are an ecosystem: a community of interacting cells
- Trillions of bacteria live in our body
- They outnumber our own cells 10:1
- Killing them off may make us sick or fat
- 5-8 million microbial genes in our bodies (3 million in digestive system from more than 1000 species)
- They play very essential roles in our bodies
  - Digest foods, absorb nutrients, provide enzymes, make vitamins and antiinflammatories
  - Regulate appetite and brain function
- Vaginal birth matters
- Everyone has unique set of gut bacteria

* NPR's On Point: Your Inner Ecosystem, http://onpoint.wbur.org/2012/06/20/bacteria-2

# "Our microbes are under threat — and the enemy is us"*

"Microbiome"

- Microbes in our body outnumber us 10:1
  - 99% of them are benign or offer protection
  - Synthesize vitamins B12 and folate
- Birth through birth canal (v.s. C section)
  - Infant picks up mother's microbiome
- Colostrum provides food for gut bacteria
- Industrialized countries are too clean
  - Drastically altered microbiome
    - H. pylori 90% --> < 10% in last hundred years in U.S.
    - Esophageal cancer and childhood asthma have risen in step



* Liz Szabo, USA Today,
http://www.usatoday.com/news/health/story/2012-07-13/body-bugs-microbes/56255904/1

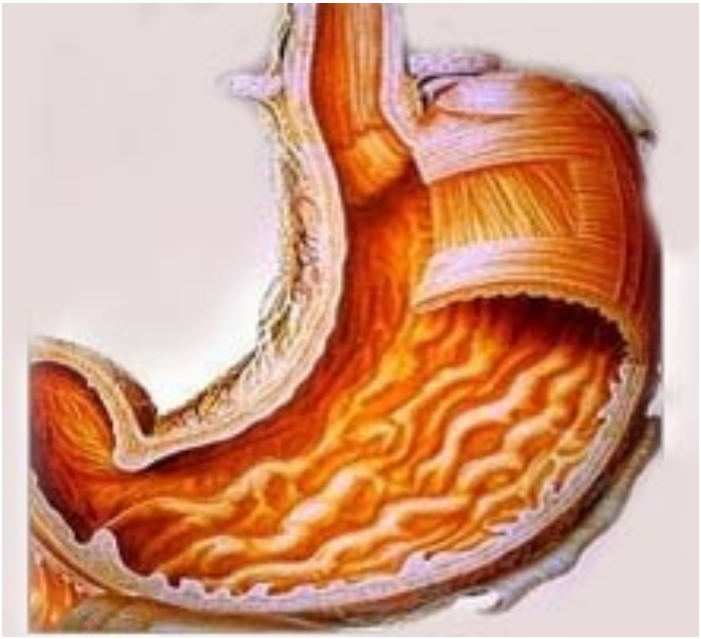
# H. pylori: There are Benefits!*

- The Western world has now significantly reduced the presence of H. pylori in the gut
  - This has reduced the incidence of stomach ulcers and stomach cancer
  - But it has also increased the incidence of esophageal reflux disease and esophageal cancer
- H. pylori serve a useful role in reducing acid production by stomach: excess acid aggravates esophagus
- We host a colony of bacteria and they work together with us for a jointly beneficial solution

*Caroline Hadley, EMBO *reports 7(5), 471-473, 2006*

# "Folate synthesized by bacteria in the human upper small intestine is assimilated by the host"*

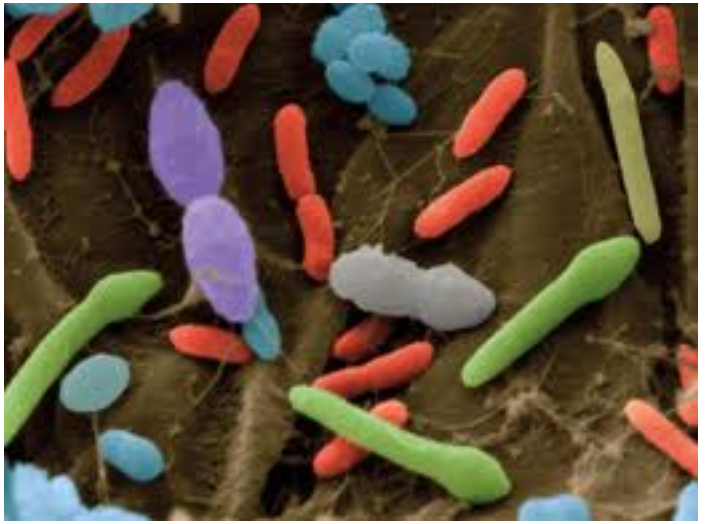
- Folate synthesized by flora in the small intestine is absorbed through the gut
- Atrophic gastritis associated with higher duodenal pH
  - Leads to bacterial overgrowth
  - Increased supply of folate
- This could be an important *benefit* to assure adequate folate



* E. Camilo et al., Gastroenterology 110(4), 991-998, 1996

# Wild Speculation

- Leaky gut and leaky skin and leaky blood brain barrier lead to infiltration of microbes from gut or skin into brain
- Bacteria are “lured” into the body & into the brain and then killed and harvested to renew important nutrients
- Possible nutrients include:
  - Vitamin B12 (cobalamin), vitamin B9 (folate), thiamine, vitamin K and heparan sulfate



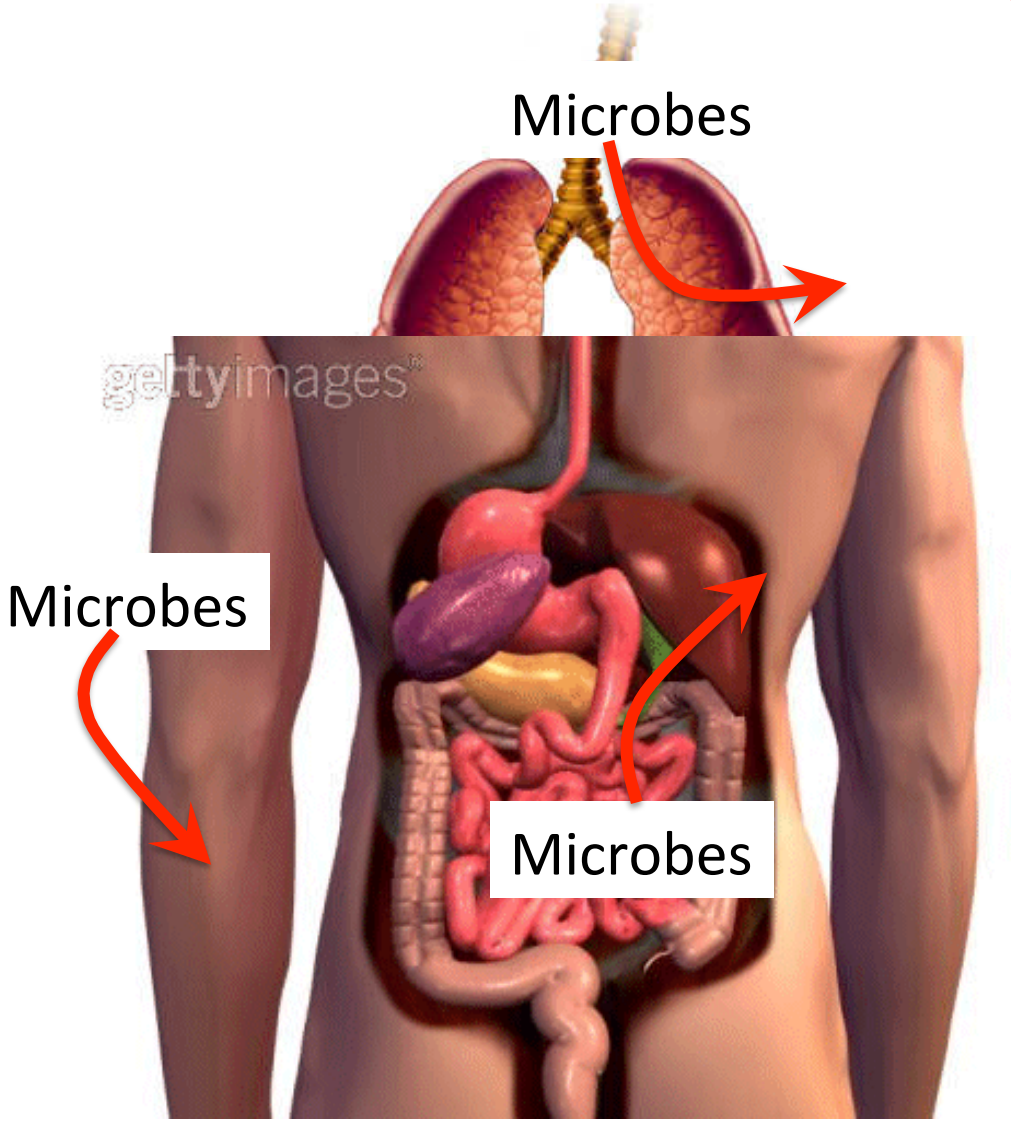
# "The Infection Connection"*

- Barry Marshall drank H. pylori broth and developed gastritis
  - Proved that H. Pylori "causes" stomach ulcers
- Other examples:
  - Cervical cancer & HPV
  - Liver cancer & Hepatitis B and C
  - Lymphoma & Epstein-Barr virus
  - Cardiovascular disease & chronic infection?
  - Alzheimer's & brain infection?

*Caroline Hadley, EMBO *reports 7(5), 471-473, 2006*

# What Causes Increased Infection?

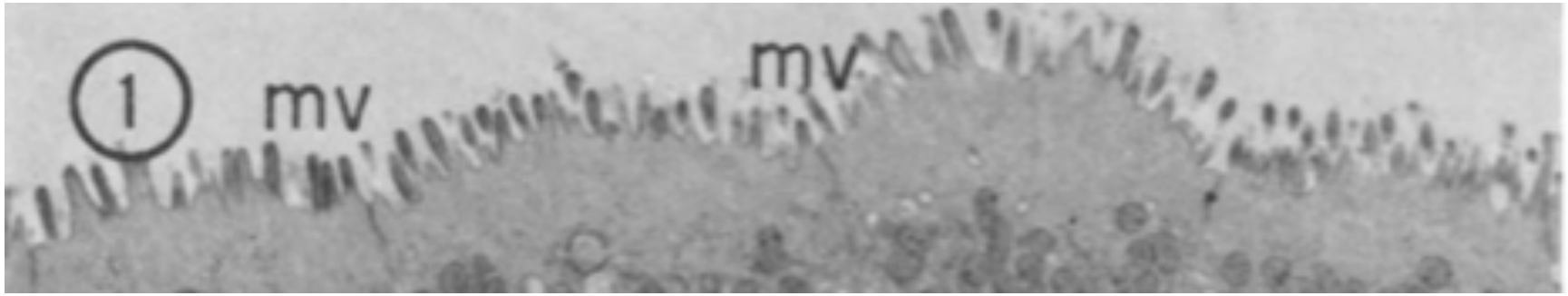
## Impaired Cholesterol Sulfate Synthesis!



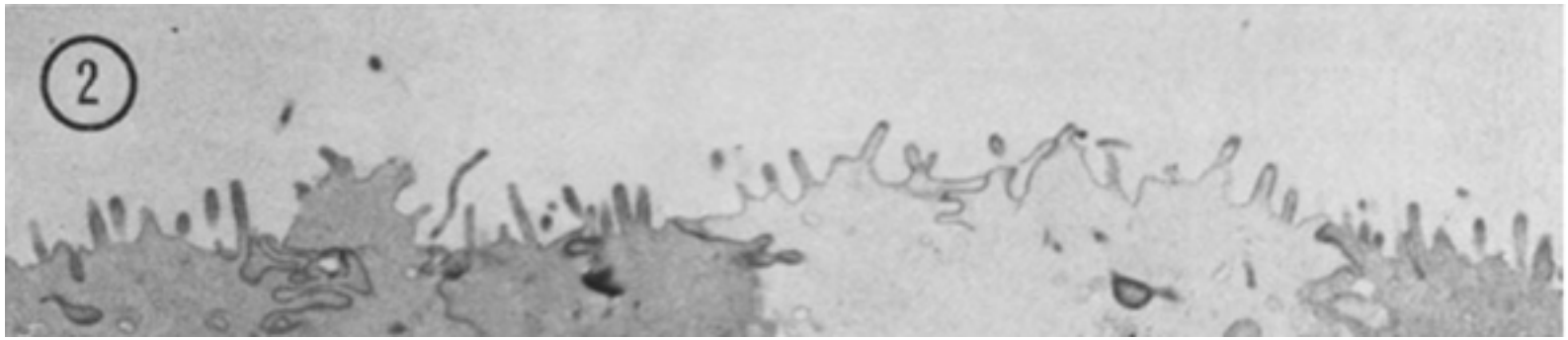
# Leaky Gut

- Collitis and Crohn's disease are associated with significantly reduced sulfate in the GAGs in the walls of the intestines
- Cells are defective in transporting nutrients
  - Leads to severe vitamin deficiencies (e.g., B12)
- Tight junctions between cells become loose
- Microbes can penetrate the gut wall and enter the blood stream
  - They can be harvested by macrophages!

# Microvilli in Gall Bladder Epithelium*



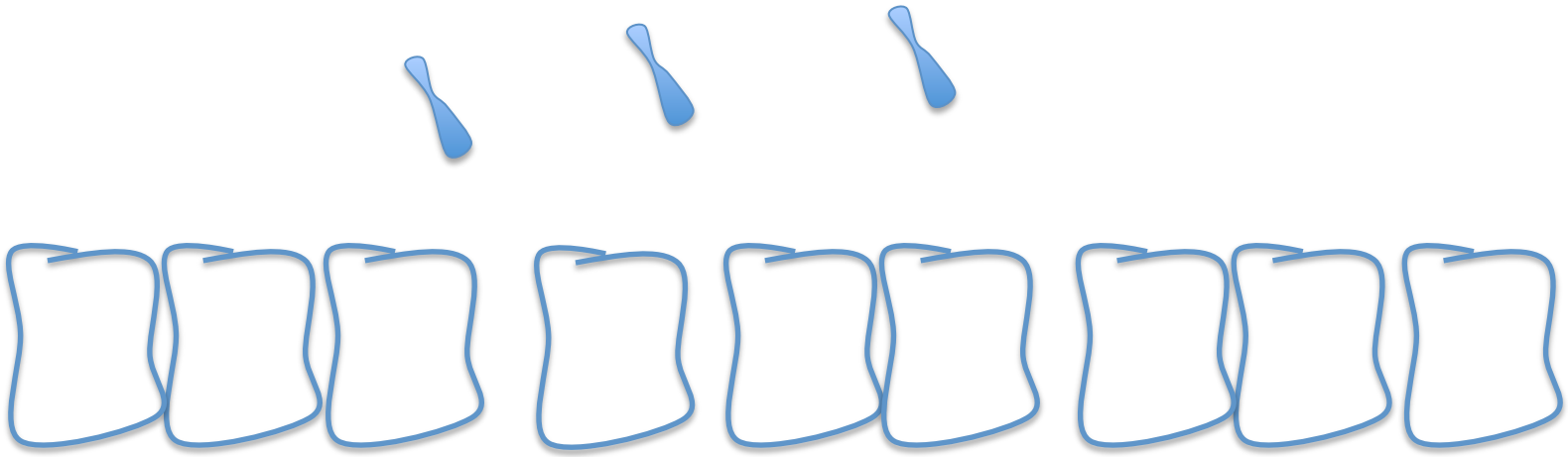

Healthy – negative charge makes them stand tall



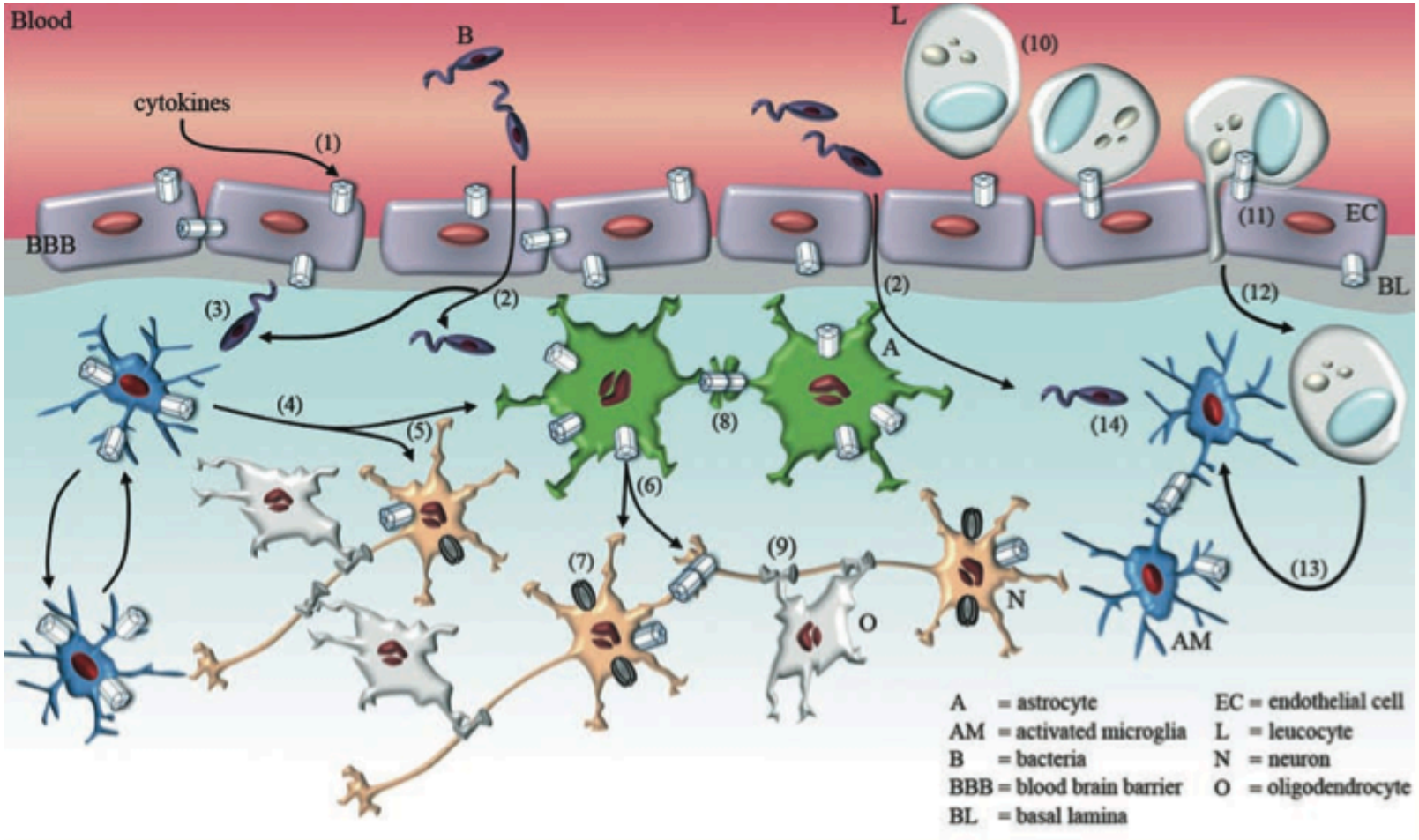

Unhealthy – addition of cationic polymer destroys them

* Figures 1 and 2 in Quinton and Philpott, J. Cell Biol. 56, 1978, 787-796.

# Bacteria can Swim Across Gut Barrier!



# Microbial Invasion through BBB*




* Figure 5, p. 380, J.A. Orellana et al., Antioxidants and Redox Signaling 11(2), 2009

# The Good Side of Infection and Inflammation in the Brain

- Inflammation activates microglia to phagocytose dying cells and Amyloid beta
- Bacterial exposure promotes phagocytosis of dying cells
- *Both dying cells and bacteria can be recycled into new raw materials!*
  - *These nutrients may be essential to brain health*

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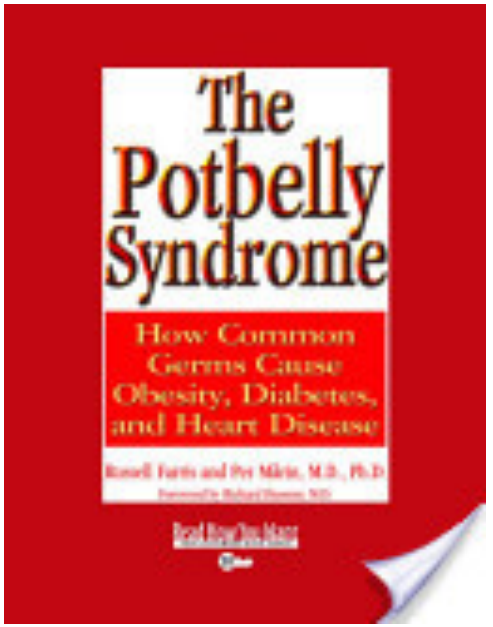
# Infection

Susceptibility to infection is a consequence of impaired cholesterol sulfate synthesis, which introduces widespread pathology

Infection serves a useful role in resupplying critical nutrients such as cobalamin, folate, and heparan sulfate

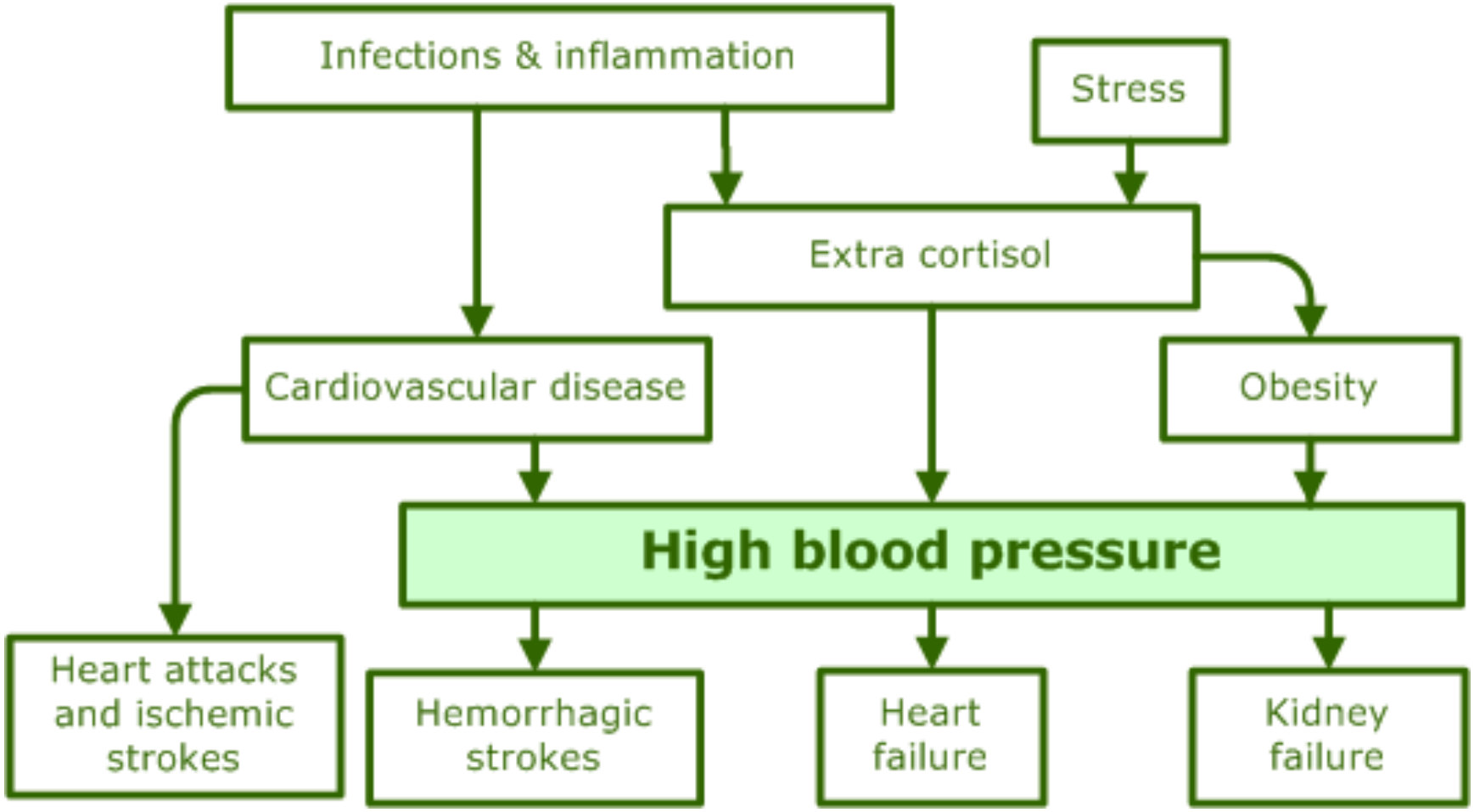
# The Falling Apart Syndrome*

- The key message: All chronic diseases can be linked to infections
- The author, Russell Farris, previously published another book:
  - The Potbelly Syndrome: How Common Germs Cause Obesity, Diabetes and Heart Disease.




* Russell Farris, http://www.polymicrobial.com

# Figure from Chapter 6 in The Potbelly Syndrome*

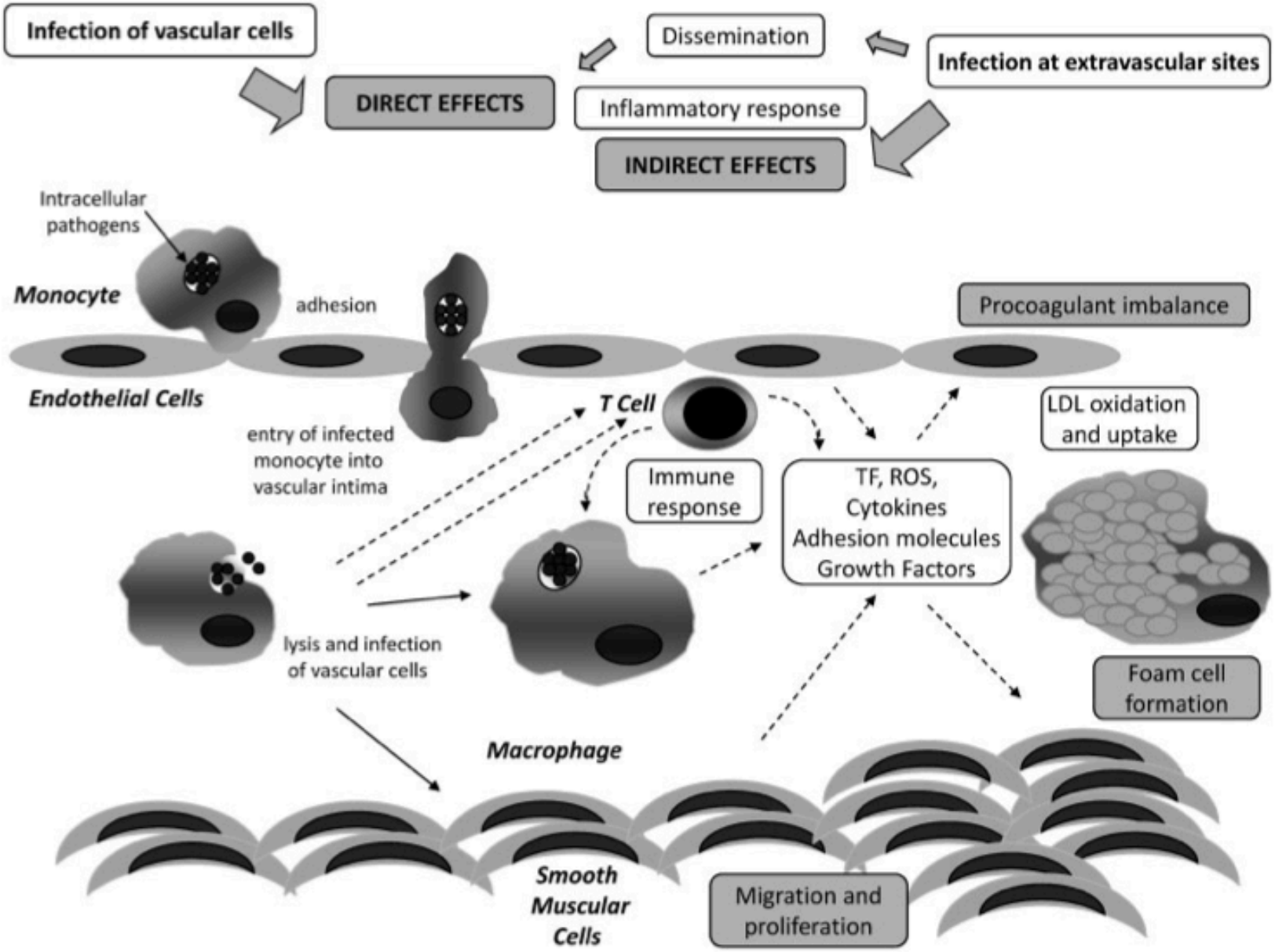



* By Russell Farris

# A Possible Cause-and-Effect Relationship

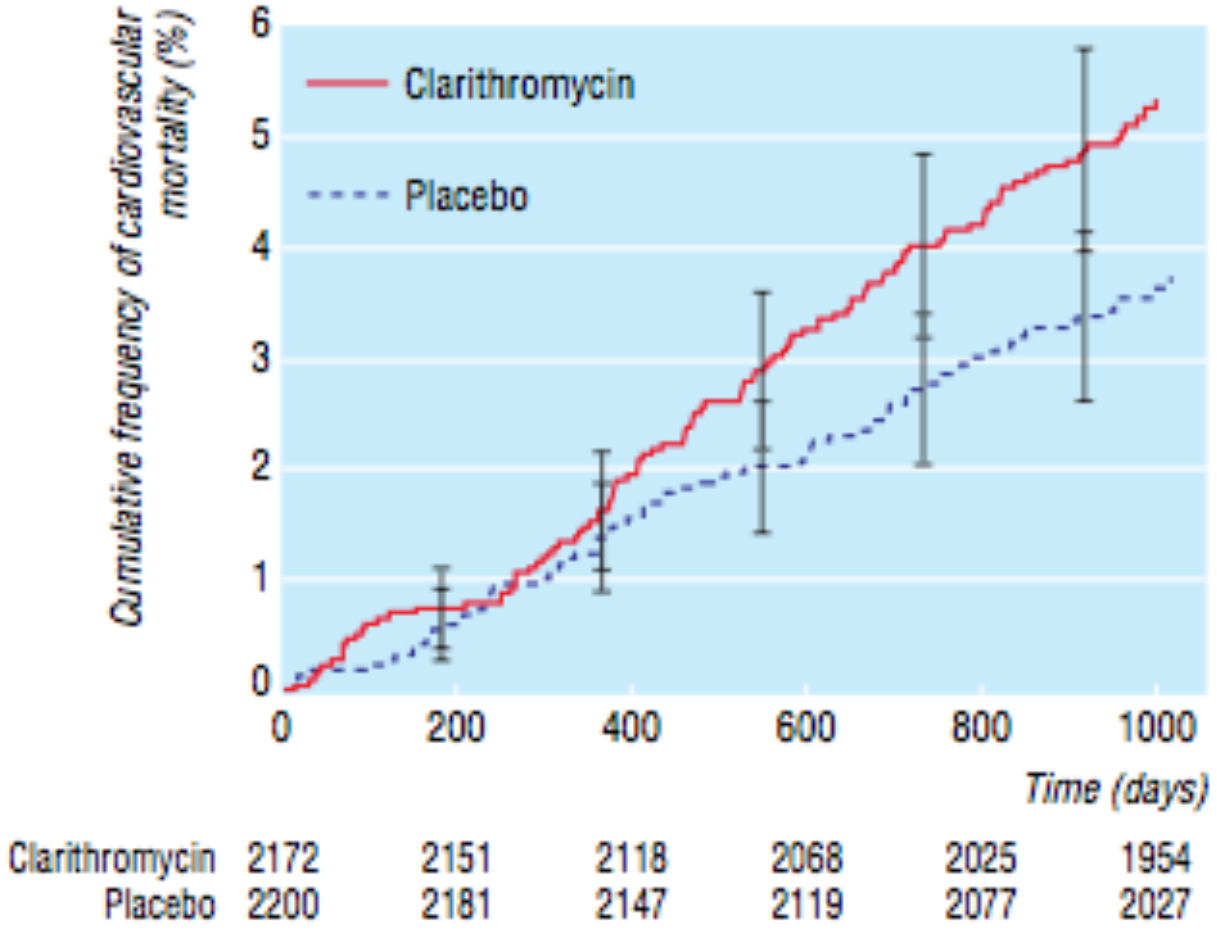
- First comes impaired barriers, impaired nutrient transport and impaired blood colloidal suspension
- Certain nutrients are desperately needed to fix the problems: e.g., cobalamin, vitamin K, heparan sulfate
  - Bacteria are invited in to provide those nutrients!
- The disease may be uncomfortable, but the alternative may be worse!

# Infection and Atherosclerosis*




* A. Tufano et al., Semin Thromb Hemost 2012(38), 515–523.

# What Happens if you Treat with Antibiotics?*




* A. Tufano et al., Seminars in Thrombosis & HemostasisVol. 38(5), 2012, 515-523.

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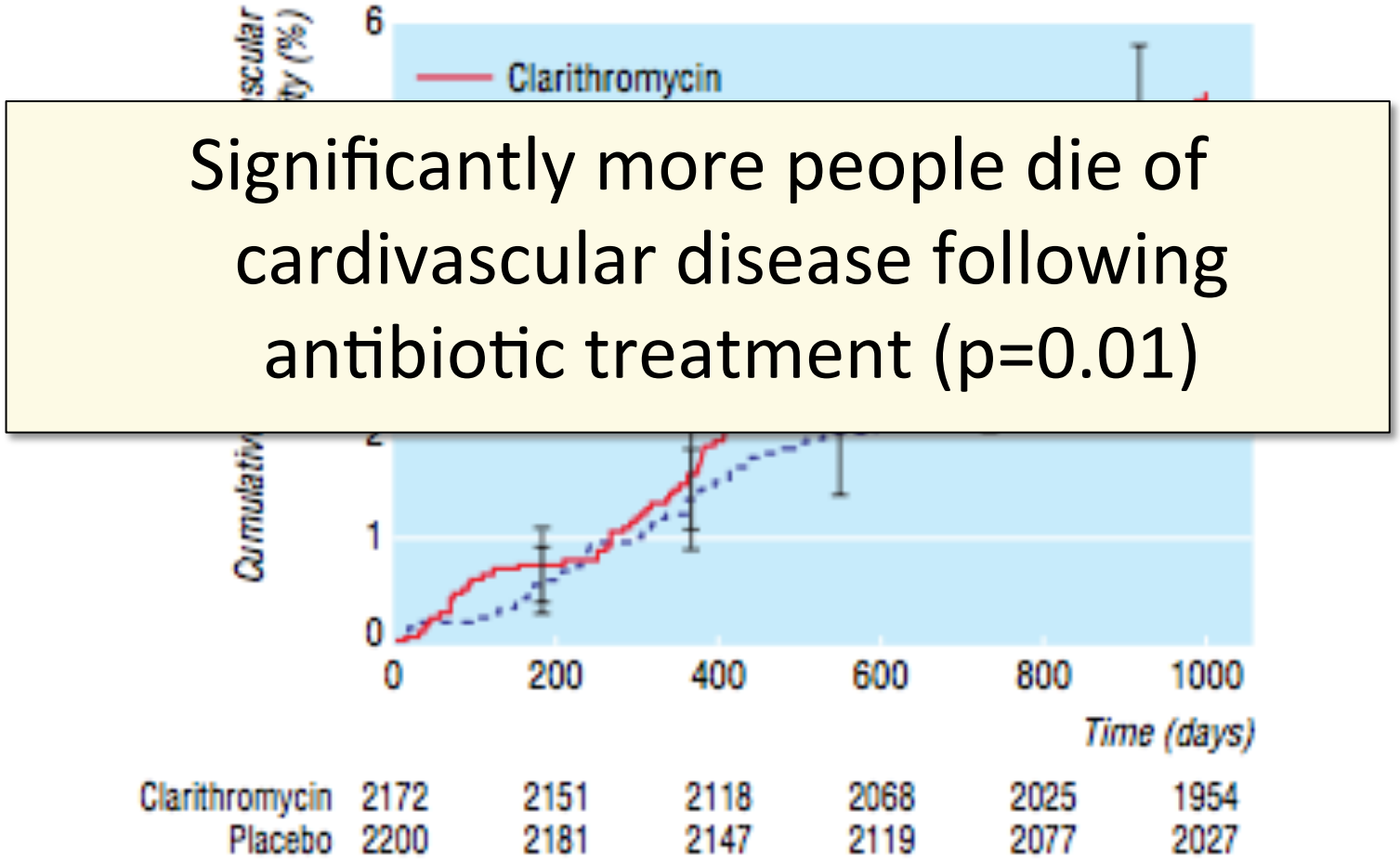
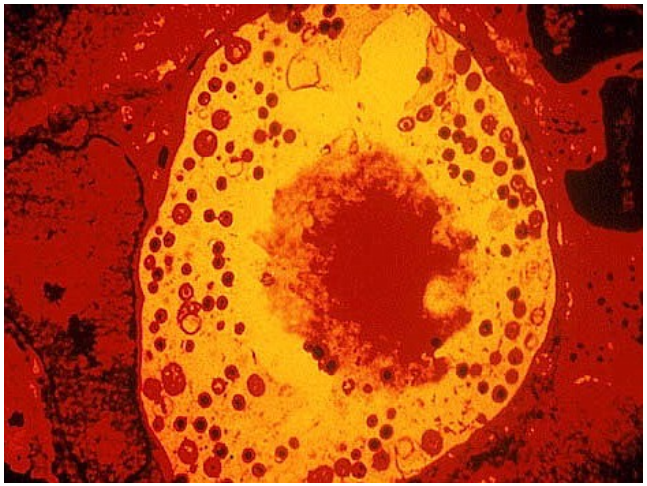



Significantly more people die of cardivascular disease following antibiotic treatment (p=0.01)

* A. Tufano et al., Seminars in Thrombosis & HemostasisVol. 38(5), 2012, 515-523.

# Chlamydia Produce Heparan Sulfate!*



- Chlamydia are viable only inside host cells
- They set up housekeeping in vacuoles within the cell (e.g., a macrophage in the plaque)
- They produce a glucosamine-containing sulphated polysaccharide that is nearly indistinguishable from heparan sulfate

* S.J. Rasmussen-Lathrop et al, Cell Microbiol. 2000 Apr, 2(2), 137-44.

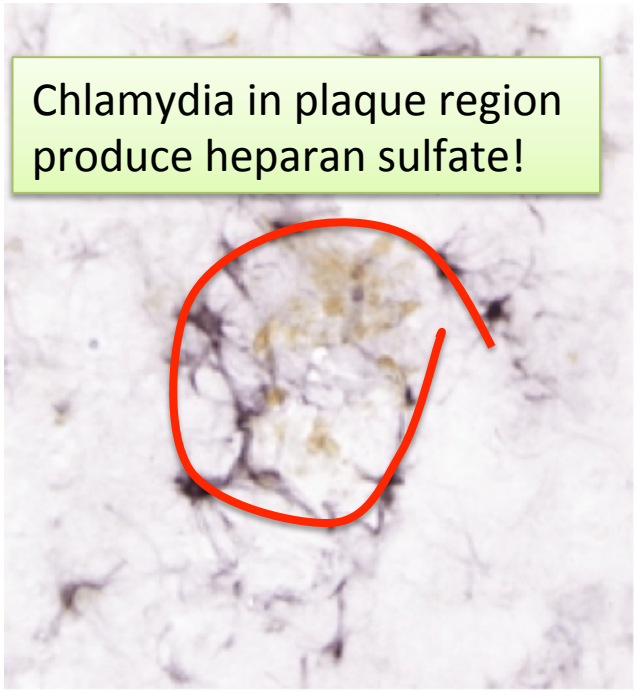
# Special Issue Devoted to Alzheimer's and Infection:* Key Points

- Pathogens can produce progressive chronic diseases like Alzheimer's, asthma, and heart disease
- Alzheimer himself proposed involvement of infective agents in Alzheimer's 100 years ago
- Pathogens stimulate inflammation
- Pathogens evade host defenses and establish chronic latent infections
- Persistent superoxide, nitric oxide and peroxynitrite (ROS) cause DNA damage and apoptosis and alter gene expression
- Environmental toxins and poor nutrition weaken immune system and provide opportunity to bacteria and viruses

* Special Issue of the Journal of Alzheimer's Disease, 2008

# Amyloid Plaque Structure*

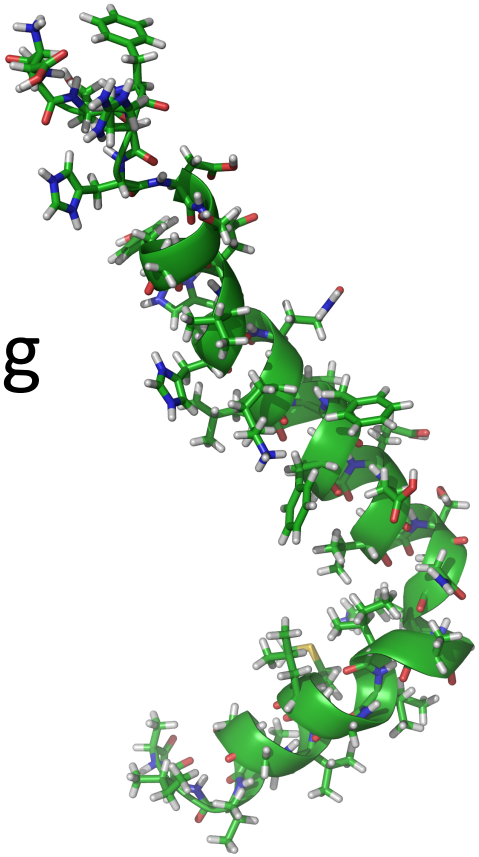
- Microglia and amyloid plaque accumulate side-by-side in a central region surrounded by astrocytes
- Microglia harbor dormant bacteria
- Should the bacteria leave, they will encounter the plaque, which will kill them
- The astrocytes guard the gates and shield the neurons from the damaging plaque




* Microphotograph from Schwab et al., Journal of Alzheimer's Disease 13 359–369, 2008.

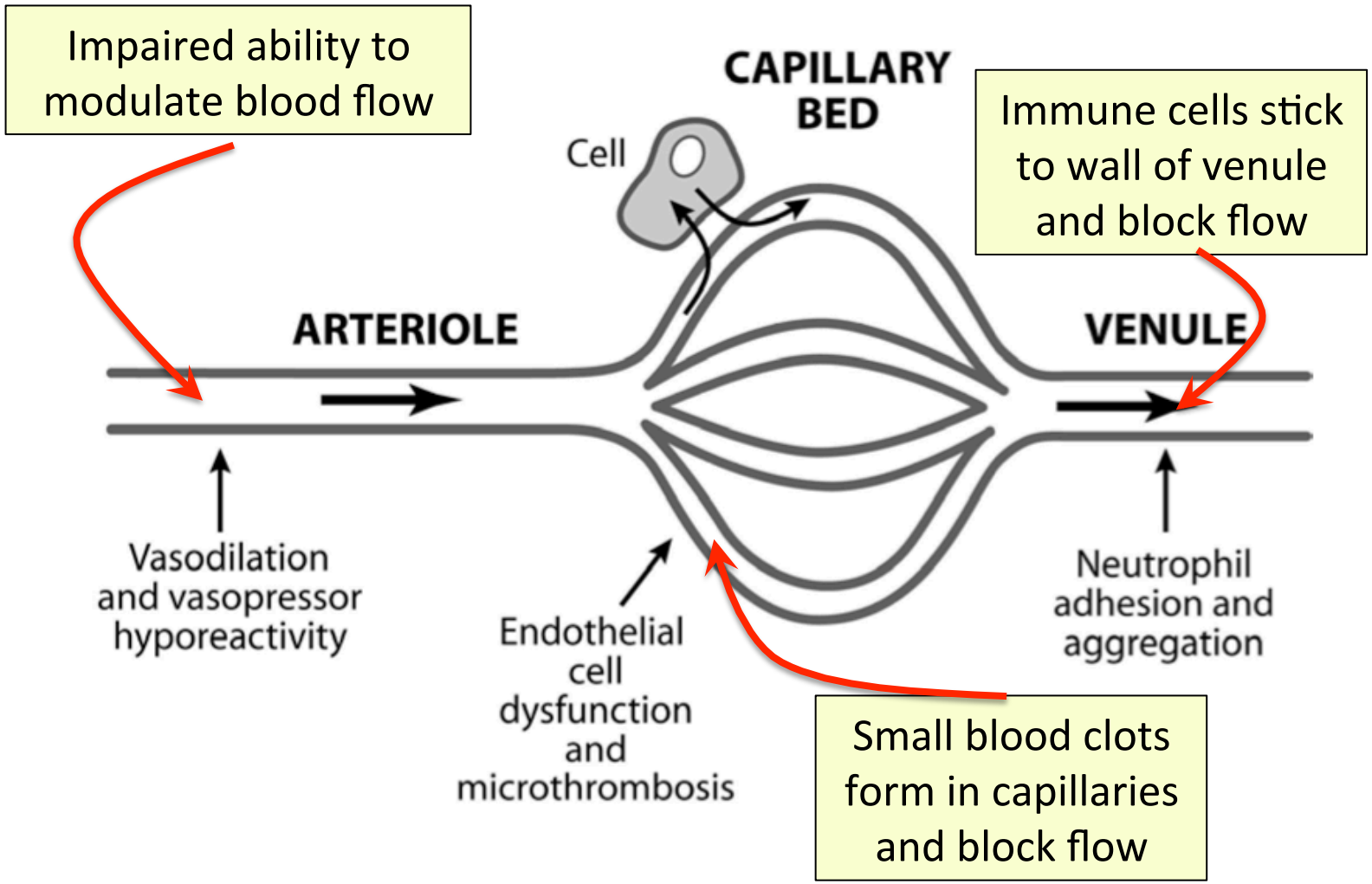
# Amyloid Beta and Nitric Oxide*

- Aβ produced by the neuron induced increased synthesis of nitric oxide
- Nitric oxide reacts with superoxide to produce peroxynitrite
- Peroxynitrite is extremely damaging to mitochondria and disrupts their function
- Cell commits apoptosis due to insufficient energy supply



* U. Keil et al., J. Biol. Chem, 2004 279(48), 50310-50320

# Sepsis and the Vasculature*




* S. Trzeciak et al., Acad Emerg Med. 2008 May ; 15(5): 399–413

# Hypothesis

Blood flow needs to nearly shut down during sepsis; organs need to shut down; the entire body focuses on harvesting heparan sulfate from the invasive microbes to recover blood stability.

# GSK-3: Inducing Inflammation*

- GSK-3 is a protein that interferes with insulin-mediated glucose uptake and with glucose storage into glycogen
- It also promotes inflammation in association with mood disorders, Alzheimer's disease, diabetes, and cancer
- This shows a clear link between glucose impairment and inflammation

* R.S. Jope et al., *Neurochem Res. 2007 ; 32(4-5): 577–595.*

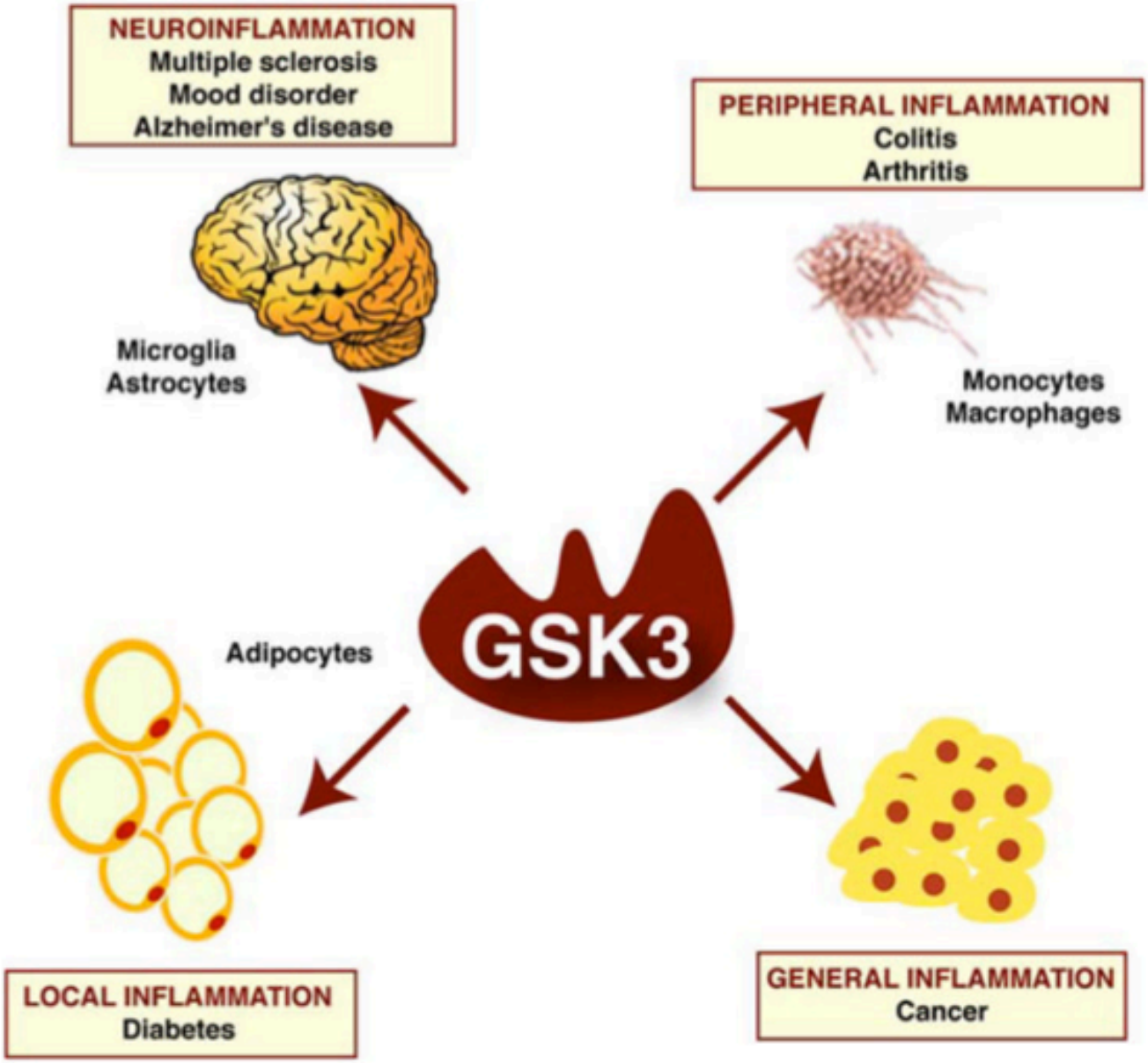



Figure 2 from R.S. Jope et al., *Neurochem Res. 2007 ; 32(4-5): 577–595.*

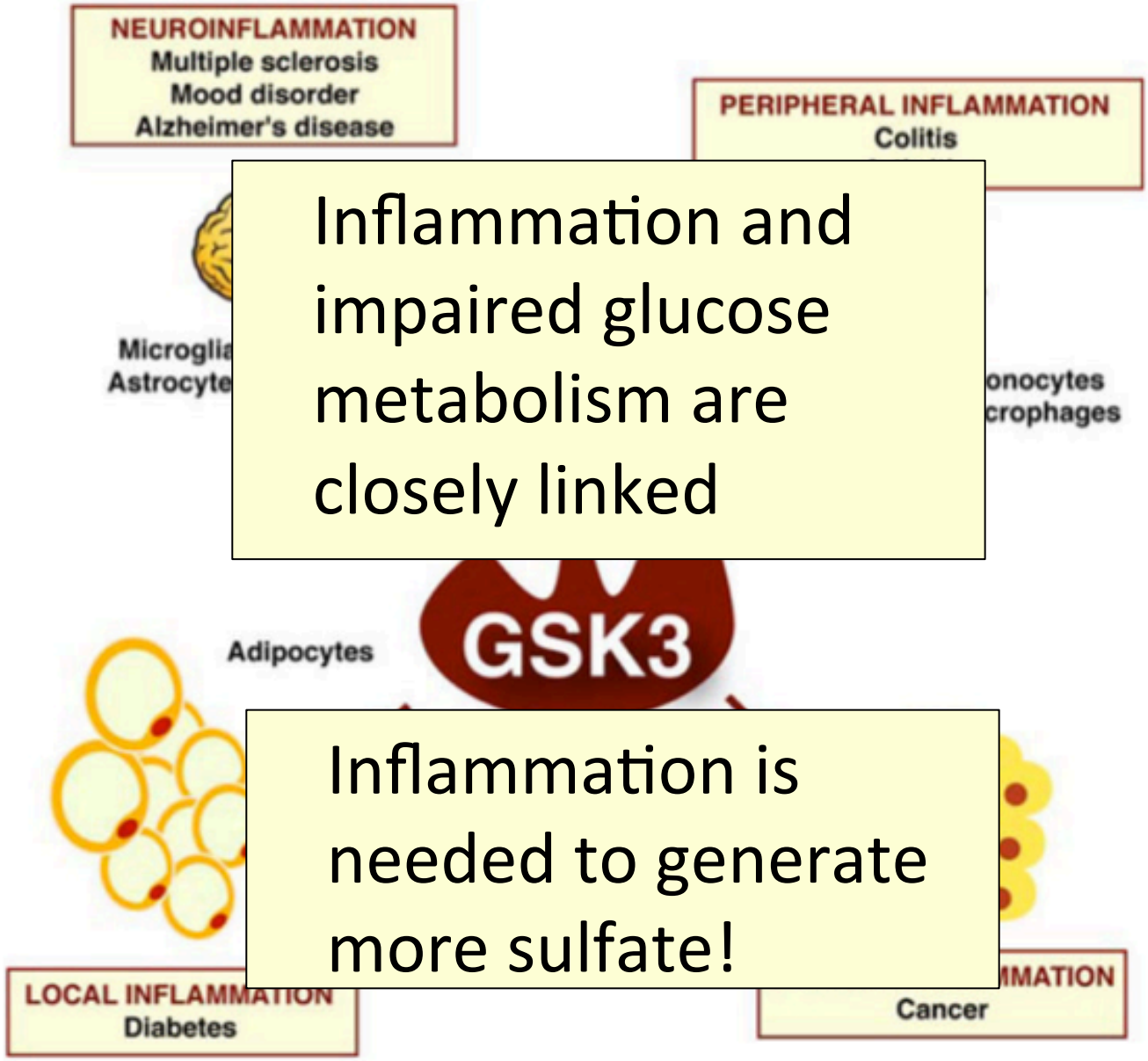



Figure 2 from R.S. Jope et al., *Neurochem Res. 2007 ; 32(4-5): 577–595.*

# Recapitulation

- Infection is associated with many chronic diseases like heart disease and Alzheimer's
  - Treating with antibiotics doesn't help
  - Infection serves useful role in supplying nutrients?
  - Support comes from Chlamydia's ability to produce heparan sulfate
  - Amyloid beta plaque provides home for microbes
  - Sepsis reflects blood melt-down due to sulfate deficiency
- Inflammation and impaired glucose metabolism are closely linked
  - Linking element is sulfate deficiency?

# Outline

- Introduction
  - Big Ideas: Dysfunction and Consequences
- Dysfunction
  - Cholesterol Transport
  - Sulfate Deficiency
  - Obesity
  - Endothelial Nitric Oxide Synthase (eNOS)
  - SiNiC
- Consequences
  - Blood Clots and Hemorrhages
  - Cardiovascular Disease
  - Impaired Gut Bacteria
  - Infection
  - Impaired Autophagy
- The Environment
  - Environmental Toxins
  - Polyphenols
- Summary

# Impaired Autophagy

Autophagy is the process by which cells dispose of the garbage that accumulates with living - they can recycle misfolded proteins into new proteins and replace broken mitochondria with fresh ones.

Excess nitric oxide leads to impaired autophagy, which results in accumulation of debris and busted mitochondria. Over time, the cell becomes so impaired that it has to shut down.

# Inflammation and Repair*

- Inflammation is at the root of a host of diseases, from diabetes to cancer
  - Induces permeability of vascular walls and movement of immune cells into the tissues
  - Leads to production of ROS and tissue damage
- Acute inflammation follows infection or tissue injury
- Chronic inflammation characterizes diseases like heart disease, diabetes, and Alzheimer's disease
- Cell debris is *phagocytized* by leukocytes

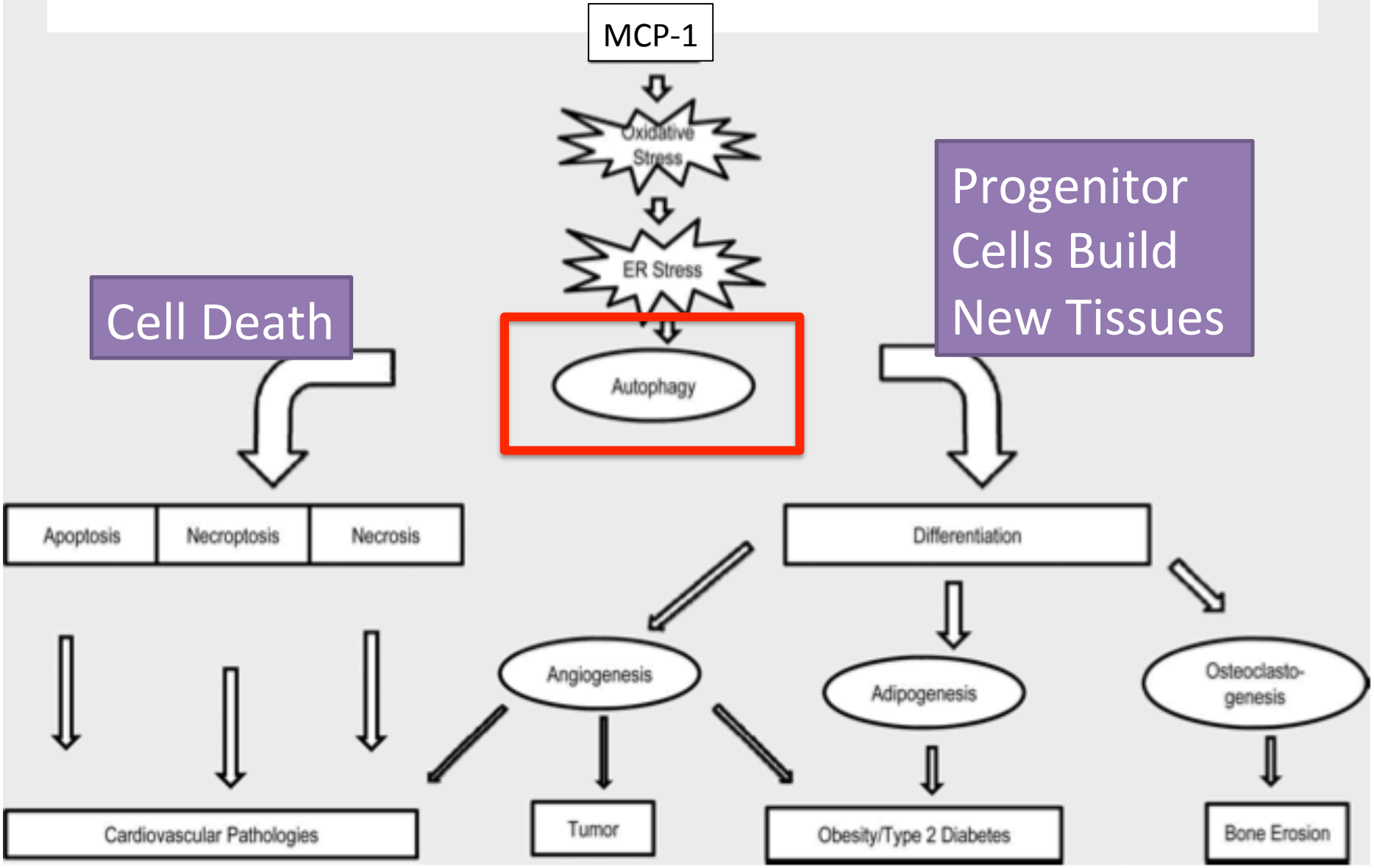
* Kolattukudy, and Niu, Circulation Research 2012, 110:174-189

# MCP-1 is a Key Signaling Molecule*

"The increased level of serum MCP-1 found in humans correlated with markers of the metabolic syndrome, including obesity, insulin resistance, type 2 diabetes, hypertension, and increased serum concentration of triacylglycerol."

* p. 181, Kolattukudy, and Niu, Circulation Research 2012, 110:174-189

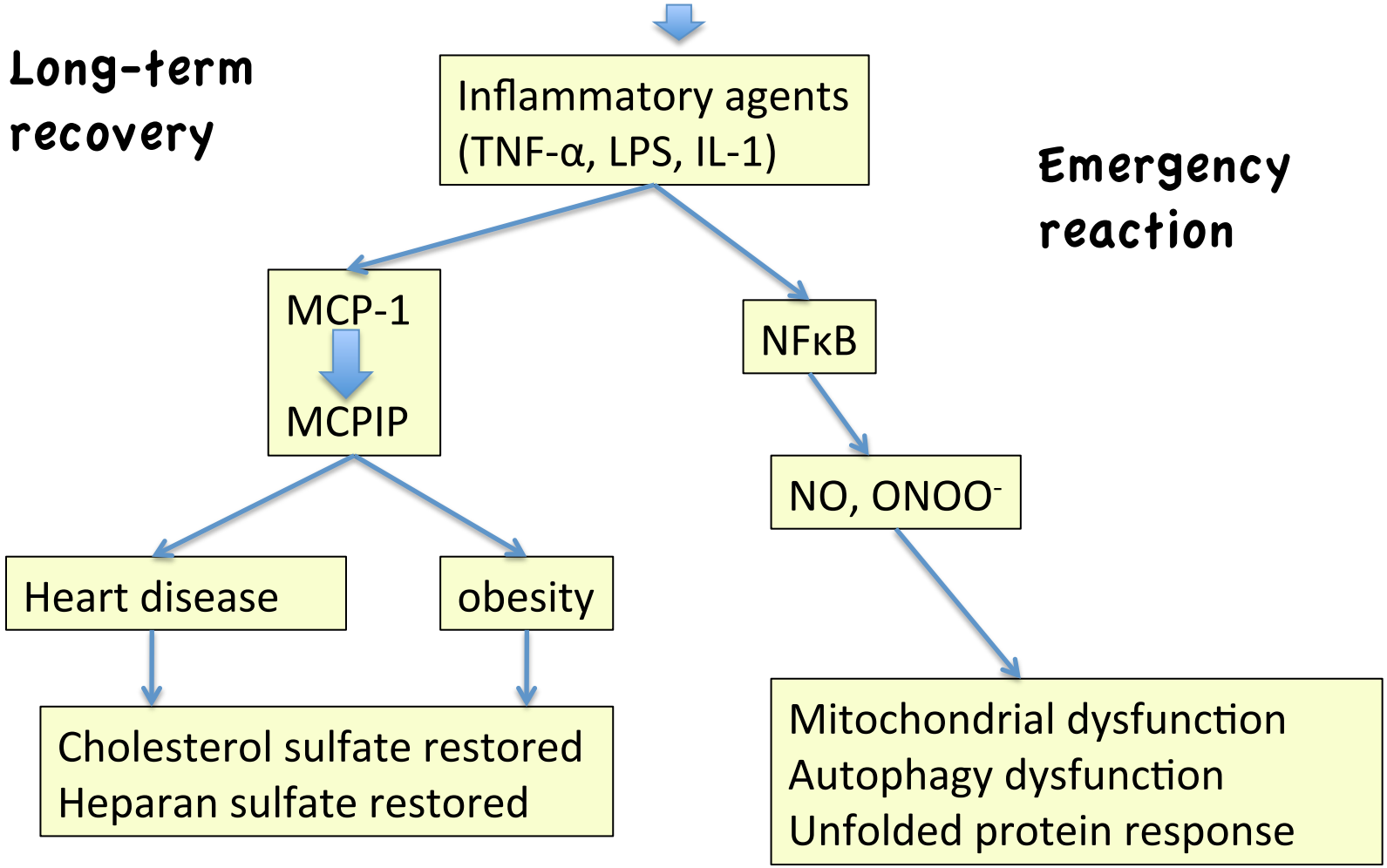
# Inflammation and ER Stress*



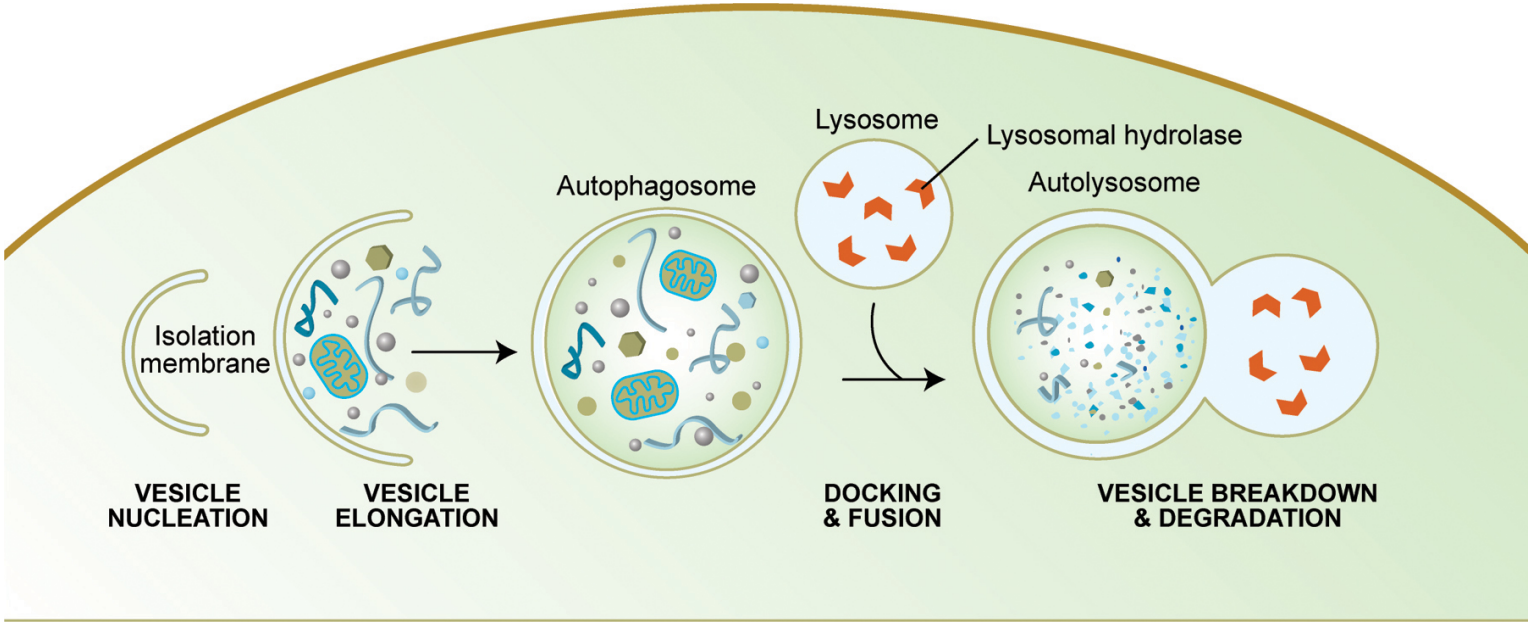

* Figure 3, Kolattukudy, and Niu, Circulation Research 2012, 110:174-189

# A Picture Emerges …

Insufficient cholesterol sulfate synthesis in the skin



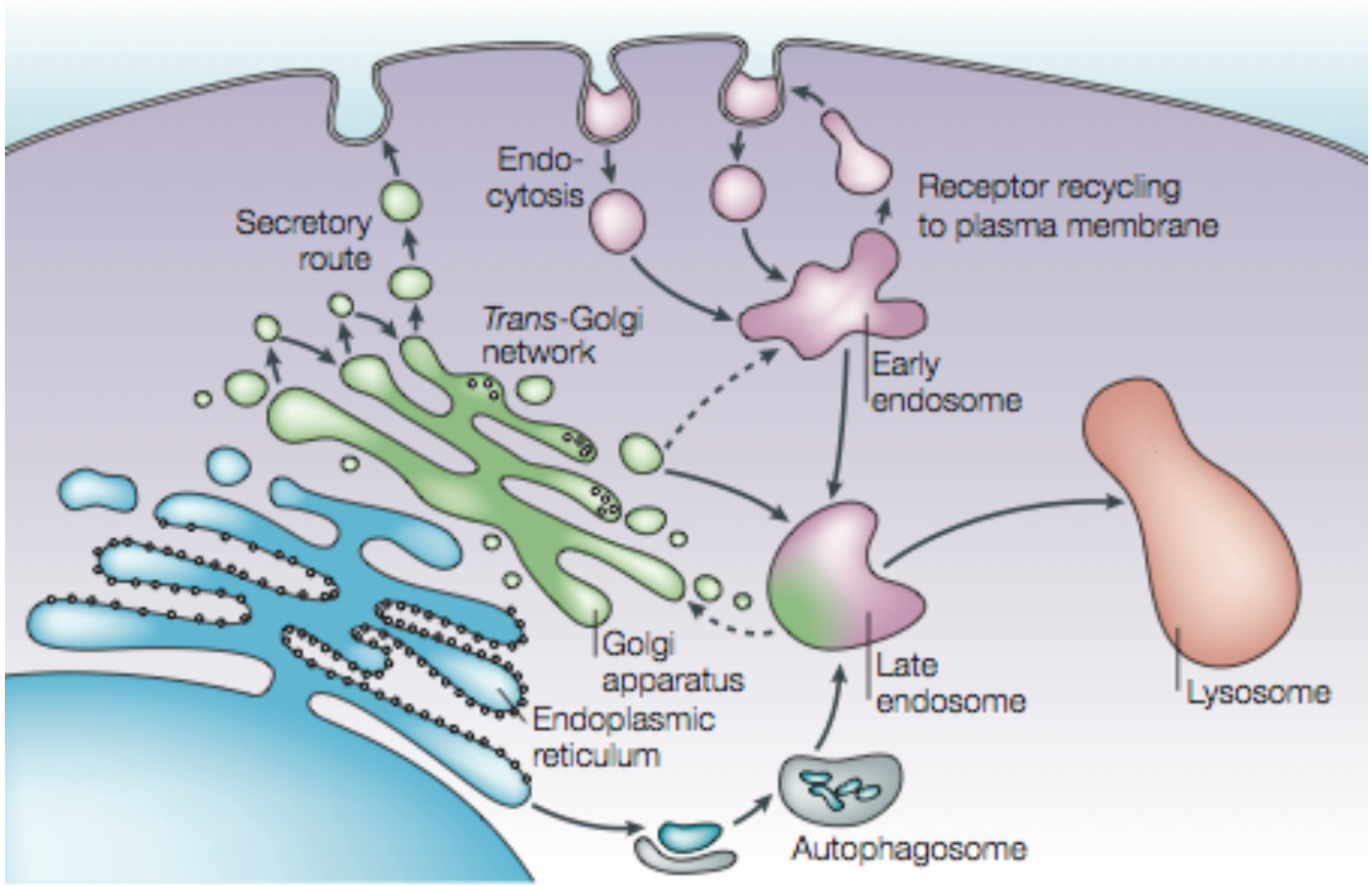
# Autophagy




- Allows cell to recycle damaged goods
- Both damaged mitochondria and damaged endoplasmic reticulum are absorbed into the lysosome and broken down
- Autophagy depends on *sulfates in HSPGs*

http://www.wormbook.org/chapters/www_autophagy/autophagy.html

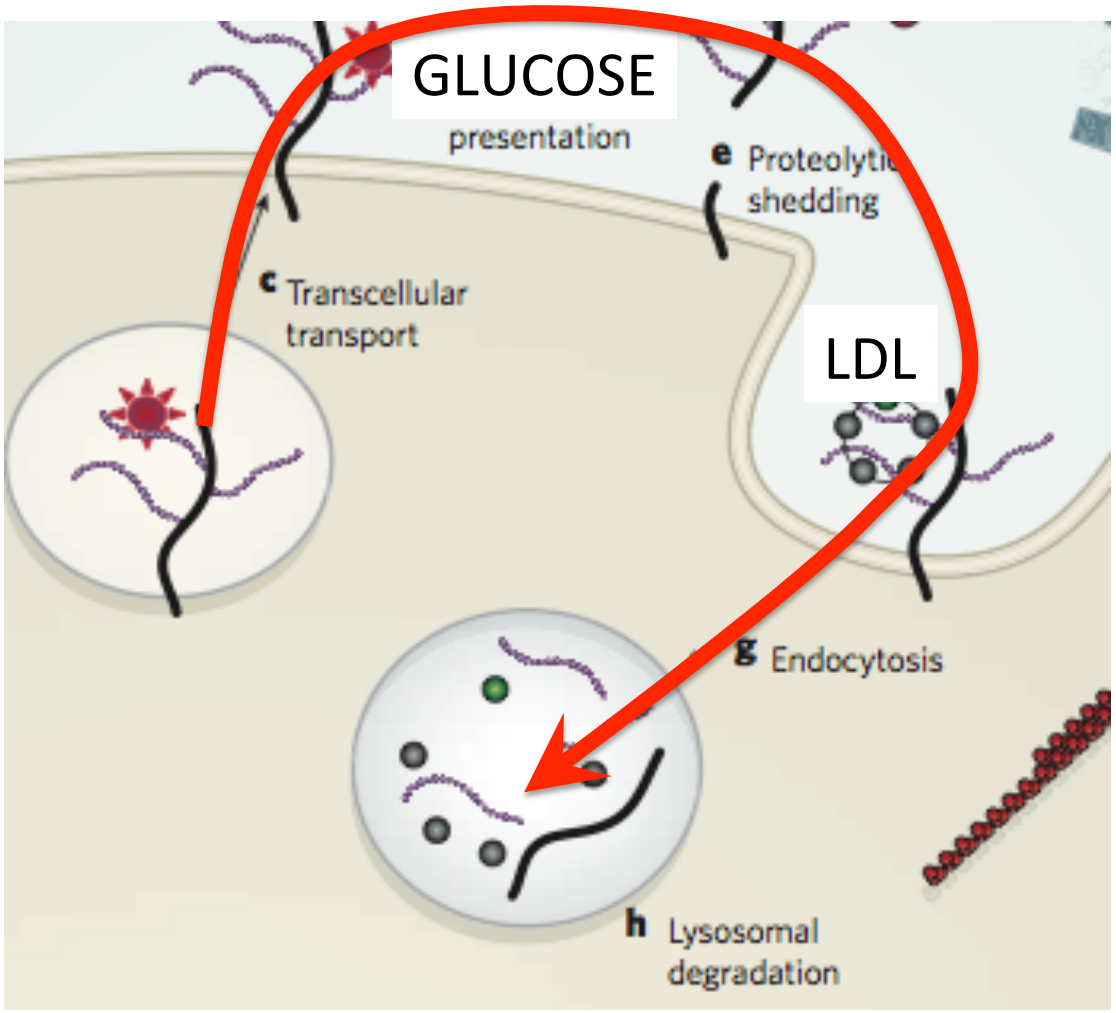
# The Lysosome is Central to the Cell*




* M. Jeyakumar, et al., Nature Reviews 6, Sep. 2005, Box 1, p. 2

# Recycling HSPGs w/ LDL*

- Hypothesis: Sugars are temporarily housed in HSPGs
- Hypothesis: sulfate in HSPGs is critically needed to recycle LDL




* J.R. Bishop et al, Nature 446, 1030-1037, Apr. 2007

# Fasting Induces Autophagy*

- Upregulation of autophagy is neuroprotective
- Much effort has been expended trying to develop drugs that pass through the blood brain barrier and enhance autophagy in the brain
- Short-term fasting leads to a dramatic upregulation in neuronal autophagy

* M. Alirezaei et al., Autophagy 6(6):702-710, Aug 16, 2010.

# Fasting Induces Autophagy*

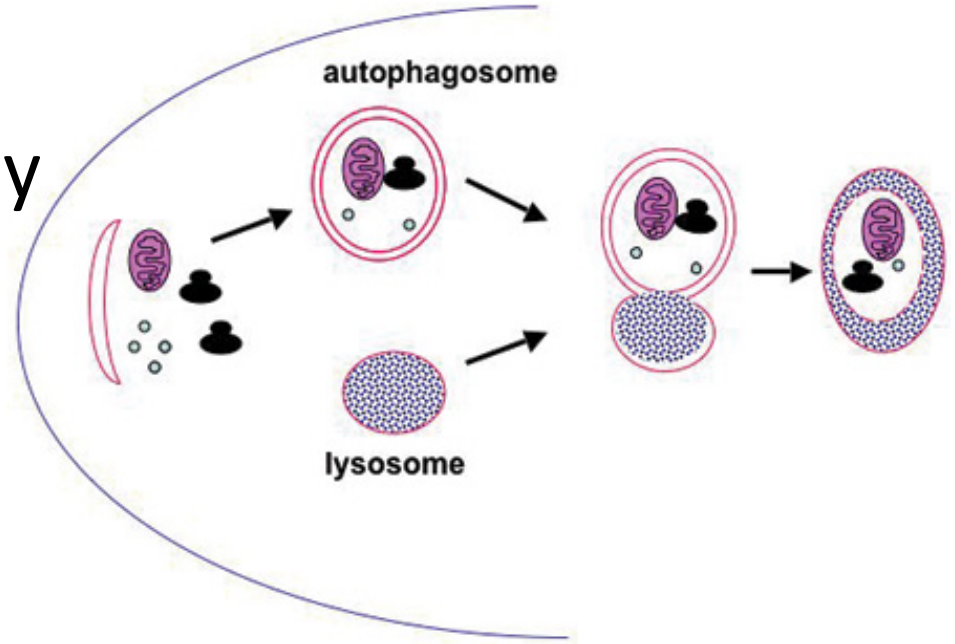
- Upregulation of autophagy is neuroprotective
- Much
exper
drugs
blood
enhar
brain
- Short
dramatic upregulation in neuronal autophagy

"Our data lead us to speculate that sporadic fasting might represent a simple, safe and inexpensive means to promote this potentially therapeutic neuronal response"

* M. Alirezaei et al., Autophagy 6(6):702-710, Aug 16, 2010.

# Autophagy and Insulin*

- Autophagy is suppressed by insulin
  - Insulin resistance leads to impaired autophagy
- Impaired autophagy may explain build-up of amyloid beta and tau protein in senile plaque of Alzheimer's disease



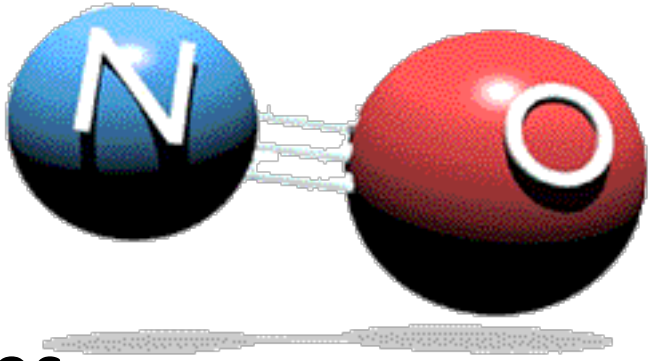

- Impaired autophagy is also associated with metabolic syndrome, cancer, asthma, and cardiac hypertrophy

* H.-Y. Liu et al., JBC 284(45):31484–31492,2009

# Nitric Oxide and Autophagy

- Autophagy is a natural mechanism by which cells clean up debris and dysfunctional organelles
- Excess nitric oxide inhibits autophagy
- Switchover to nitric oxide by eNOS leads to autophagy dysfunction and accumulation of garbage in the cell
- Long-term autophagy inhibition leads to cellular dysfunction and cell death

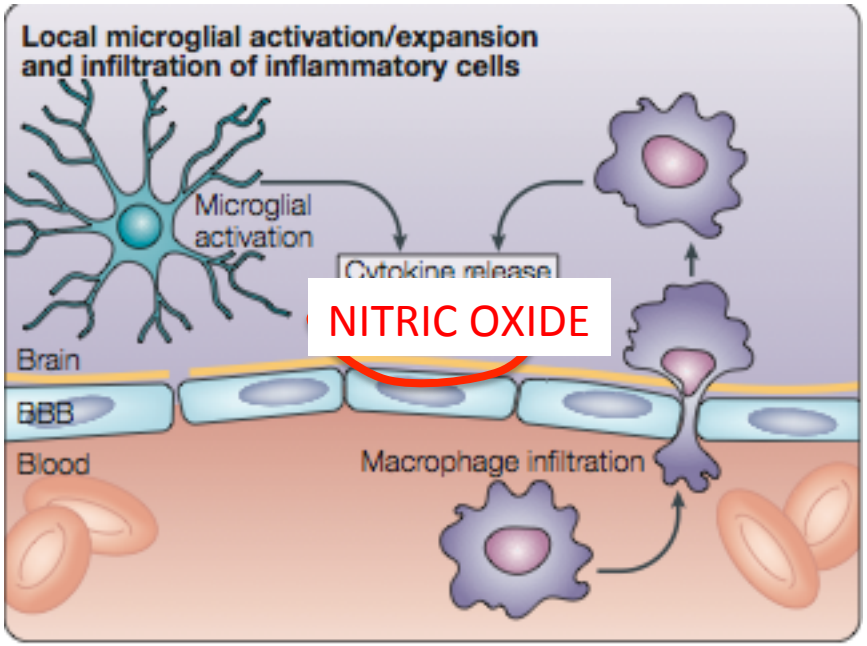
# Some Effects of Excess Nitric Oxide on the Brain*

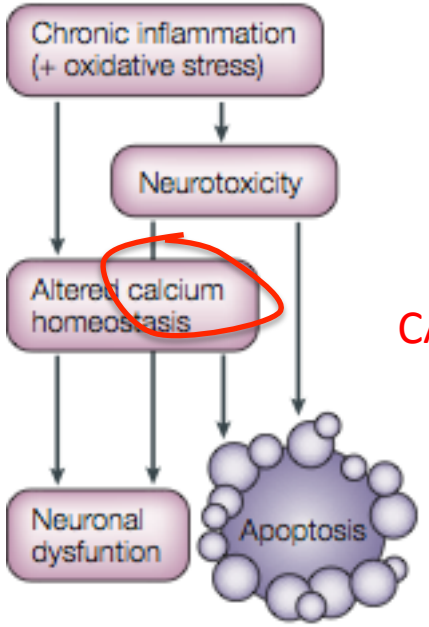



- Impairs autophagy
- Damages lipids in cell membranes
- Disrupts mitochondrial synthesis of ATP
- Interferes with reuptake of serotonin, dopamine, and epinephrine

*O. Akyol et al., In Vivo 18: 377-390, 2004

# Common Underlying Pathology*




CALCIUM

Applies to autism, Alzheimer's disease, Parkinson's disease,
ALS, depression and seizures,
as well as lysosomal storage disorders (rare genetic diseases)

* Jeyakumar et al., Nature Reviews:Neuroscience 6, Sep. 2005.

# Recapitulation

- Autophagy is the process by which cells clean up their accumulating debris
  - Insulin and nitric oxide suppress autophagy
  - Fasting enhances it
- Autophagy and phagocytosis are closely related in that both take place in the lysosome, and the lysosome depends on both cholesterol and sulfate to function properly
- Autophagy failure is a  common pathology associated with many diseases
  - Leads to broad restructuring of process of cell maintenance and explains the onset of these diseases

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  - Polyphenols
- Summary

# Environmental Toxins

# Some Environmental Toxins

- PCB's (plastic)
- Pesticides
- Perfluorooctanoic Acid (PFOA) (teflon)
- Glyphosate (Roundup) → hydroxybenzoic acid
- Arsenic
- Aluminum

# Synergy between Environmental Toxins and Epigenetics*

- Epigenetics is interface between inherited genome and environment
- Epigenetic dysregulation is increasingly implicated in disease
  - Dutch Hunger Winter (1944-45) associated with hypomethylation of IGF2 leading to diabetes in offspring several decades later
  - Hypomethylation also associated w/ arthritis, heart disease autism, and cancer
- We are exposed to a growing number of environmental toxins
  - Particularly lipophilic xenobiotics like PCB's, dioxins, pesticides

* D.-H. Lee et al., Environmental Health Perspectives 117(12) Dec. 2009, 1799-1802.

# Synergy between Environmental Toxins and Epigenetics* (Cont'd)

- Dietary deficiencies compound effects
  - S-adenosyl methionine (SAM) (methyl donor) requires methionine, folate, choline, betaine, and B12
  - Mother's deficiencies may induce epigenetic bias
- Chronic low-grade exposure to environmental toxins depletes glutathione
  - Methionine is precursor to glutathione → methionine drain → methylation impairment → cancer, etc.

* D.-H. Lee et al., Environmental Health Perspectives 117(12) Dec. 2009, 1799-1802.

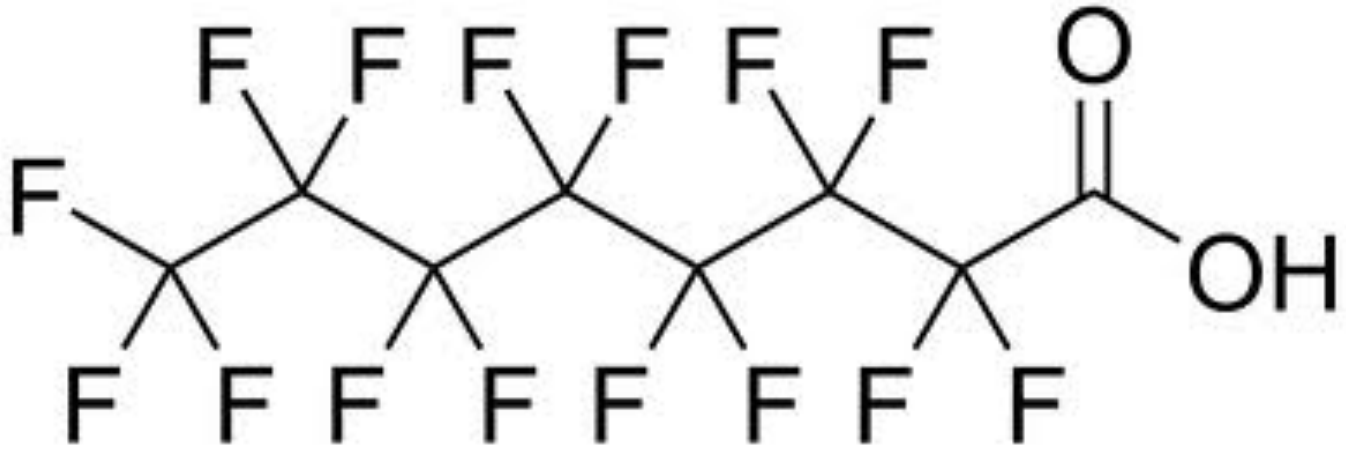
# Impaired Sulfate Metabolism and Epigenetics: Is There a Link in Autism?*


- Methionine sits at the crossroads of the pathway to sulfate and to DNA methylation
- Decreased sulfate synthesis in the skin leads to depletion of methionine (drain to sulfate)
- This induces hypomethylation of DNA in utero and reprogramming towards a model of a sunless world → autism

* S. Hartzell and S. Seneff, , Entropy, 14, 1953-1977.

# "Perfluorooctanoic Acid and Cardiovascular Disease in US Adults"*

- PFOA is a toxin and carcinogen found in Teflon and Gore-Tex
- Increasing serum levels of PFOA are associated with increased risk to cardiovascular disease




* A. Shankar et al., Archives of Internal Medicine, 2012.
sciencedaily.com/releases/2012/09/120903221128.htm

# Glyphosate

- Glyphosate is now the #1 herbicide in the U.S. and is increasingly used around the world
  - Developed and patented by Monsanto in the 1970's
  - Came out from patent in 2000
  - Inhibits an enzyme involved in synthesis of tyrosine, tryptophan and phenlalanine (the three aromatic amino acids)
- Surfactant POEA (polyethoxylated tallow amine) is often included in products, and is highly toxic to animals and people

# Benzene Toxicity!

- Glyphosate disrupts synthesis of aromatic amino acids
  - Leads to build-up of intermediate product: shikimate → 4-hydroxybenzoic acid
  - This is a toxic compound!
- Glyphosate is claimed to be relatively safe because humans don't have shikimate pathway
- However, gut bacteria do!!!!

# Sulfate-reducing Bacteria can Detoxify Benzene Compounds*

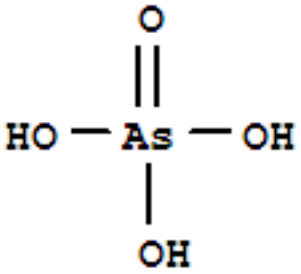
- Study involved anaerobic bacteria in marine sediment
- Analogy can be drawn with human colon
  - Sulfate-reducing bacteria are overabundant in association with autism
  - Are they needed to detoxify benzene?

Benzene + sulfate + water → carbonate + hydrogen sulfide

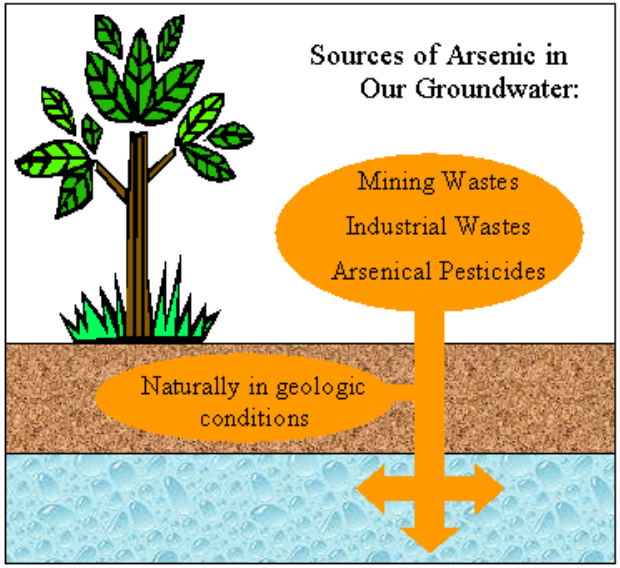
$$4C_6H_6 + 15SO_4^{2-} + 12H_2O \rightarrow 24HCO_3^- + 15HS^- + 9H^+$$

$$\Delta G^{o'} = -186.2\,\text{kJ (mol benzene)}^{-1}$$

* F. Musat and F. Widdel, Environmental Microbiology 10(1), 10–19, 2008.




# Arsenic




- Arsenic exposure leads to increased risk to cardiovascular disease*
- Soil bacteria chelate arsenic through sulfate synthesis**
- My hypothesis: glyphosate (Roundup) kills soil bacteria and permits arsenic accumulation


* Y..C. Hsieh et al., Environ Res. 2011 Aug, 111(6), 804-10.
** M.F. Kirk et al. Geology; Nov. 2004, 32(11), 953-956

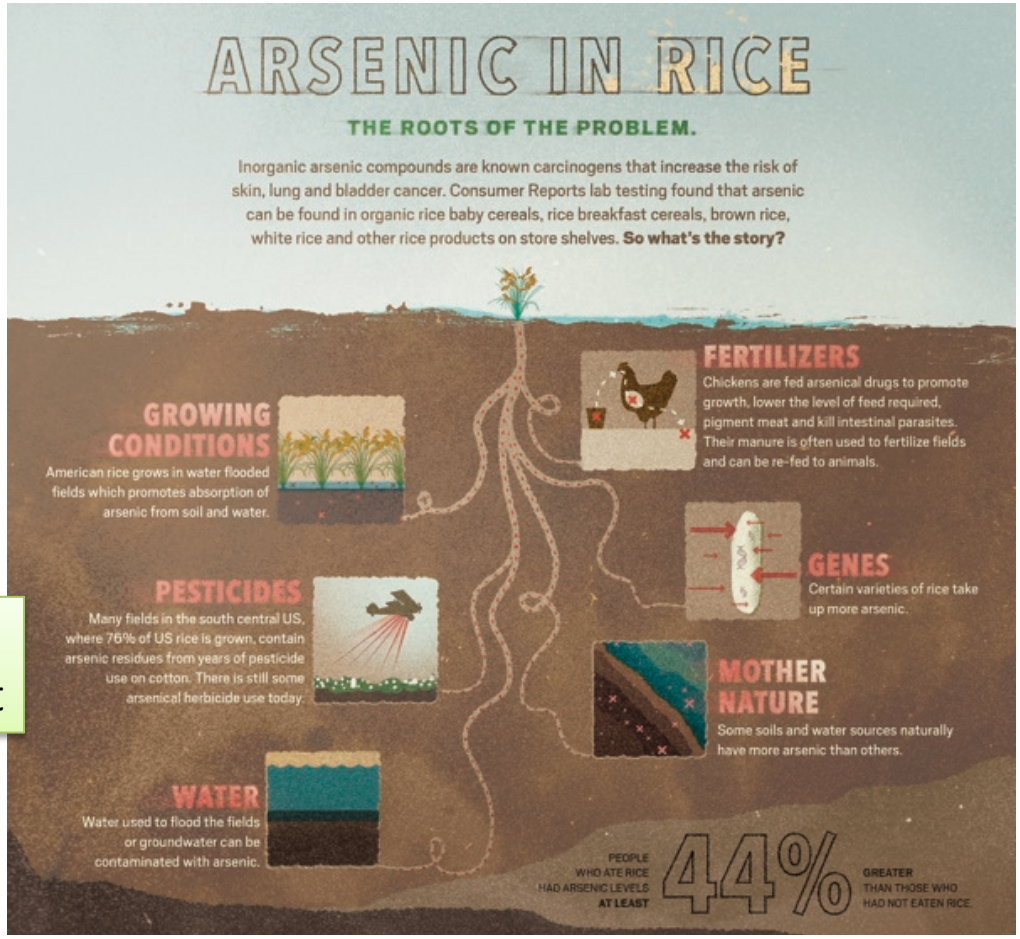
# Quote From Abstract*

**"Where sulfate-reducing bacteria are active, the sulfide produced reacts to precipitate arsenic, or coprecipitate it with iron, leaving little in solution. In the absence of sulfate reduction, methanogenesis is the dominant type of microbial metabolism, and arsenic accumulates to high levels."**

* M.F. Kirk et al. Geology; Nov. 2004, 32(11), 953-956

# Consumer Reports (CR) Study on Rice*

76% of US Rice
Grown in Cotton Belt

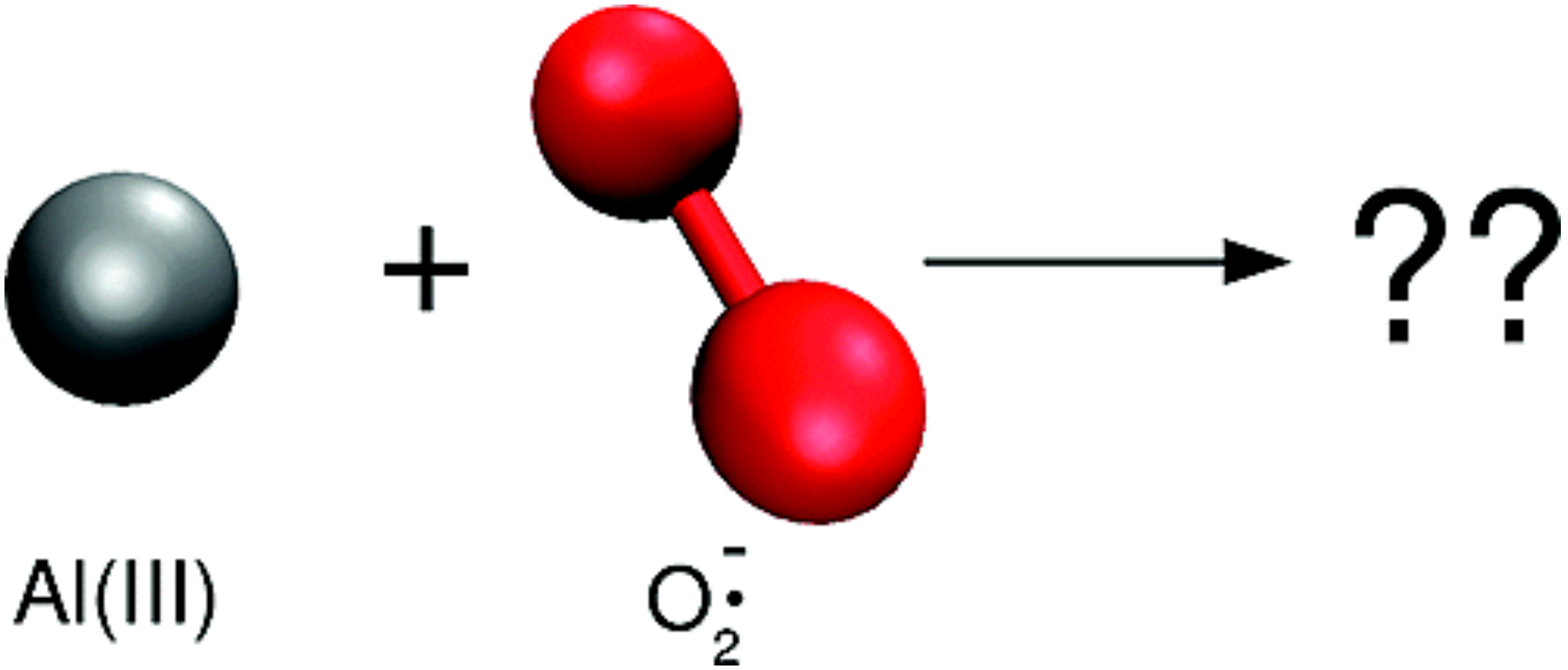



* http://www.consumerreports.org/cro/arsenicinfood.htm

# Summary of CR Study

- Samples from American Cotton Belt had higher levels than those from other countries
- Brown rice higher than white rice
- People who ate rice had 44% greater body burden of arsenic
- Action: Asking Food and Drug Administration to set limits in rice products and fruit juices


# Aluminum: The Worst Toxin?



# We are Too Comfortable with Aluminum!


"Aluminum is not perceived, I believe, by the public as a dangerous metal and, therefore, we are in a much more comfortable wicket in terms of defending its presence in vaccines"

-- Dr. John Clement, World Health Organization, San Juan, Puerto Rico, May 11th - 12th, 2000, p. 64

# Aluminum and Alzheimer's Disease*

- Aluminum is a neurotoxin that inhibits more than 200 biologically important functions
- Aluminum has been associated with multiple diseases affecting the nervous system:
  - Dialysis encephalopathy
  - ALS (Lou Gehrig's disease)
  - Parkinsonism dementia in Guam
  - Alzheimer's disease
- Aluminum may play crucial role as a cross-linker in amyloid beta oligomerization

* Kawahara and Kato-Negishi, International J. Alzheimer's Disease, Article ID 276393,2011

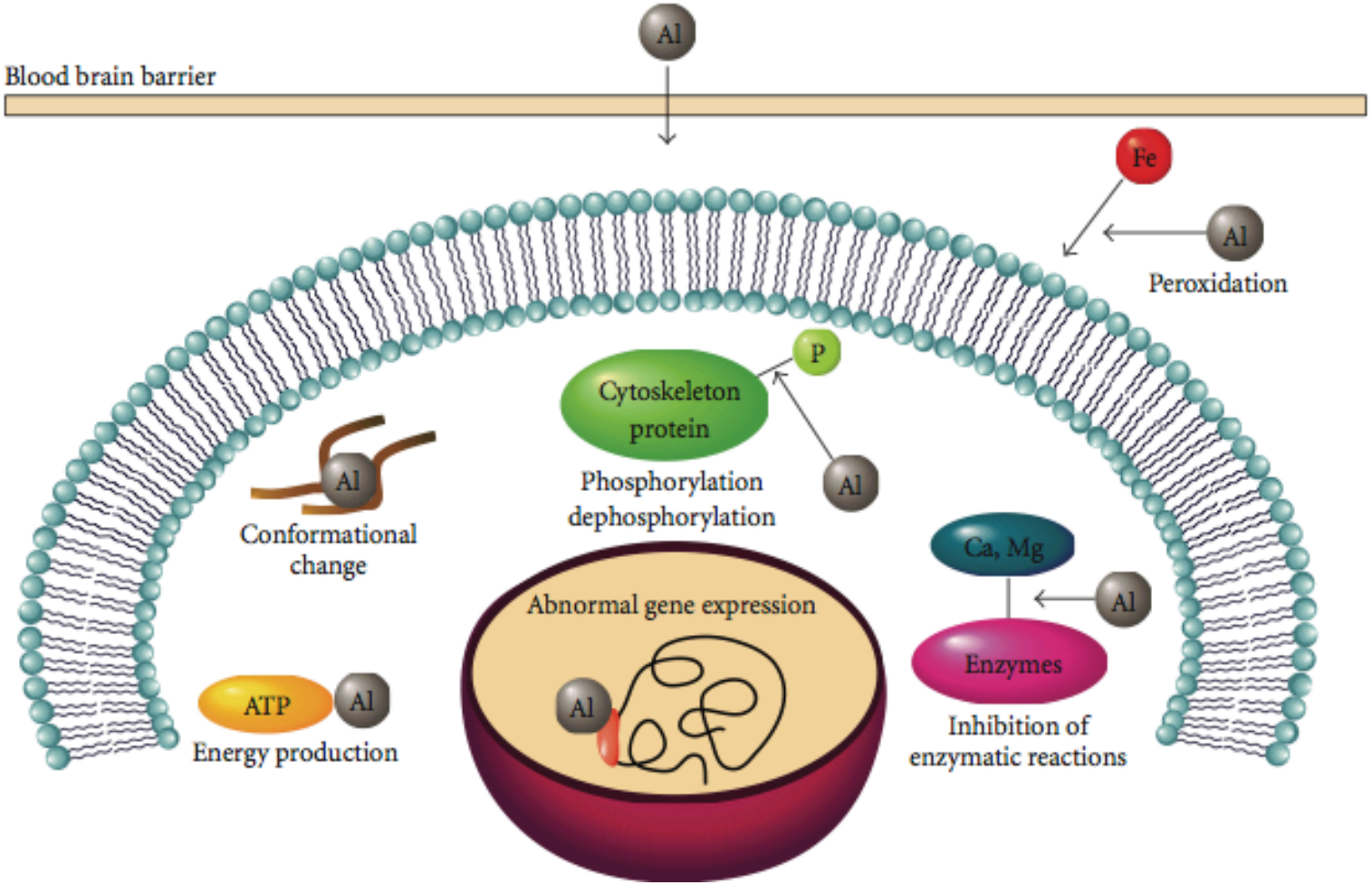
# Aluminum, Dialysis, and Dementia*

- Patients with end-stage kidney disease
- Aluminum in water in the dialysate
- Leads to severe dementia
- Occurs after three to five years of dialysis

- Can be avoided by careful filtering of water supply

* Wills and Savory, Environmental Health Perspectives 63, 141-147, 1985

# Aluminum's Many Effects in the Brain




Kawahara and Kato-Negishi, International J. Alzheimer's Disease 2011, Article ID 276393

# Aluminum Exposure and Memory, Depression*

- 25 symptomatic workers from the same aluminum smelting plant
  - 22 (88%) reported frequent loss of balance
  - 21 (84%) reported memory loss
  - 21 (84%) showed physical signs of incoordination
- 19 were tested for depression on the Minnesota Multiphasic Personality Inventory
  - 17 (89%) tested positive for depression

*White et al., Arch Intern Med. 152(7):1443-1448, 1992.

# Our Studies with VAERS Database on Aluminum & Depression

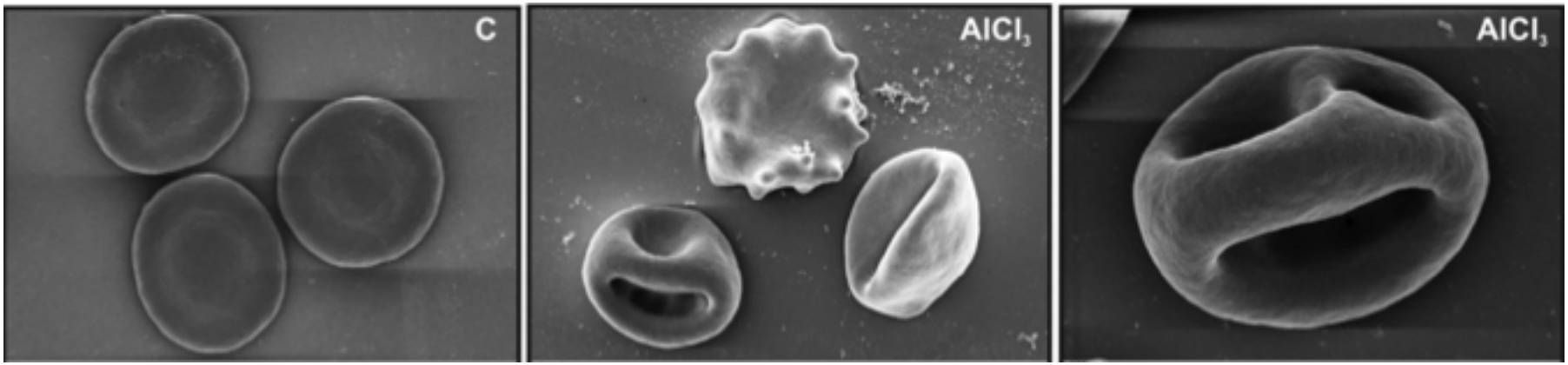
- VAERS: Vaccine Adverse Event Reporting System, maintained by CDC
- Tabulate word frequencies for mentions of "depression" in adverse reactions to aluminum-containing vaccines versus non-aluminum-containing vaccines.
  - 231 mentions in aluminum-containing vaccines versus 85 in age-matched controls
  - Highly significant result ($p = 0.005$)

# Aluminum's effect on Red Blood Cells*

- Aluminum causes microcytic anemia
  - Induces severe morphological changes in RBCs
  - Leads to eryptosis – cell dies
- N-acetyl cysteine (a source of sulfur) affords some protection

Normal

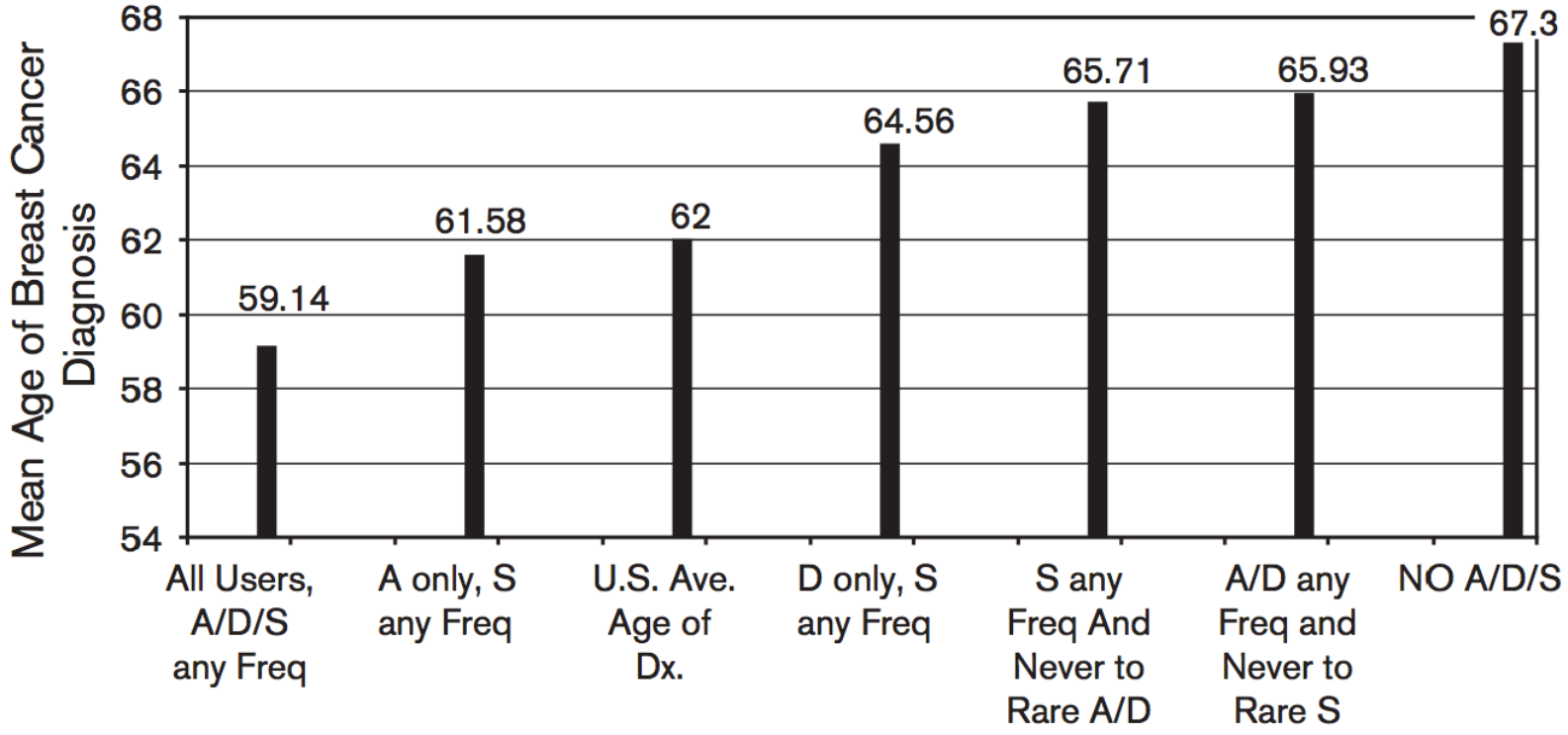
With Aluminum




* D.M. Vota et al., Journal of Cellular Biochemistry 113:1581–1589, 2012.

# Antiperspirants and Breast Cancer *

**Fig. 4**

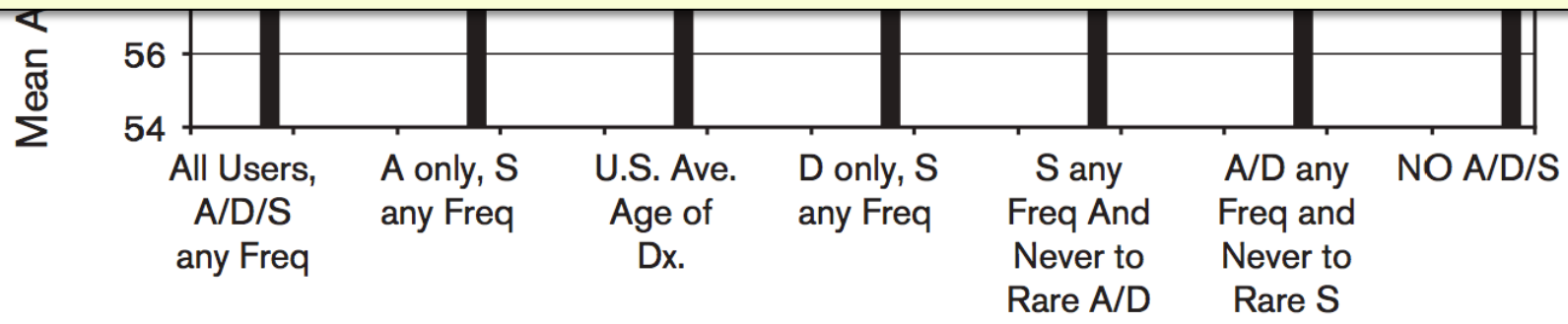



* K.G. McGrath, European Journal of Cancer Prevention 2003, 12:479–485

# Antiperspirants and Breast Cancer *

**Fig. 4**

"Ninety per cent of the United States population regularly uses antiperspirants and deodorants, with their daily use ($ sales) only exceeded by toothpastes"




* K.G. McGrath, European Journal of Cancer Prevention 2003, 12:479–485

# Aluminum Foil in Cooking*

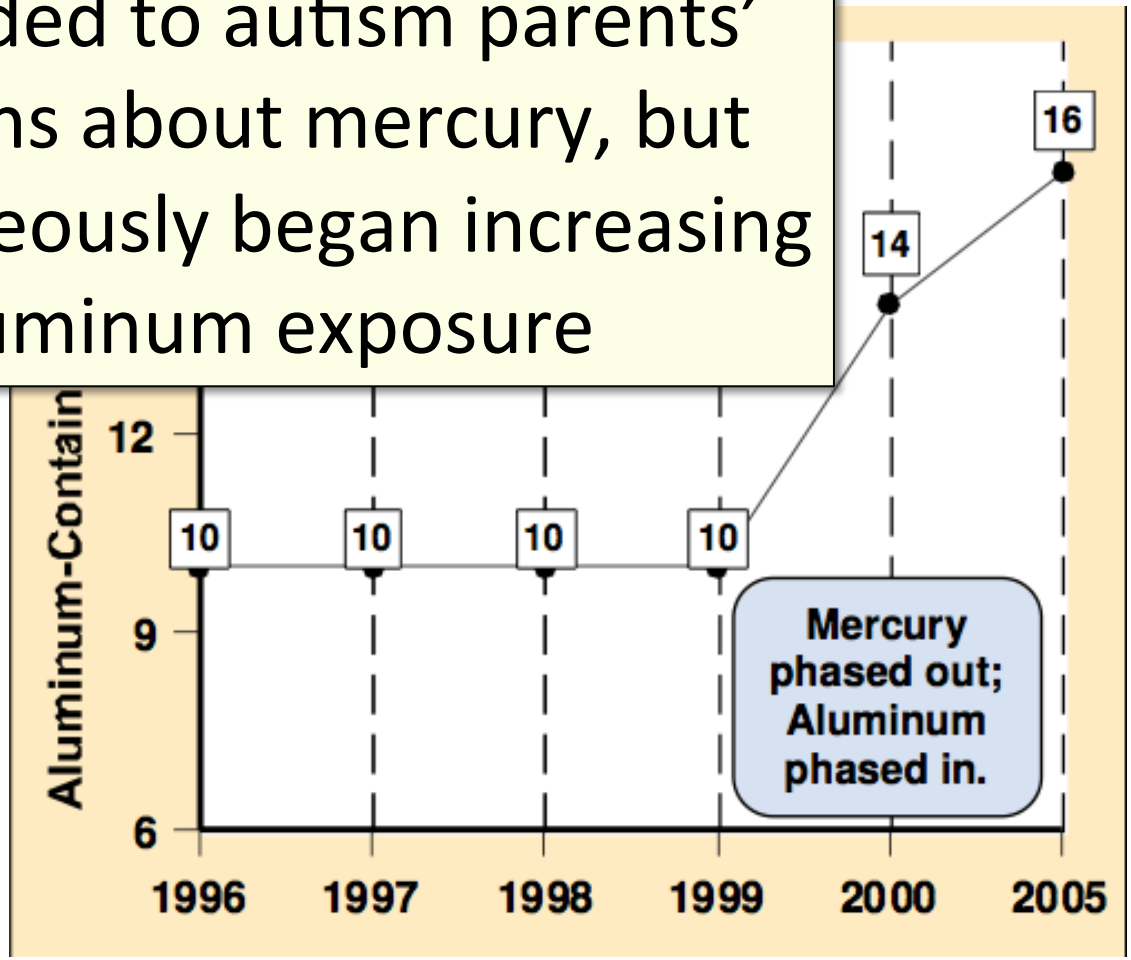
- Leaching from aluminum foil into foods during cooking may carry significant health risks
  - Provides an easy channel for metal entry into the body
- Factors leading to more leaching
  - Higher temperature
  - Higher acidity
  - Salt and spices
- Particularly problematic for people with leaky gut syndrome

* G. Bassioni, Int. J. Electrochem. Sci., 7 (2012) 4498 - 4509

# Aluminum in Vaccines:

# mble*

In 1999, vaccine industry responded to autism parents' concerns about mercury, but simultaneously began increasing aluminum exposure




* Neil Z. Miller, Thinktwice Global Vaccine Institute, www.thinktwice.com

# "Empirical Data Confirm Autism Symptoms Related to Aluminum and Acetaminophen Exposure"*

- Hypothesis: Vaccine industry removed mercury from vaccines but simultaneously augmented aluminum burden
- This is what prevented autism rates from falling following mercury reduction
- MMR association with autism is in part attributable to administering Tylenol to curb fever
  - Tylenol detoxification depletes sulfate

* S. Seneff, R.M. Davidson, and J. Liu, Entropy, Special Issue on Semiotics, 2012

# Recapitulation

- We live with multiple environmental toxins
- Two that I am particularly worried about are aluminum and glyphosate
- Aluminum likely plays a role in autism, Alzheimer's disease, depression and breast cancer
- We have become complacent with these toxins, and they are slowly eroding our collective health

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# Polyphenols

# Polyphenols

- Tannin in tea
- Flavonoids in fruits
- Resveratrol in wine
- Curcumin in curries
- Polyphenols in coffee and chocolate

These compounds all have significant health benefits, but it's not clear why!

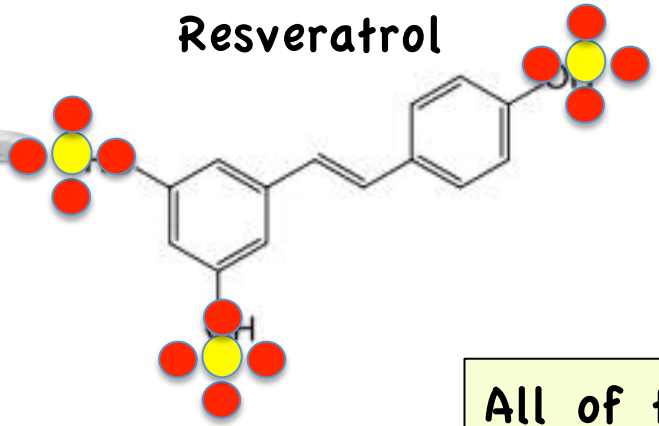
# Polyphenols in Coffee*

"Supplementation with CPP [Coffee Polyphenols] significantly reduced body weight gain, abdominal and liver fat accumulation, and infiltration of macrophages into adipose tissues."

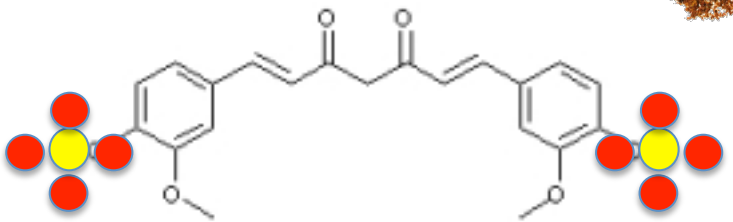
* T. Murase et al., Am J Physiol Endocrinol Metab 300: E122–E133, 2011

# Four Related Molecules

Resveratrol

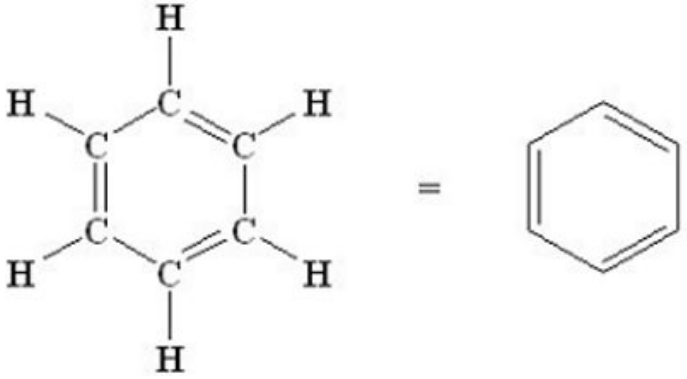


Curcumin

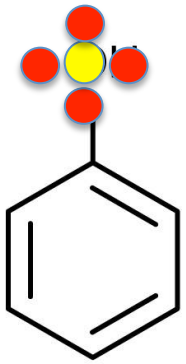


All of the OH groups can become sulfates

Benzene



Phenol

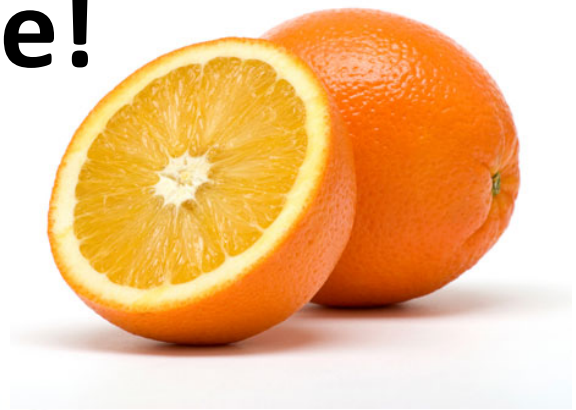


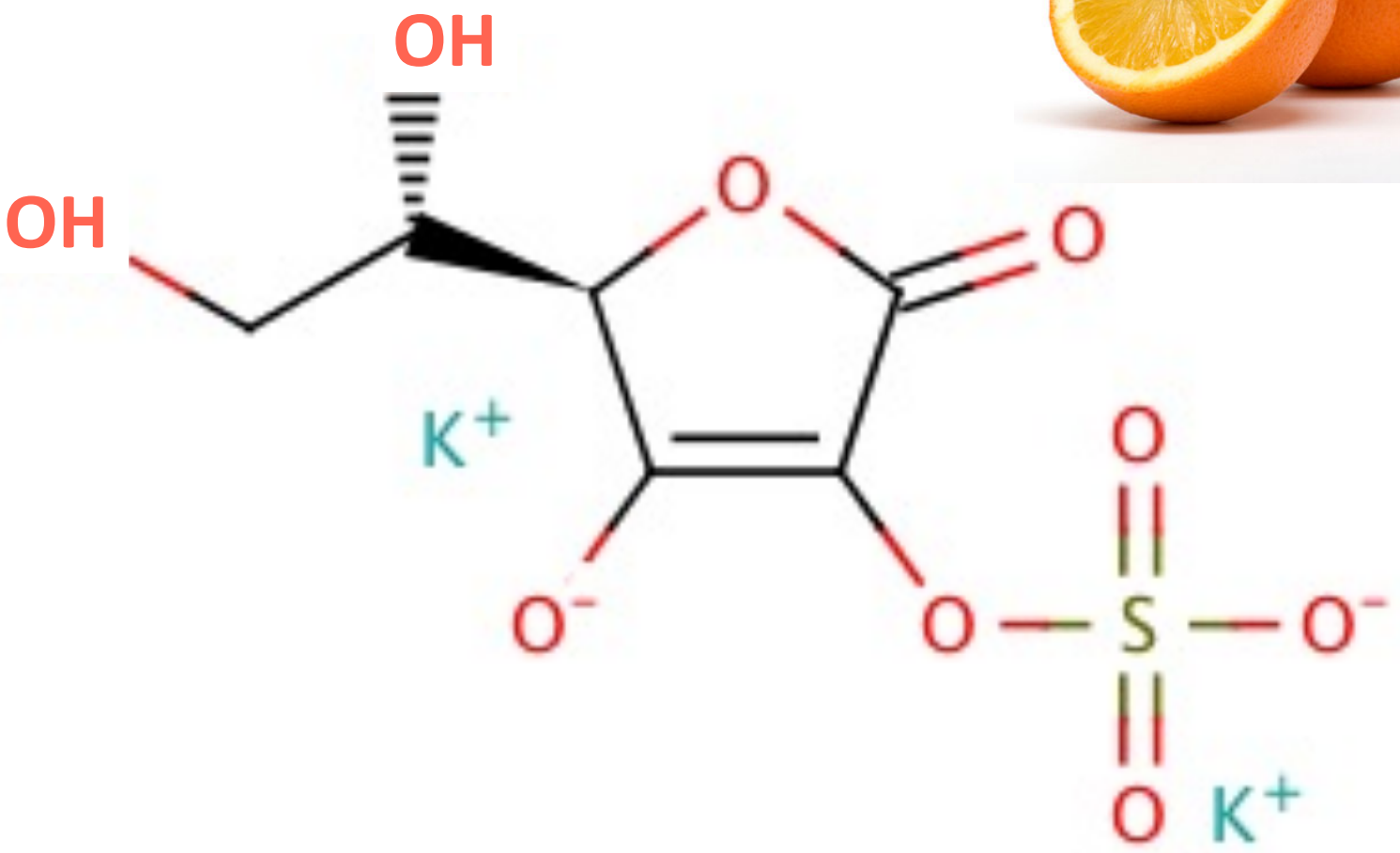
# "High Absorption but Very Low Bioavailability of Oral Resveratrol in Humans"*

- Researchers studied absorption of resveratrol
- Wanted to understand its role in biology
- Found that it was sulfated in the gut, went into the bloodstream and promptly disappeared
- I hypothesize that its health benefit has solely to do with its ability to transport sulfate

*T. Walle et al., Drug Metabolism and Disposition, 32(12), 1377-1382, 2004

# Vitamin C Sulfate!



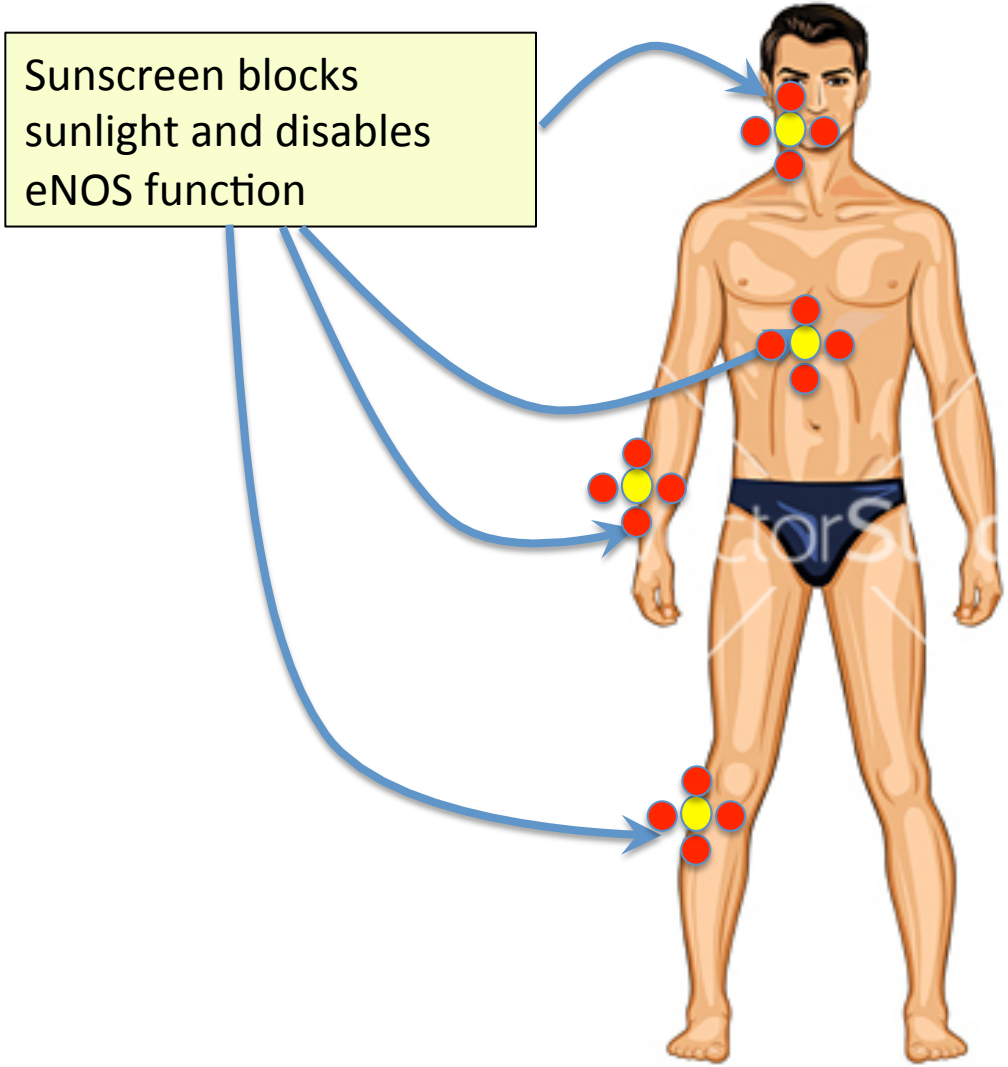


# Recapitulation

- A variety of “healthy” compounds in foods have the interesting property that they can be sulfated
- These include coffee, tea, resveratrol (wine), flavonoids in berries and other fruits, curcumin in curries and vitamin C
- I maintain that the health benefits of these foods are solely due to their ability to transport sulfate in the blood stream

# Summary

# Modern Environment
# Depletes Sulfate Everywhere



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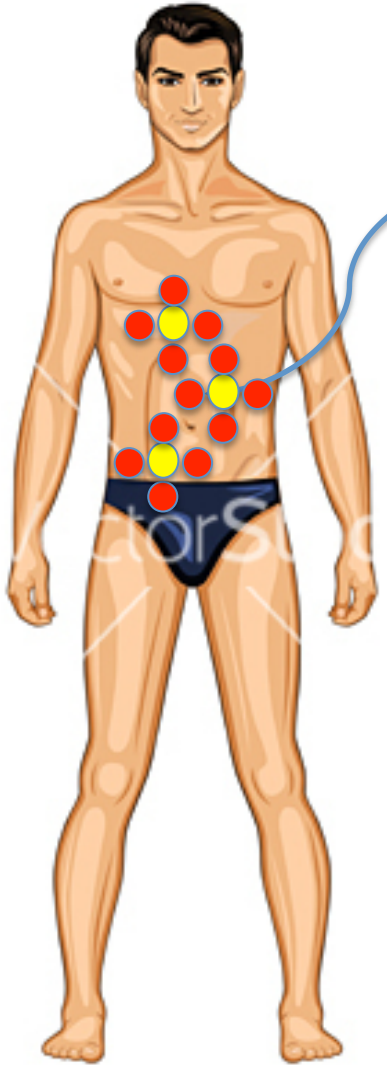

Glyphosate induces toxic phenols that deplete sulfate in their breakdown by colon bacteria

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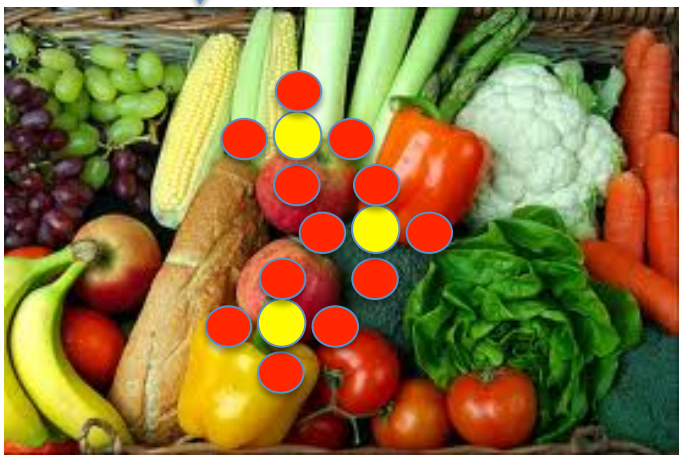
Depleted sulfur in our food supply due to modern farming practices



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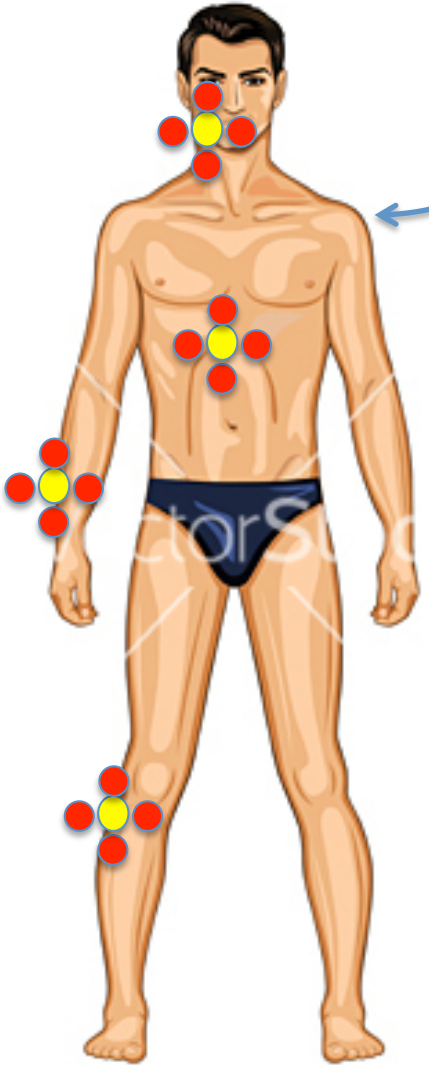

Vaccines deplete sulfate via aluminum and mercury detoxification; these toxins also interfere with eNOS function

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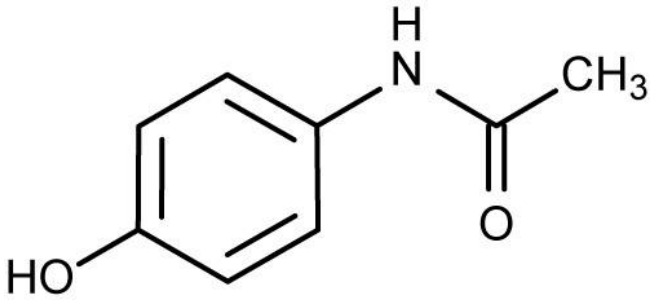


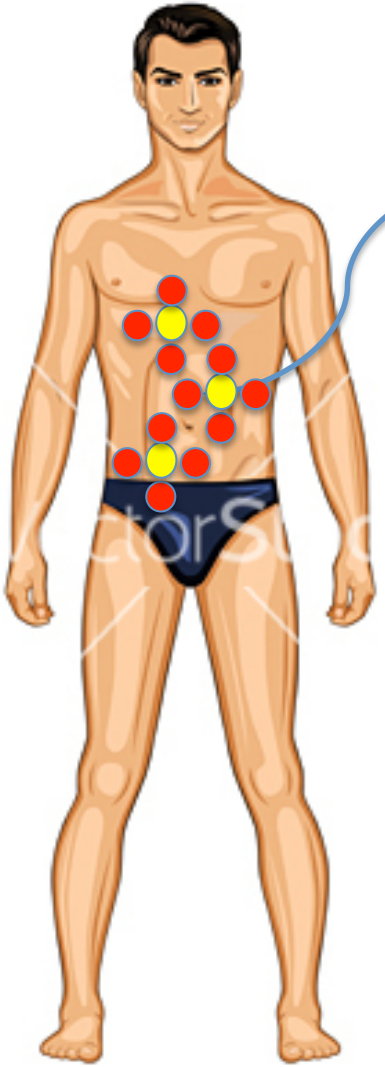

Various pharmaceutical drugs like acetaminophen deplete sulfate in their detoxification process

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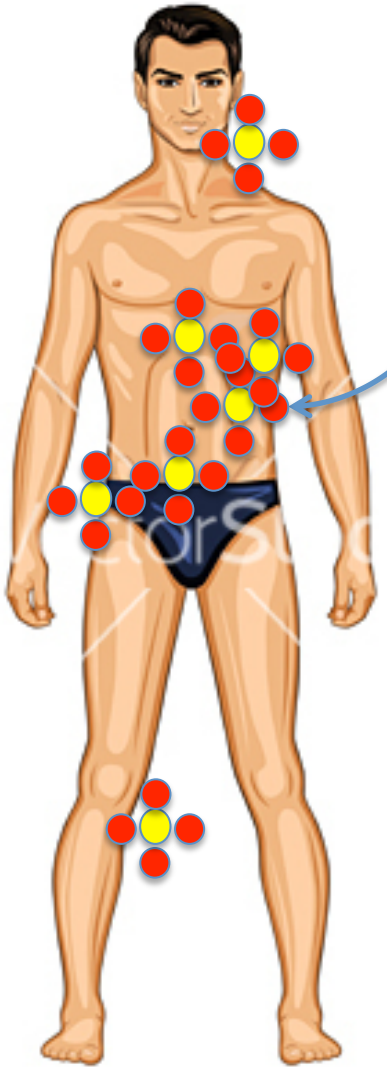
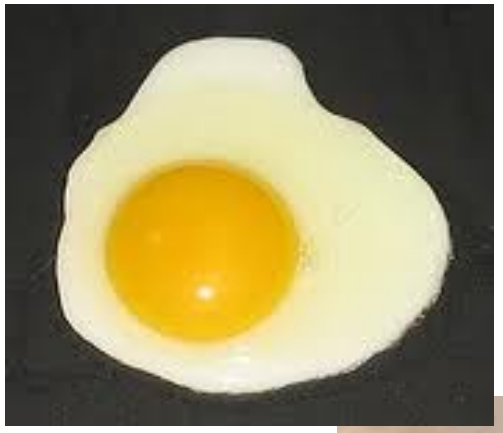


Antibiotics kill gut bacteria that we depend on for critical bioactive molecules like cobalamin and vitamin K

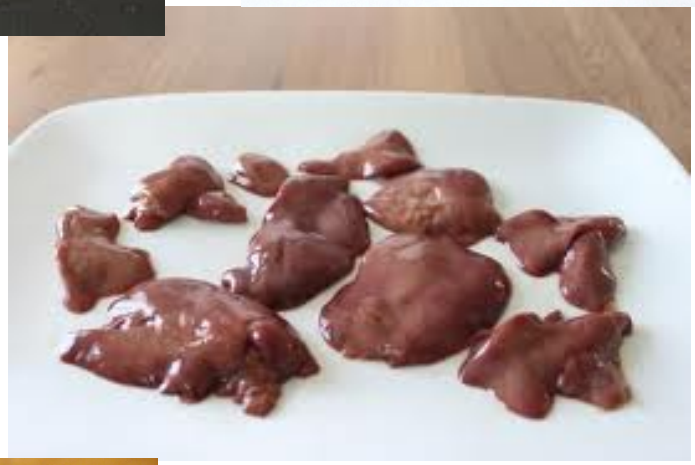
# What You Can Do!

# Eat Foods Containing Sulfur!








# Go Organic!

PALO ALTO'S
CALIFORNIA AVENUE
Farmers' Market
URBAN VILLAGE FARMERS' MARKET ASSOCIATION

# Probiotics!

# Escape to a sunny place in winter!

# If you Live in Canada, Use a Sunlamp!

# Epsom Salts!

Magnesium Sulfate in hot bath water is a cheap and easy way to get sulfate supply to the skin


# Don’ts!




# Summary

- I have identified impaired cholesterol sulfate synthesis as a key factor in the diseases of modern times
  - I have shown how this can lead to diabetes, obesity, autism, Alzheimer's disease and heart disease
- Microbes are often unsung heroes who help rescue us from pathological situations of our own making
- Environmental toxins play an important role in our current state of health crisis

Thank you!