Fatty Acid and Triacylglycerol Biochemistry
- Omega-3 Fatty Acids -

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Lipid Classification

- Lipids are biologic substances that are generally hydrophobic or amphipathic in nature and in many cases soluble in organic solvents.
- Lipids are produced, transported and recognized by the concerted actions of numerous enzymes, binding proteins and receptors
- Lipids include: fatty acids, phospholipids, sterols, sphingolipids, terpenes and others:

  - Fatty acids
  - Glycerolipids
  - Glycerophospholipids
  - Sphingolipids
  - Sterol lipids
  - Prenol lipids
  - Saccharolipids
  - Polyketides

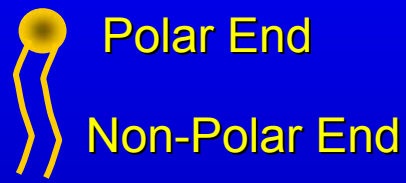
Fathy E et al. J Lipid Res 2005;46:839-861

Phospholipids

- Contain one or more fatty acid molecule and one phosphoric acid radical and usually contain a nitrogenous base

Major types:

- Lecithins: phosphatidylcholine
- Cephalins: phospatidylethanolamine
  - Some formed from inositol
- Sphingomyelin



- 90% are formed in the liver, but all cells can make them
- They are all lipid soluble and **transported in lipoproteins** and used throughout the body for structural purposes
- Phospholipids are donors of phosphate radicals which are needed for different chemical reactions in tissues

Guyton & Hall Textbook of Medical Physiology Unit XII pp756-757
WB Saunders Philadelphia 2000

Lipid Classification

- Lipid Nomenclature
  - Stereospecific numbering (*sn*) method is used in describing glycerolipids and glycerophospholipids
  - The glycerol group is typically acylated or alkylated at the *sn*-1 or *sn*-2 position
  - Core names are used for sterols (cholestane, androstane and estrane)

Fathy E et al. J Lipid Res 2005;46:839-861

Lipid Classification

- Fatty acids (acyl) group: repeating series of methylene groups that impart hydrophobic character
  - First subclass is the straight chain saturated group with a terminal carboxylic acid
- Glycerolpids are abundant as membrane constituents, metabolic fuels (acylglycerols) and signaling molecules
- Glycerophospholipids are ubiquitous and key components of the lipid bilayers of cells
  - Phospholipids may be divided by the nature of the polar head group at the *sn*-3 position of the glycerol backbone
- Sphingolipids are a complex family with a sphingoid base: ceramides, phosphosphingolipids & glycosphingolipids

Fathy E et al. J Lipid Res 2005;46:839-861

Lipid Classification

- Sterol Lipids are important membrane lipids
  - Cholesterol and derivatives
    - Cholesteryl esters
    - Phyto, marine and fungal sterols
  - Steroids (C_{18}, C_{19}, C_{21})
  - Secosteroids (Vitamin D_2 and D_3)
  - Bile acids and derivatives
  - Steroid conjugates
  - Hopanoids

Fathy E et al. J Lipid Res 2005;46:839-861

Lipid Classification

- Prenol lipids are synthesized from the five carbon precursors isopentyl diphosphate and dimethylallyl diphosphate from the mevalonic acid pathway.
- Saccharolipids (Glycolipids): Fatty acids are linked directly to a sugar backbone forming structures compatible with membrane bilayers. They exist as glycan or phosphorylated derivatives

Fathy E et al. J Lipid Res 2005;46:839-861

Fatty Acid Nomenclature

- Trivial and shorthand nomenclature have been in common usage.
- Gas liquid chromatography separates FA by chain length and saturation: nomenclature using this technology consists of two numbers separated by a colon.
  - The number before the colon gives the carbon number and the number after denotes the number of double bonds
  - Sometimes it is useful to number the double bonds from the methyl end: ie n-6
    - Older literature uses ω instead of n
  - Δ refers to a double bond: Δ-9 is a double bond at #9 position
  - The isomeric configuration around a double bond can be cis/trans or as it is now called Z/E
    - In the cis form the two hydrogen substituents are on the same side of the molecule and in trans form they are on opposite sides. Trans FA are rare.
  - Older systems numbered carbon atoms with Greek letters:
    - α refers to C2, β to C3 and ending with ω at the last atom, furthest from the carboxyl chain

Gurr MI et al. Lipid Biochemistry 5th Ed 2002 Blackwell Science Malden, MA

Fatty Acid Nomenclature

- Commonly occurring acids

# Carbons	Systematic Name	Common Name
2	n-Ethanoic	Acetic
3	n-propanoic	Propionic
4	n-butanoic	Butyric
6	n-hexanoic	Caproic
10	n-decanoic	Capric
12	n-dodecanoic	Lauric
14	n-tetradecanoic	Myristic
16	n-hexadecanoic	Palmitic

Gurr MI et al. Lipid Biochemistry 5th Ed 2002 Blackwell Science Malden, MA

Fatty Acid Nomenclature

- Commonly occurring acids

# Carbons	Systematic Name	Common Name
18	n-octadecanoic	Stearic
20	n-eicosanoic	Arachidic
22	n-docosanoic	Behenic
24	n-tetracosanoic	Lignoceric
26	n-hexacosanoic	Cerotic
28	n-octacosanoic	Montanic

Gurr MI et al. Lipid Biochemistry 5th Ed 2002 Blackwell Science Malden, MA

Saturated Fatty Acids

- Major dietary determinant of LDL-C
  - For every 1% increase in calories from SF, serum LDL-C raises 2% or vice versa
- DELTA and beFIT studies have demonstrated benefit in improving LDL-C
- Reduced intakes show no compromised growth or development issues in children

NCEP ATP-III Circulation 2002;25:3260

Unsaturated Fatty Acids

- **Monoenoic** (monounsaturated)
  - The more common have an even number of carbon atoms and a chain length of 16-22 carbons and a double bond with the cis configuration
  - Often the cis begins at the Δ9 position
  - The most common monoenoic acid is an 18 chain:
    - cis-9-octadecenoic acid or Oleic Acid

Gurr MI et al. Lipid Biochemistry 5th Ed 2002 Blackwell Science Malden, MA

Unsaturated Fatty Acids

- **Polyenoic** (polyunsaturated)
  - Unsaturated FA are very susceptible to oxidation; the more double binds the more the susceptibility

Gurr MI et al. Lipid Biochemistry 5th Ed 2002 Blackwell Science Malden, MA

Unsaturated Fatty Acids

- Commonly occurring acids

# Carbons	Systematic Name	Common Name
Dienoic		
18	Δ-6,9 octadecadienoic	Linoleic
Trienoic		
18	Δ-6,9,12 octadecatrienoic	γ-Linolenic
18	Δ-9,12,15 octadecatrienoic	α-Linolenic
Tetraenoic		
20	Δ-5,8,11,14 eicosatetraenoic	Arachidonic
Pentaenoic		
20	Δ-5,8,11,14,17 eicosapentaenoic	
Hexaenoic		
22	Δ-4,7,10,13,16,19 docosahexaenic	

Gurr MI et al. Lipid Biochemistry 5th Ed 2002 Blackwell Science Malden, MA

Synthesis of EPA & DHA

Linoleic acid (18:2*n*-6)

→ Δ15-desaturase (plants only) → α-Linolenic acid (18:3*n*-3)

↓ Δ6-desaturase

γ-linoleic acid (18:3*n*-6)

↓ Elongase

Di-homo-γ-linolenic acid (20:3*n*-6)

↓ Δ5-desaturase

Arachidonic acid (20:4*n*-6)

α-Linolenic acid (18:3*n*-3)

↓ Δ6-desaturase

Stearidonic acid (18:4*n*-3)

↓ Elongase

20:4*n*-3)

↓ Δ5-desaturase

Eicosapentaenoic acid (20:5*n*-3)

↓ Elongase

Docosapentaenoic acid (22:5*n*-3)

↓ Elongase

(24:5*n*-3)

↓ Δ6-desaturase

(26:6*n*-3)

↓ β-oxidation

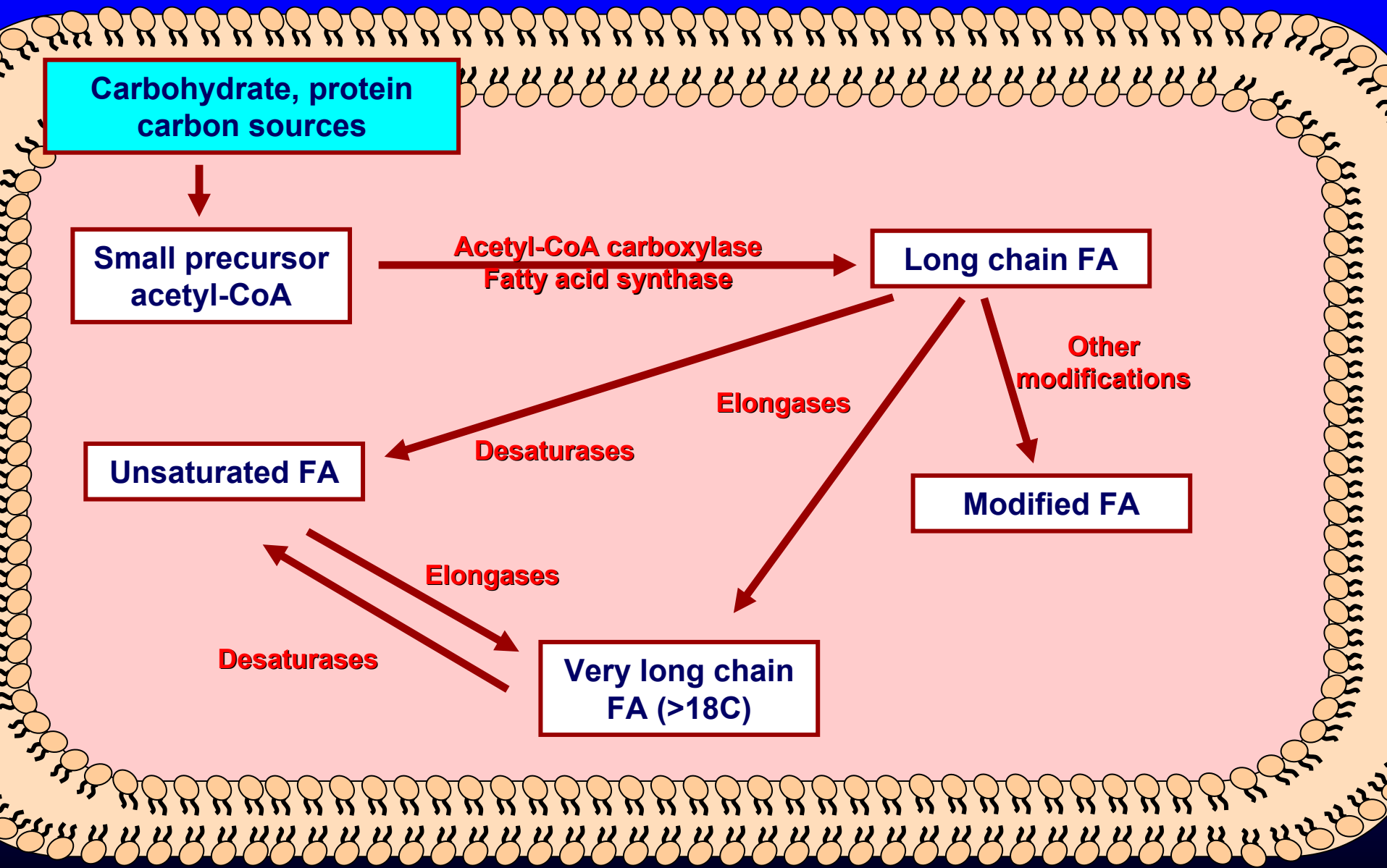
Docosahexaenoic acid (22:6*n*-3)

Neither linoleic or linolenic acid can be synthesized de novo in mammals

Conversion of α-linolenic acid into its longer chain derivatives is not very efficient in adult humans especially males

Calder P. Clinical Science 2004;107:1-11

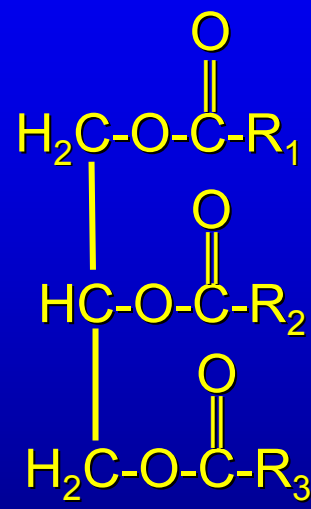
De Novo Fatty Acid Synthesis



Gurr MI et al. Lipid Biochemistry 5th Ed 2002 Blackwell Science Malden, MA

Triglycerides

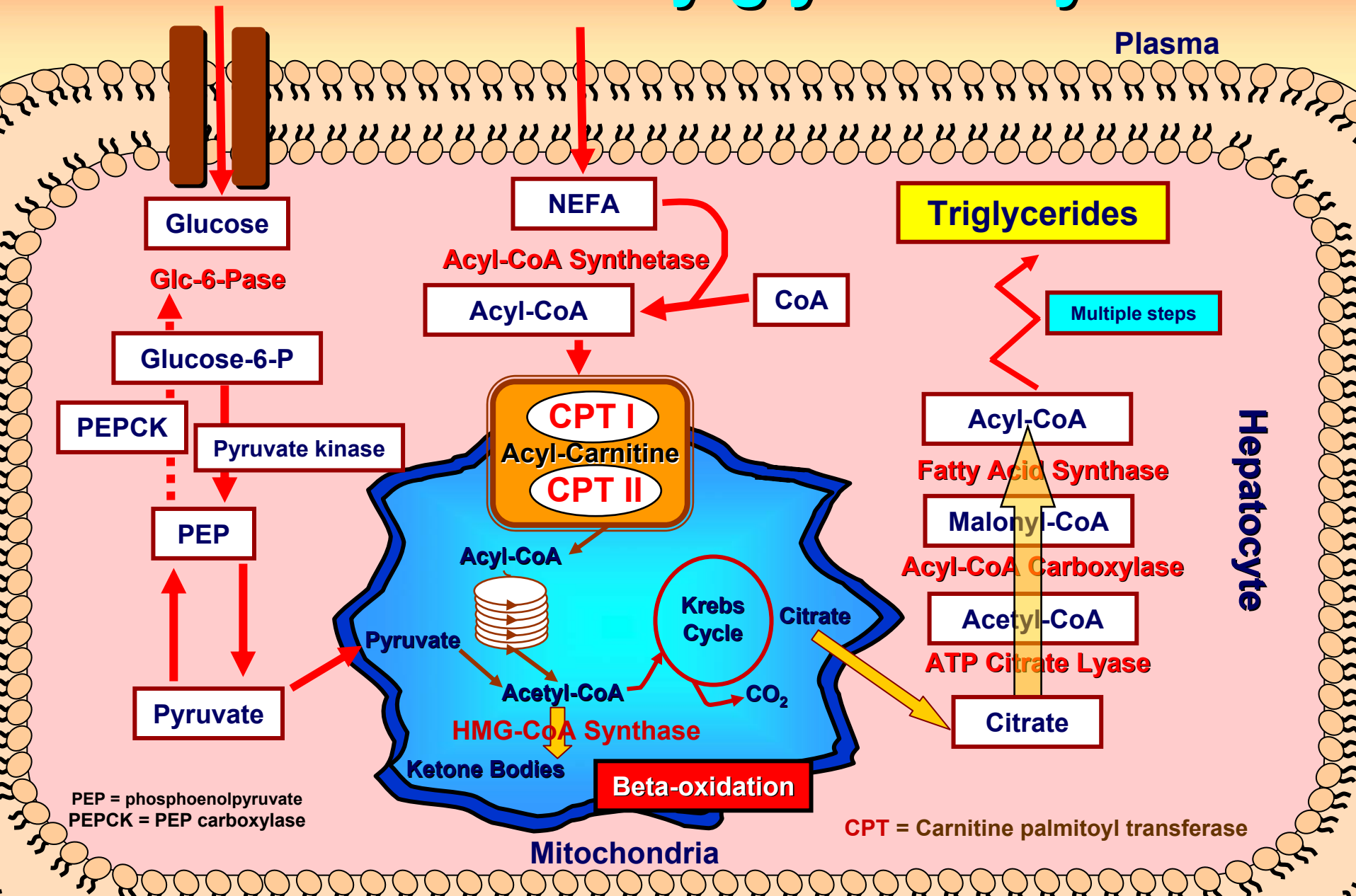
- Triglycerides are water-insoluble lipids consisting of three fatty acids linked to one glycerol molecule.
  - They represent a concentrated source of metabolic energy contributing 9 kcal/gm.
- TG are transported as core constituents of all lipoproteins, but the greatest concentration is in TG-rich chylomicrons and VLDL particles



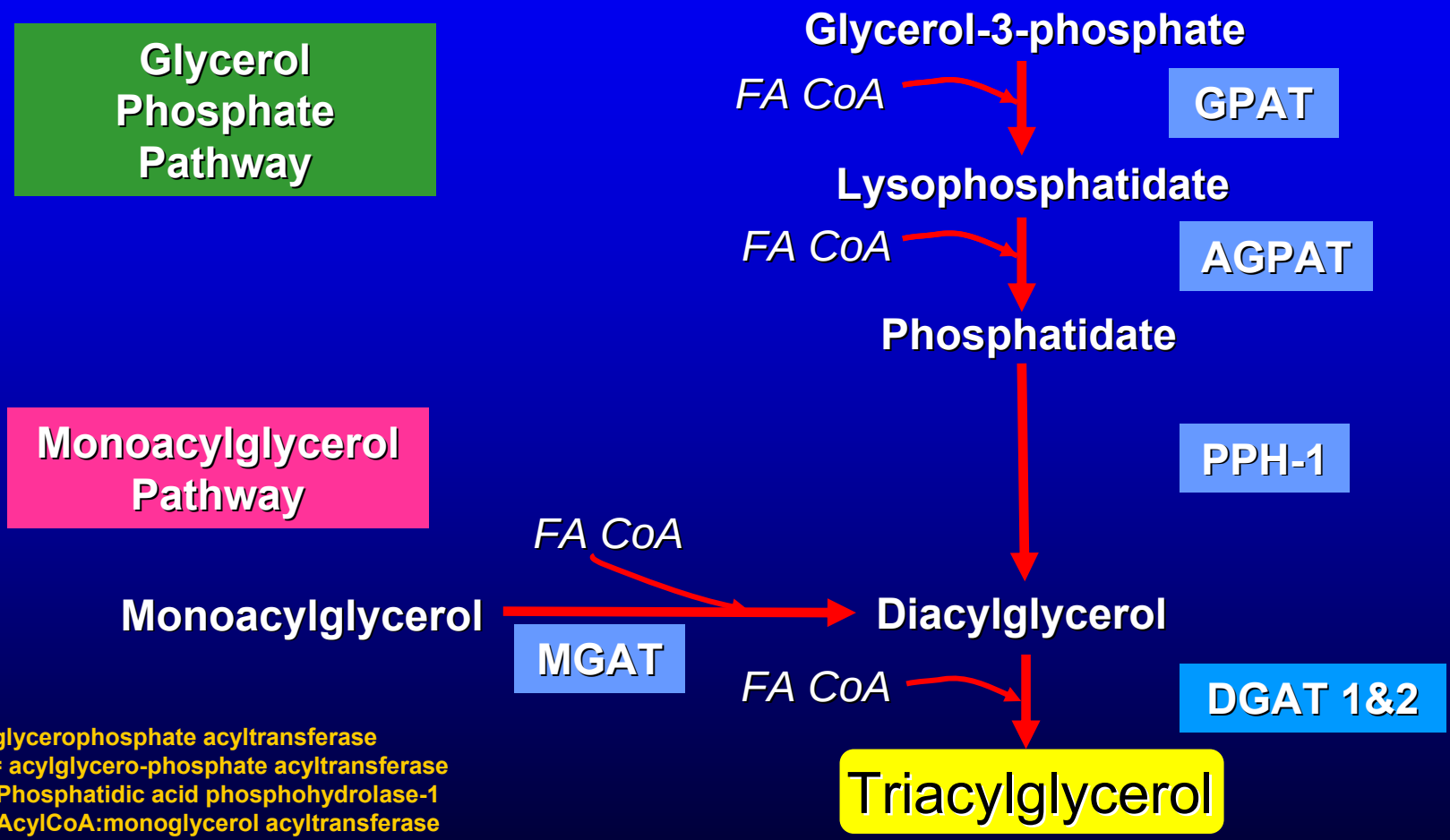
R = Fatty acid chain

Rafai, N et al. Handbook of Lipoprotein Testing AACC Press Washington DC 2nd Ed 2000

Substrates for Triacylglycerol Synthesis



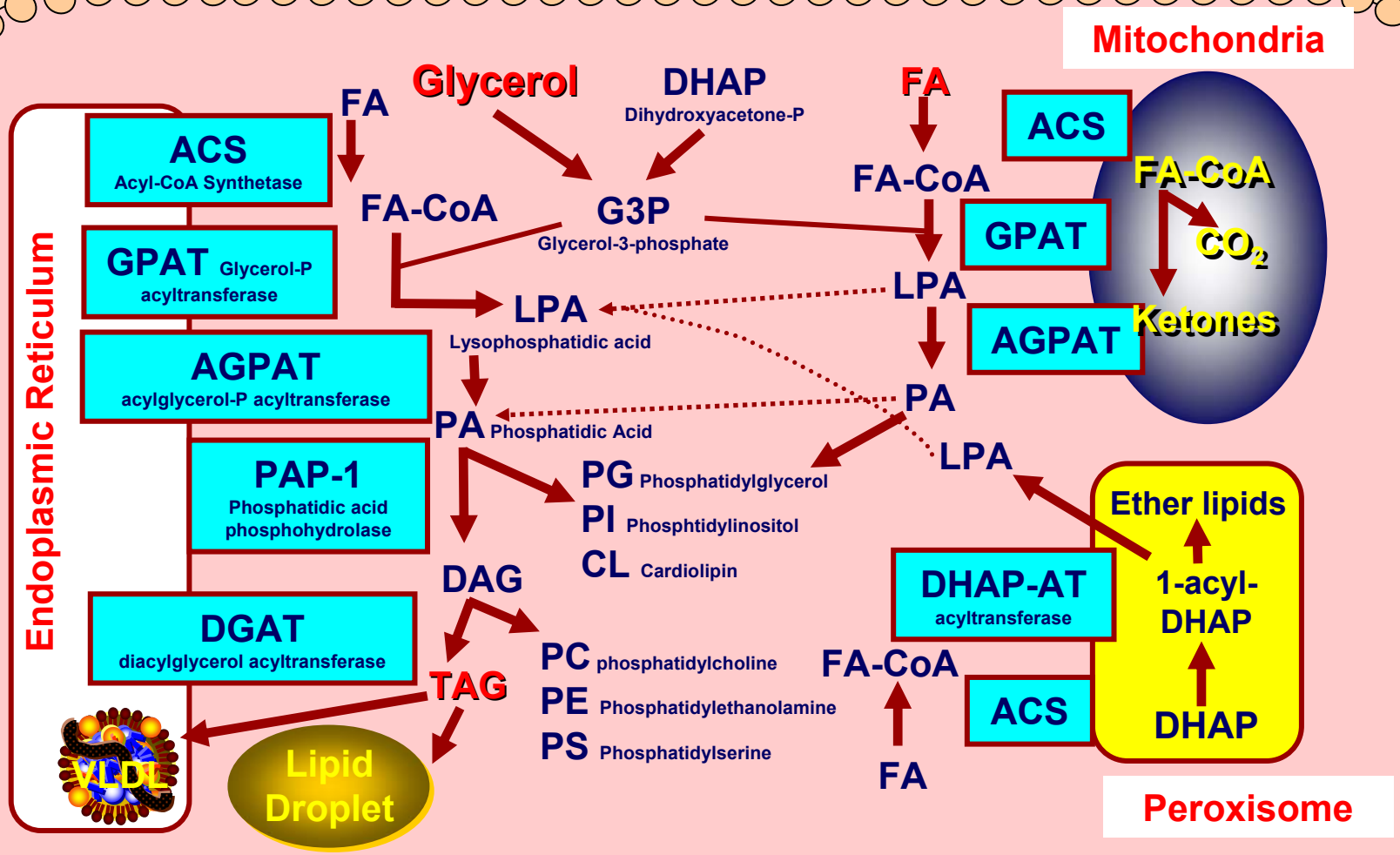
Triacylglycerol (Triglyceride) Synthesis & DGAT Enzymes



GPAT = glycerophosphate acyltransferase
AGPAT = acylglycero-phosphate acyltransferase
PPH-1 = Phosphatidic acid phosphohydrolase-1
MGAT = AcylCoA:monoglycerol acyltransferase
DGAT Diacylglycerol acyltransferase

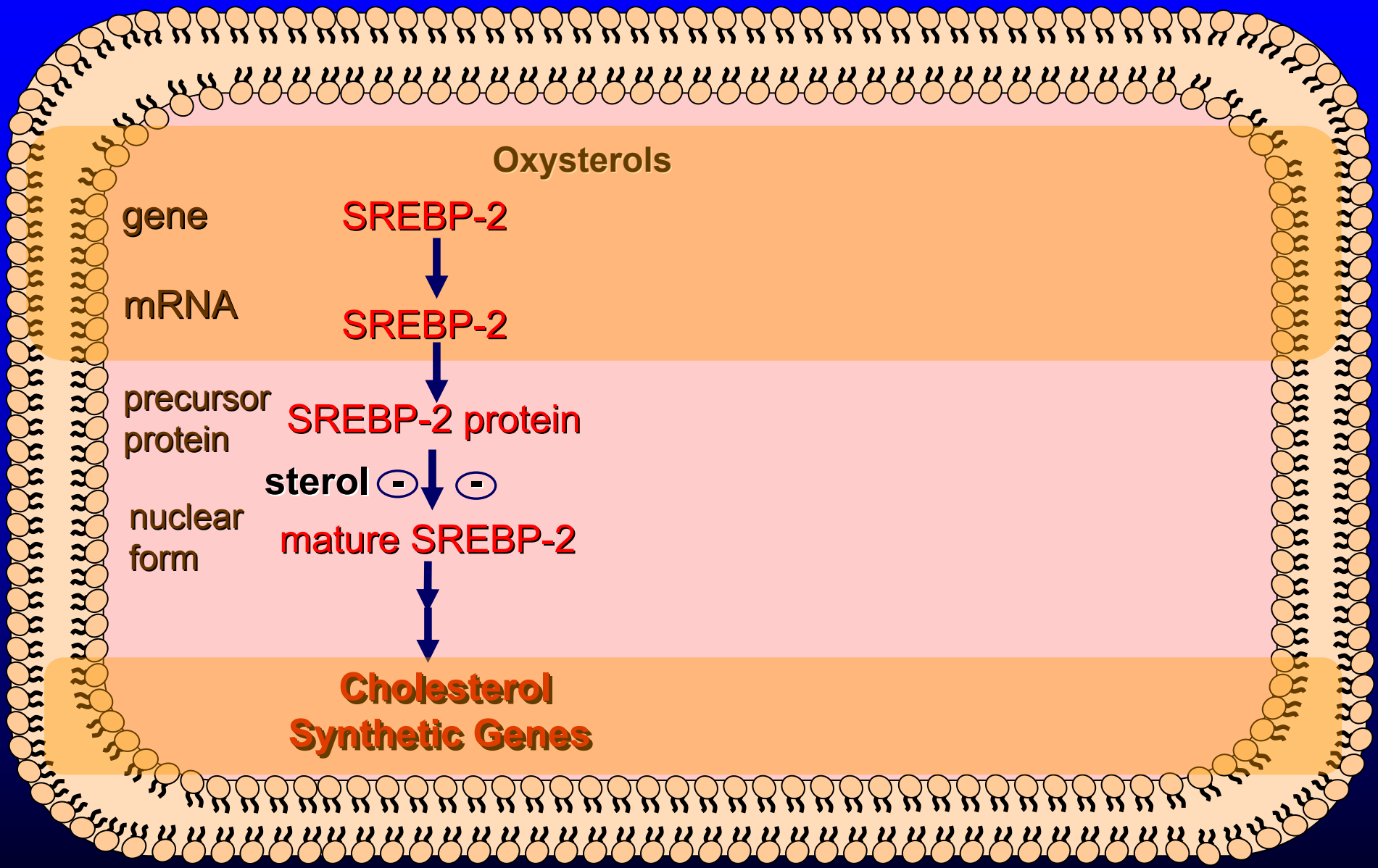
Hubert C. Chen, Robert V. Farese, Jr *ATVB* 2005;25:482-486.

Hepatic Triacylglyceride Synthesis



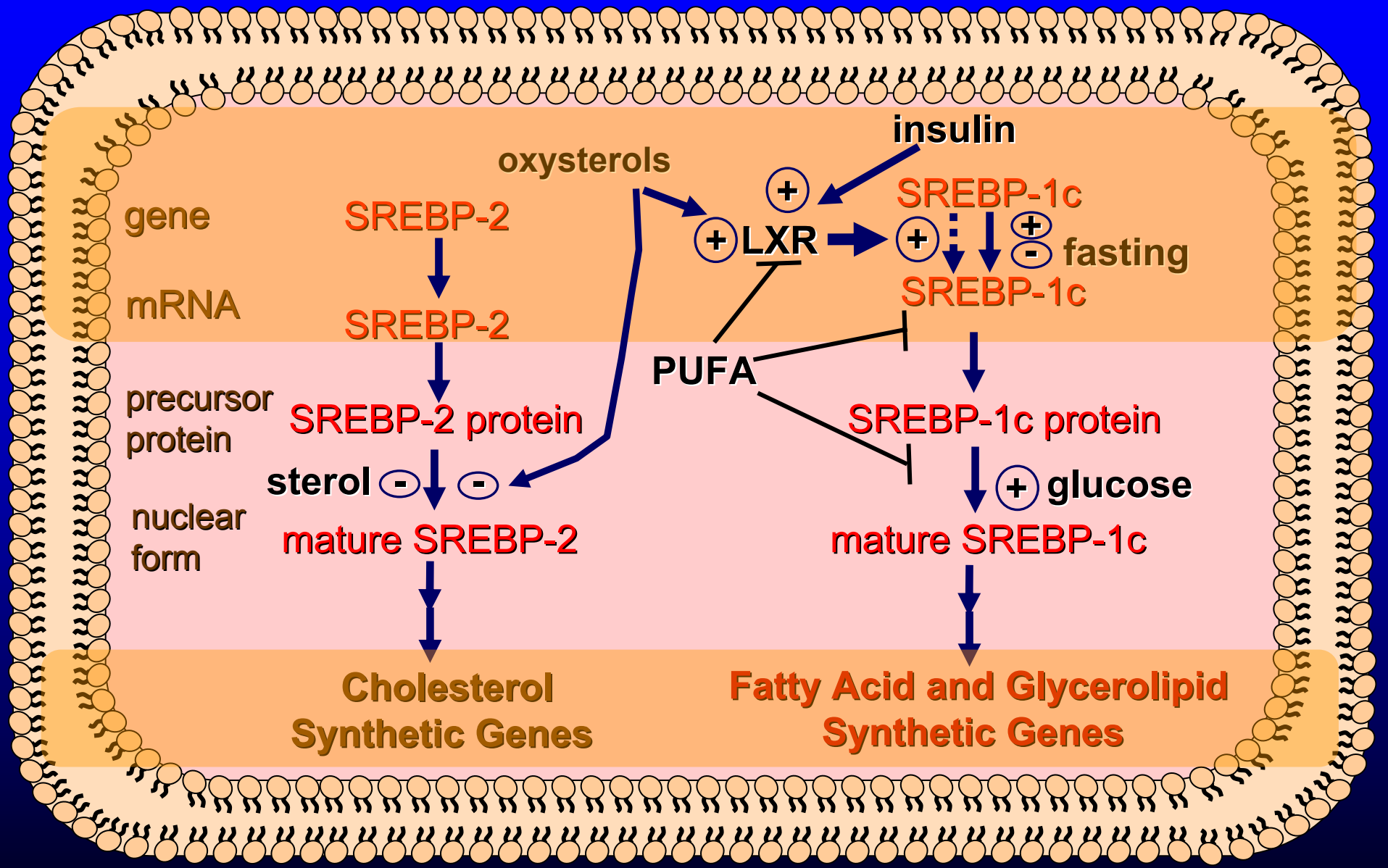
Coleman R & Douglass L. Prog Lip Res 2004 43;134-176

Regulation of Lipogenic Genes



Coleman R & Douglass L. Prog Lip Res 2004 43;134-176

Regulation of Lipogenic & TG Genes



Coleman R & Douglass L. Prog Lip Res 2004 43;134-176

Nuclear Receptors

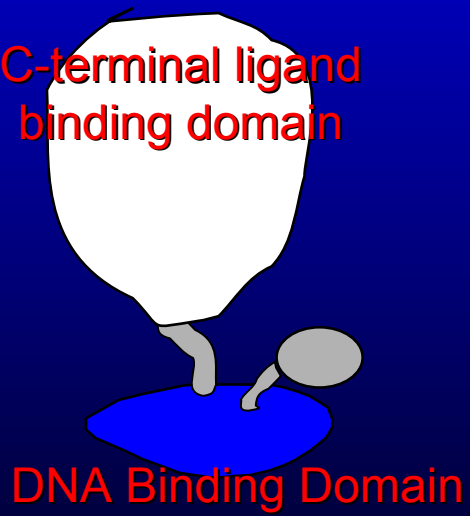
- Nuclear Receptors (NRs) exist in inactive forms as multi-protein complexes.
- Activation occurs when a ligand (fatty acid) binds to the NR and causes conformational changes
  - This alters the protein-protein interfaces of the molecule
- Heterodimerization with the Retinoid X Receptor occurs further changing the conformation

Nuclear Receptors

- At least 4 nuclear receptors (NRs) are affected by fatty acids and may regulate triglyceride metabolism.
  - Liver X receptor (LXR)
  - Hepatocyte Nuclear Factor-4-alpha (HNF-4-α)
  - Farnesol X Receptor (FXR)
  - Peroxisome Proliferator-Activated Receptors (PPARs)
- These often heterodimerize with Retinoid X Receptors (RXR)

Peroxisome Proliferator-Activated Subfamily of Nuclear Receptors (PPARs)

- PPAR and LXR subfamilies account for 5 of the 48 nuclear receptors that have been identified in human and mouse genomes
- They possess a conserved DNA binding and ligand binding domains
  - The DNA binding domain has two zinc finger motifs that mediate sequence-specific recognition of hormone-response elements in distinct target genes
  - The C-terminal ligand binding domain determines the specific binding properties of each receptor and mediates ligand-regulated interactions with effectors or repressors of transcription

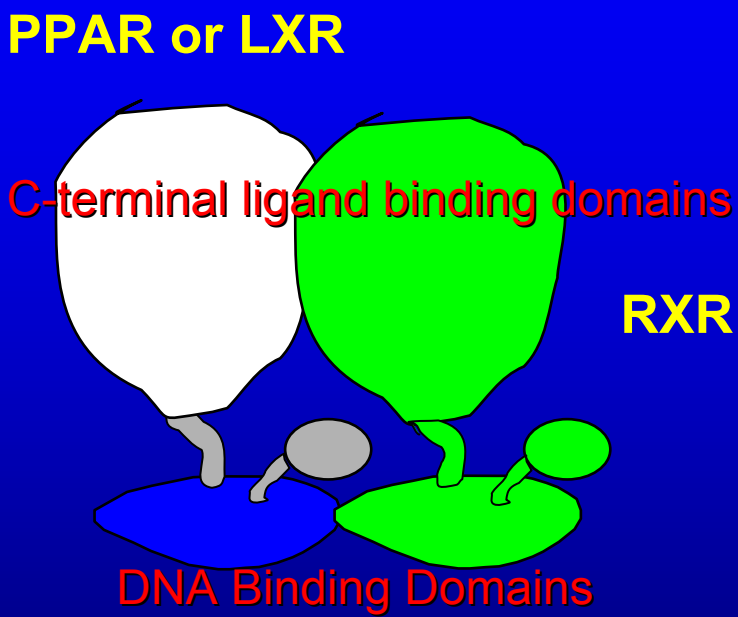


Li, AC, & Glass CH. J Lipid Res 2004;45:2161-2173

Transcriptional Activities of Peroxisome Proliferator-Activated and Liver X Receptors

PPARs and LXRs possess the conserved DNA binding domain and the C-terminal ligand binding domain characteristic of nuclear hormone receptors

PPARs and LXRs bind to specific response elements in target genes as heterodimers with retinoid X receptors (RXRs) which are also members of the nuclear receptor superfamily



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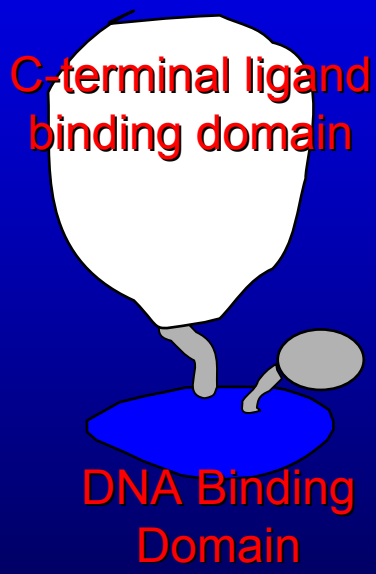
Liver X Receptors (LXRs)

LXR alpha



Tissue expression

Liver, macrophages

Biological Functions

Cholesterol absorption (intestine), cholesterol excretion (liver), cholesterol efflux (peripheral cells), fatty acid biosynthesis (liver & peripheral cells)

Ligands

24(S),25 epoxycholesterol, 22(S)-hydroxycholesterol, 24(S)-hydroxycholesterol

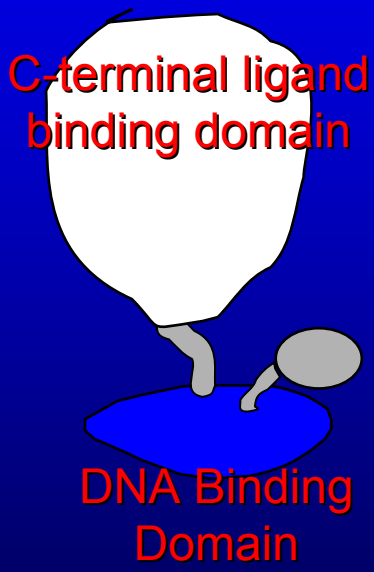
Disease Targets

Atherosclerosis

Li, AC, & Glass CH. J Lipid Res 2004;45:2161-2173

Liver X Receptors (LXRs)

LXR beta



Tissue expression

Broadly expressed

Biological Functions

Cholesterol absorption (intestine), cholesterol excretion (liver), cholesterol efflux (peripheral cells), fatty acid biosynthesis (liver & peripheral cells)

Ligands

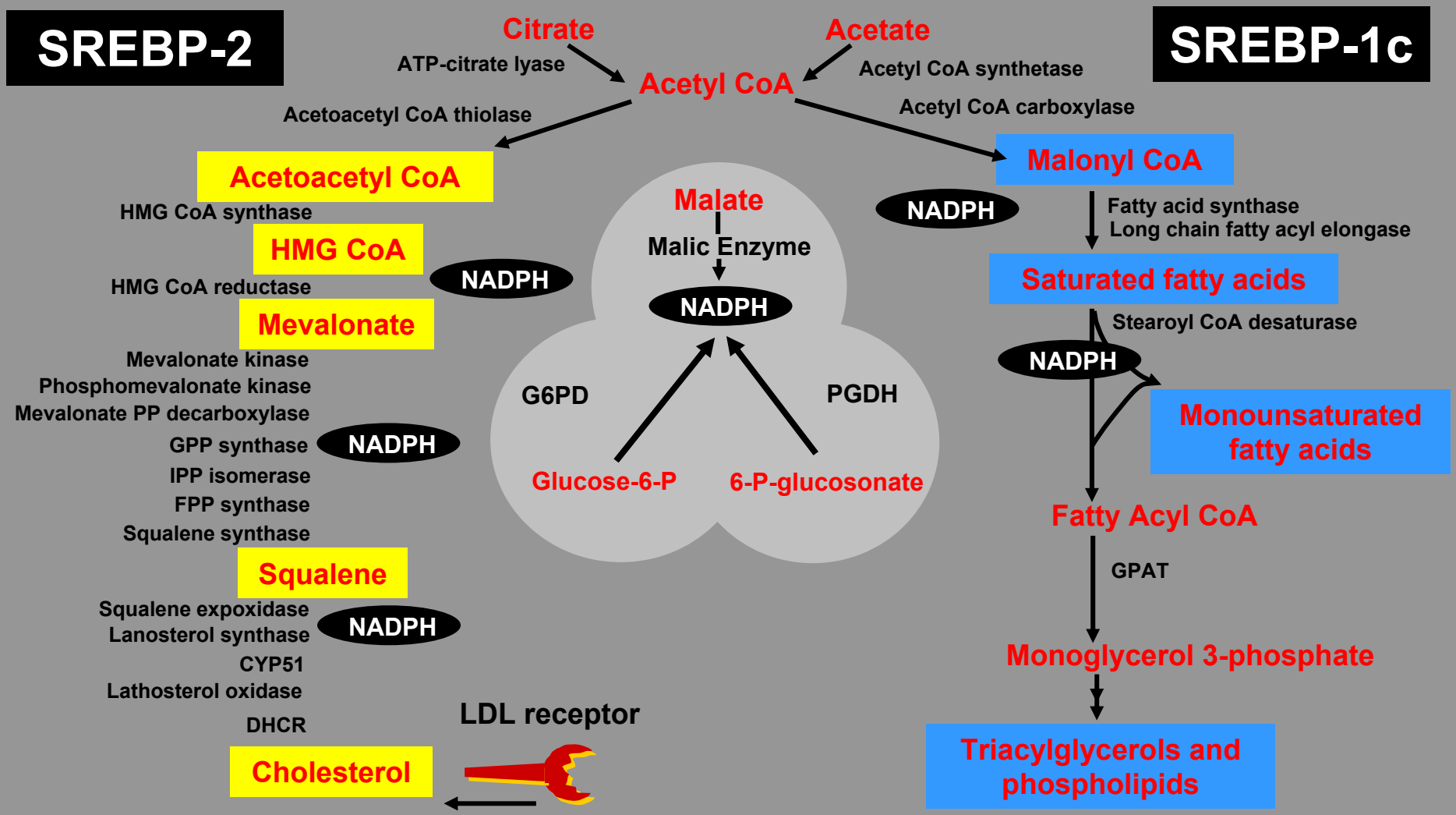
24(S),25 epoxycholesterol, 22(S)-hydroxycholesterol, 24(S)-hydroxycholesterol

Disease Targets

Atherosclerosis

Li, AC, & Glass CH. J Lipid Res 2004;45:2161-2173

LXR Controls SREBP-2 and SREBP-1c



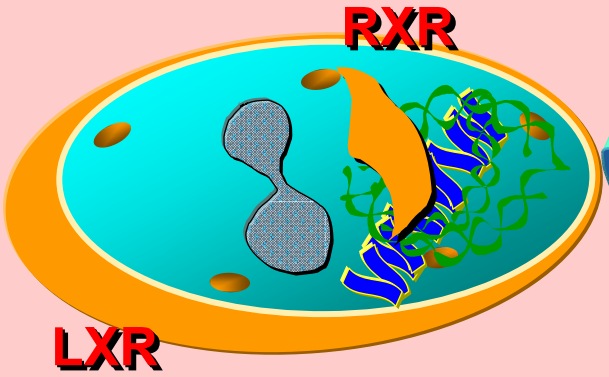
Sterol Regulatory Element Binding Protein (SREBP)-2 regulates the genes involved with cholesterol synthesis while SREBP-1C stimulates the lipogenic enzyme genes

Liver X Receptors

- LXR, which prevents excessive cellular cholesterol is activated by binding of ligands such as oxysterols
- LXR activation prevents excessive cellular cholesterol by
  - enhancing the expression of genes which stimulate bile acid synthesis (7-alpha-hydroxylase CYP7A)
  - Activates ABCA1 to promote cholesterol efflux into HDL
  - Activates ABCG5, G8 to increase cholesterol flux from hepatic cells into bile and from intestinal cells into the lumen: in effect the latter inhibits cholesterol absorption
- The net effect is reduced cellular cholesterol

LXR and Bile Acid Formation

Heterodimerization with Retinoid X Receptor



Liver X Receptor Upregulation

Increased oxysterols

Hepatocyte

Increased Free Cholesterol (FC)

↑ Oxysterol 7α hydroxylase production

Catalyzes formation of bile acids

Mitochondria

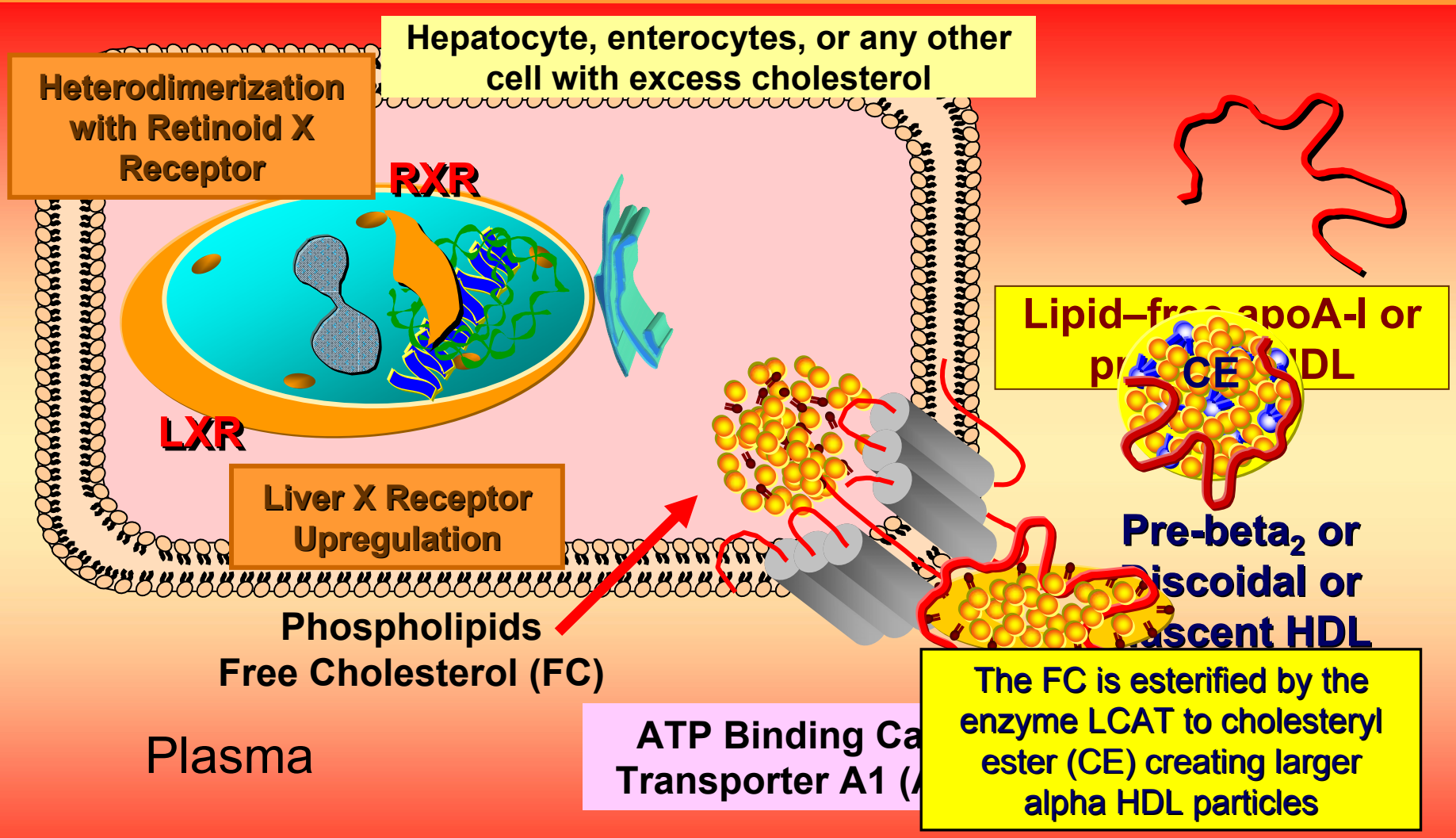
27 OH-Cholesterol

Peroxisomes

C24 acids

Cholate, Chenodeoxycholate synthesis

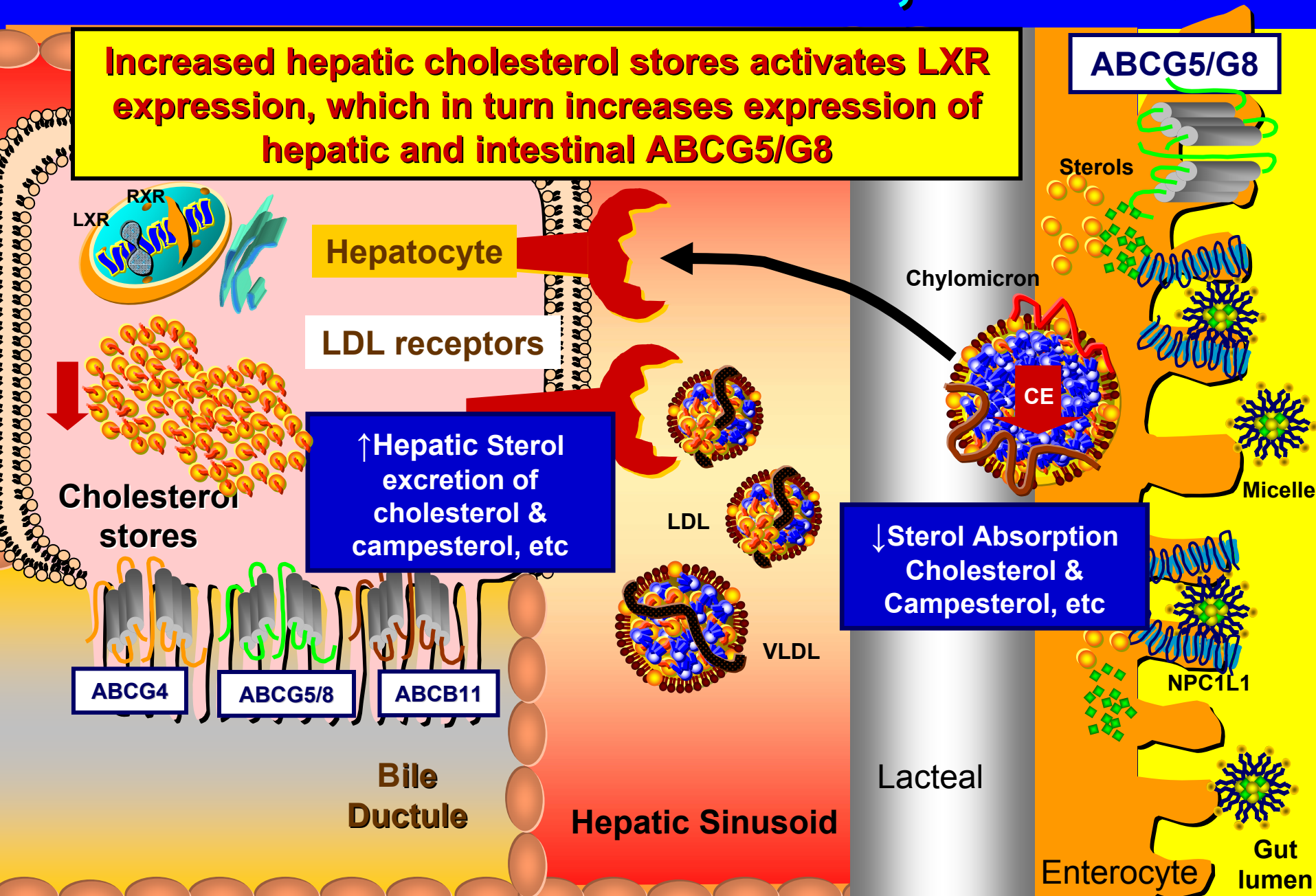
LXR & ApoA-I Lipidation



Rye K-A & Barter PJ. ATVB 2003;24:1-8 Sahoo D et al. J Lip Res 2004;45:1122-1131

LXRs Activate ABCG5, ABCG8

Increased hepatic cholesterol stores activates LXR expression, which in turn increases expression of hepatic and intestinal ABCG5/G8

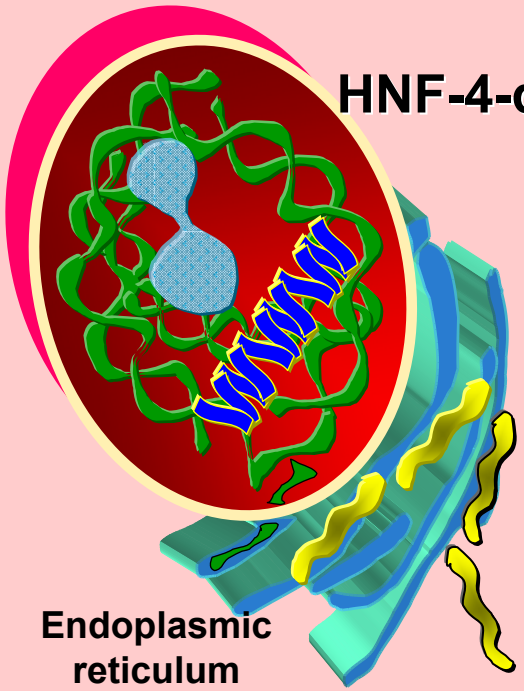


Hepatic Nuclear Factor - 4 -Alpha

- Hepatic Nuclear Factor - 4- alpha (HNF-4-α) binds to long chain fatty acyl-CoA with high affinity
- Binding of saturated FA stimulates whereas binding of polyunsaturated FA (PUFA) inhibits HNF-4-α
- HNF-4-α affects genes encoding proteins involved in both fat and carbohydrate metabolism including:
  - apoC-III, apoA-I, apoA-IV on lipoproteins
  - L-pyruvate kinase in carbohydrate metabolism
  - CYP 7A in bile acid synthesis
- Fibrates bind HNF-4-α and inhibit its transcriptional activity

Hepatic Nuclear Factor - 4 -Alpha

Hepatocyte



Long chain FA activation or PUFA inactivation of HNF-4-α

Potential Targets

apoC-III, apoA-I, apoA-IV (LP)
L-pyruvate kinase (carbs)
CYP 7A hydroxylase (BA)

Protein synthesis

Farnesol X Receptor (FXR)

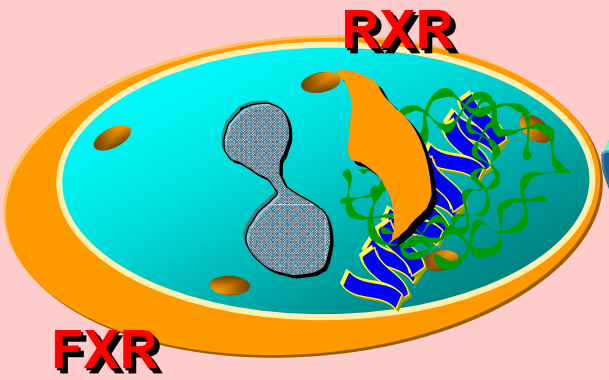
- Farnesol X Receptors are activated by bile acids and PUFA. They appear to protect cells from bile acid toxicity
- FXR controls bile acid synthesis by inhibiting expression of CYP 7A hydroxylase and other bile synthetic enzymes
- FXR controls expression of the bile acid export pump (BSEP) also termed ATP binding cassette transporter B11 (ABCB11)
- FXR expression has hypotriglyceridemic effects by:
  - Inducing PPAR-α expression
  - Modulating lipoprotein lipase
  - Inhibition of Sterol regulatory binding element protein-1C (SREB-1c) which is mediated through the short heterodimer protein (SHP) negative effect on LXR

FXR and Bile Acid Formation

Heterodimerization with Retinoid X Receptor

Catalyzes formation of bile acids

Mitochondria



↑ Oxysterol 7α hydroxylase production

27 OH-Cholesterol

Peroxisomes

C24 acids

Farnesol X Receptor Upregulation

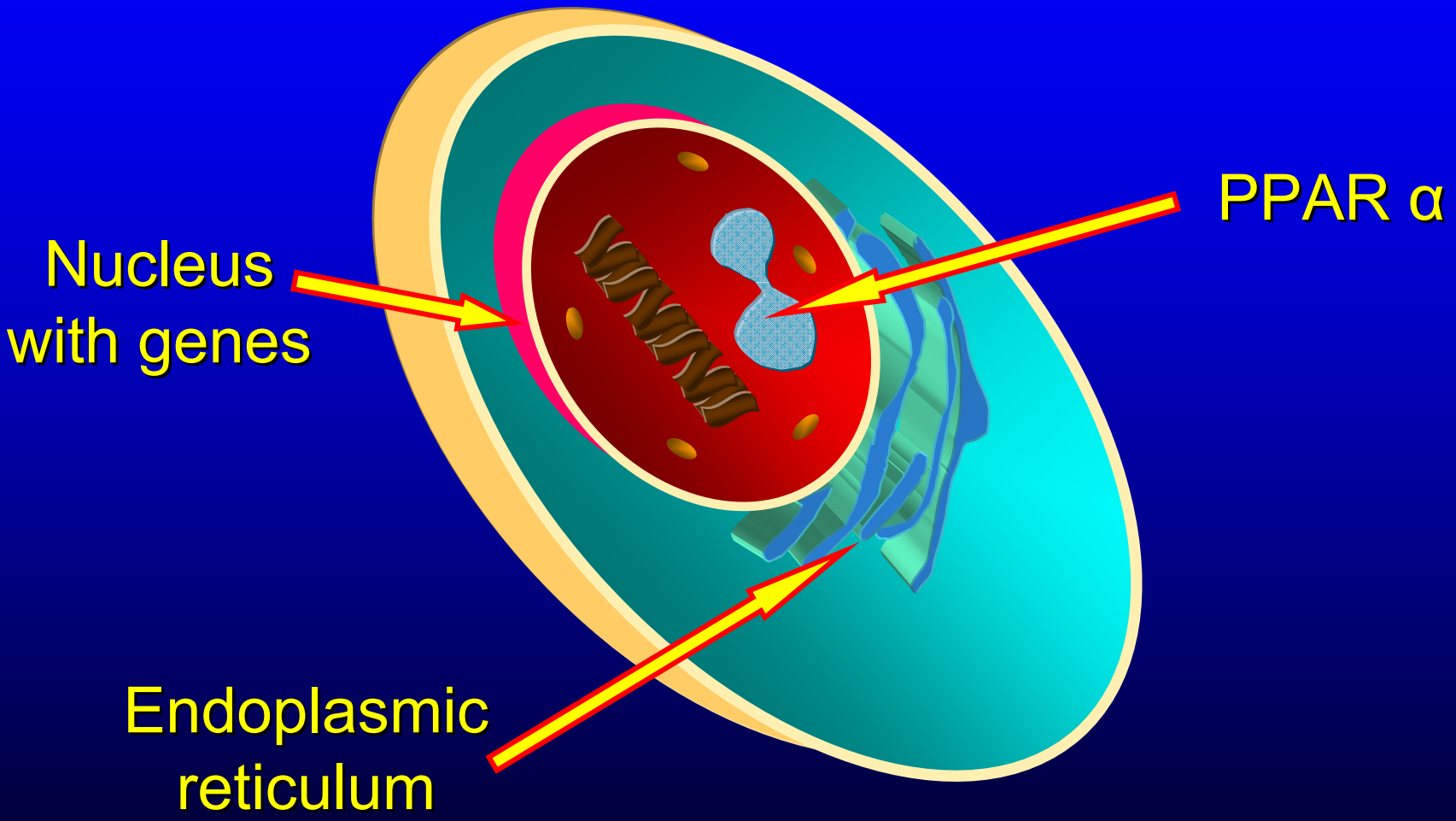
Cholate, Chenodeoxycholate synthesis

Hepatocyte

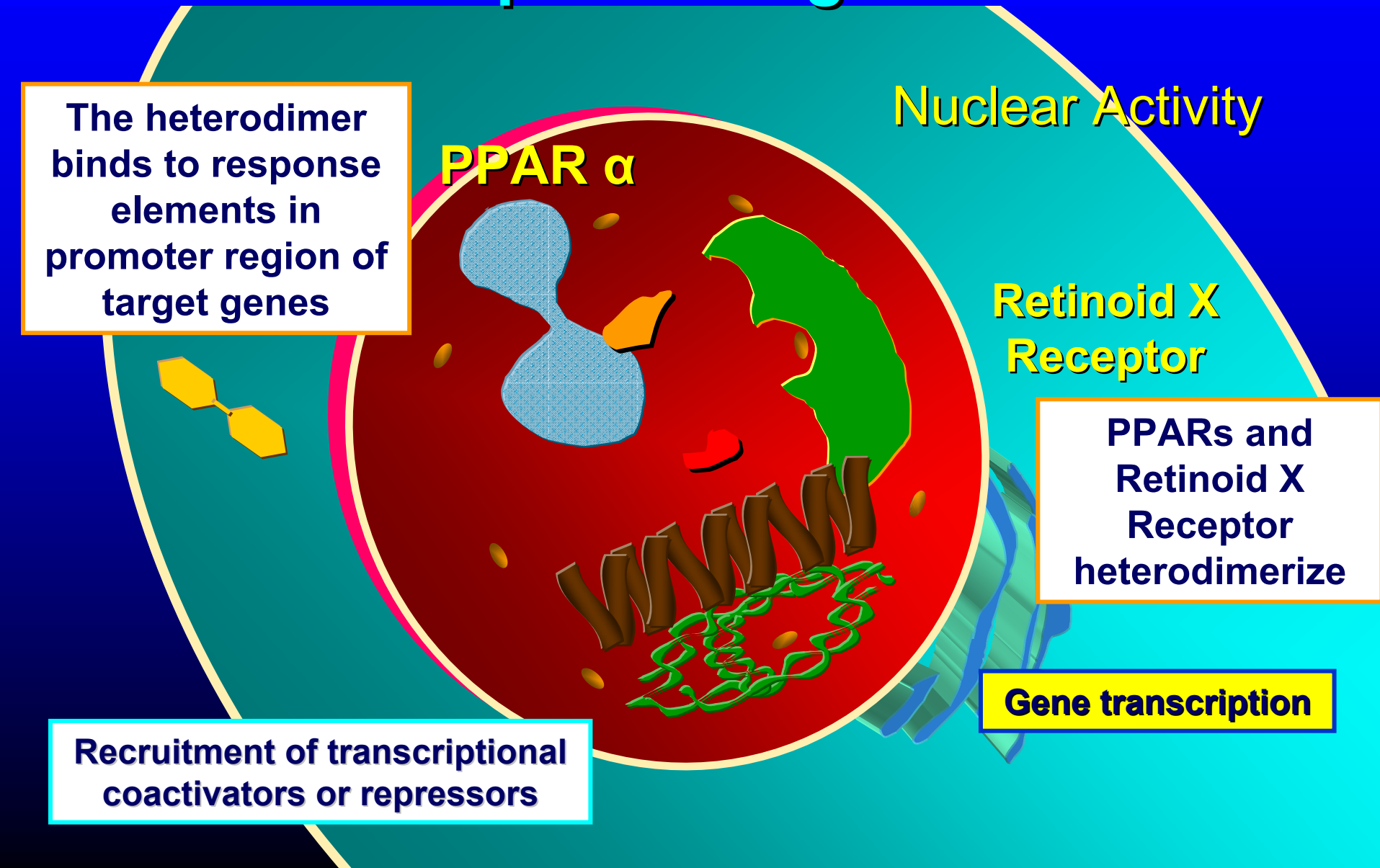
Peroxisome Proliferator-Activated Receptors (PPARs)

- Peroxisome Proliferator-Activated Receptors (PPARS) exist as three subtypes (alpha, beta or delta and gamma). They vary in their expression and biological function:
  - PPAR-α regulates genes in hepatocytes and vascular cells involved with multiple aspects of lipoprotein metabolism, fatty acid catabolism and vascular inflammation
  - PPAR-γ regulates genes in muscles and adipocytes involved with glucose control and vascular inflammation
  - PPAR-Δ is widely expressed and its function is not thoroughly understood at present

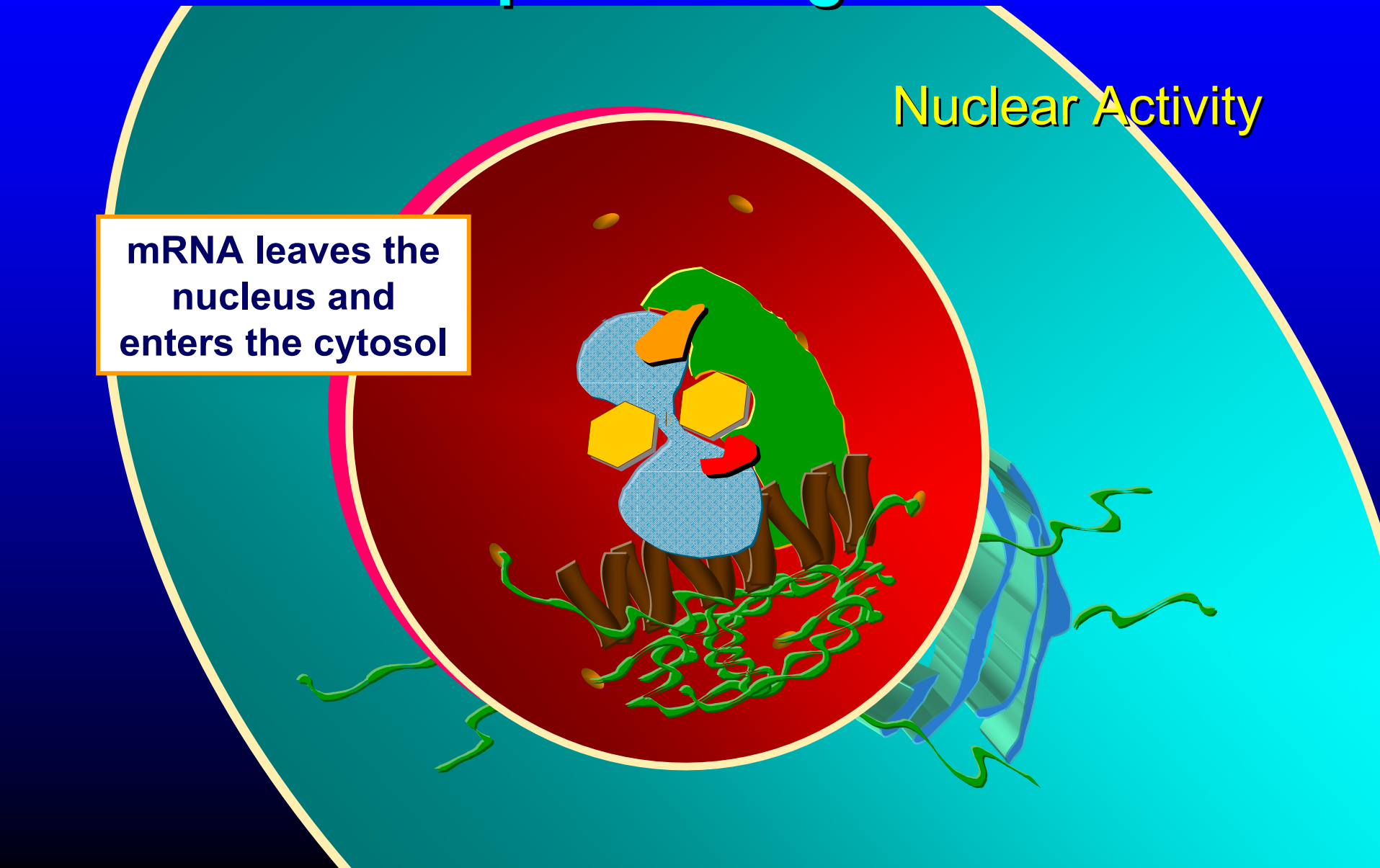
Role of Peroxisome Proliferator-Activated Receptors α (PPARs)



Peroxisome Proliferator-Activated Receptors α Agonism



Peroxisome Proliferator-Activated Receptors α Agonism



Peroxisome Proliferator-Activated Subfamily of Nuclear Receptors (PPARs)

PPAR alpha

Tissue expression

Liver, Heart, Kidney Adrenal

Cell specific expression

Endothelium, Macrophages, Smooth Muscle cells

Biological Functions

TG-rich lipoprotein synthesis and metabolism, β-oxidation of FA, anti-inflammation

Ligands

PUFAs, 8(S)-HETE

Disease Targets

Hypertriglyceridemia

Drugs

Fibrates

HETE = hydroxyeicosatetraenoic acid

Li, AC, & Glass CH. J Lipid Res 2004;45:2161-2173

Peroxisome Proliferator-Activated Subfamily of Nuclear Receptors (PPARs)

PPAR gamma

Tissue expression

Adipose tissue, spleen, adrenal, colon

Cell specific expression

Macrophages, T cells

Biological Functions

Fat cell development, Glucose homeostasis, Anti-inflammation

Ligands

PUFAs, 15d-PGJ2, 13-HETE, 9-HODE

Disease Targets

Type 2 diabetes

Drugs

TZDs

HETE = hydroxyeicosoateraenoic acid
15dPGJ2 15-deoxy$\Delta^{12,14}$-prostaglandin J_2
HODE = hydroxyoctadecadienoci acid

Li, AC, & Glass CH. J Lipid Res 2004;45:2161-2173

Peroxisome Proliferator-Activated Subfamily of Nuclear Receptors (PPARs)

PPAR delta

Tissue expression

Many tissues

Cell specific expression

Many cell types

Biological Functions

Endothelial biology,
Energy utilization,
Lipid metabolism

Ligands

PUFAs

Disease Targets

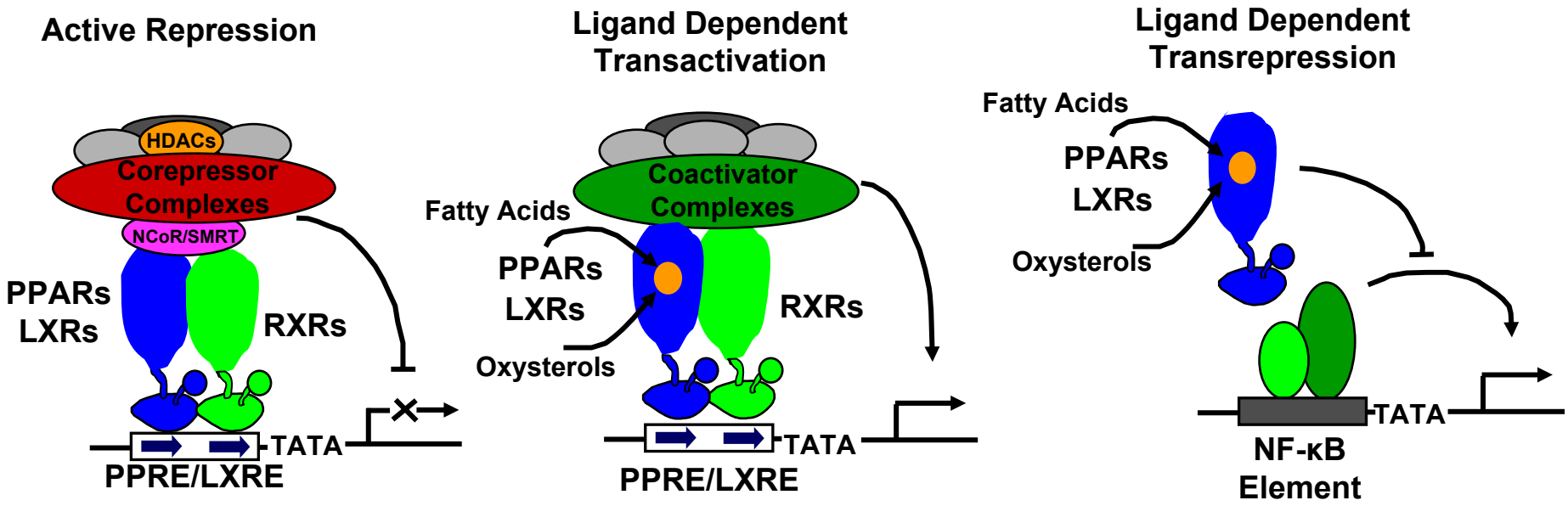
Metabolic syndrome ?

Drugs

- - -

Li, AC, & Glass CH. J Lipid Res 2004;45:2161-2173

Transcriptional Activities of Peroxisome Proliferator-Activated and Liver X Receptors



In the **absence of ligands**, PPAR/RXR and LXR/RXR heterodimers can bind to target genes and actively repress transcription through the recruitment of corepressor complexes that contain NCoR, SMRT and histone deacetylases (HDACs)

In the **presence of ligands**, PPAR/RXR and LXR/RXR heterodimers activate transcription through the recruitment of diverse coactivator complexes that include nucleosome remodeling activity, histone acetyltransferase and histone methyltransferase activities, and directly or indirectly recruit core transcriptional machinery to the promotor

PPARs and LXR agonists can inhibit the activities of other signal-dependent transcription factors, such as nuclear factor κB and activator protein-1 (AP-1). **This repression function contributes to their anti-inflammatory actions**

Li, AC, & Glass CH. J Lipid Res 2004;45:2161-2173

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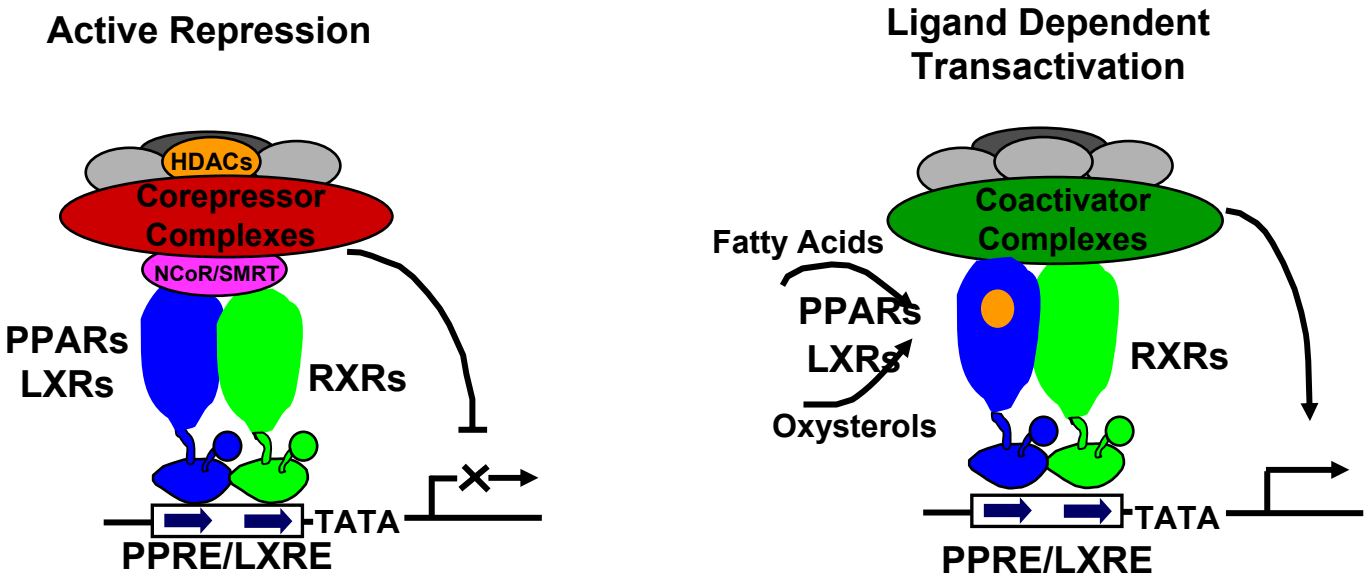

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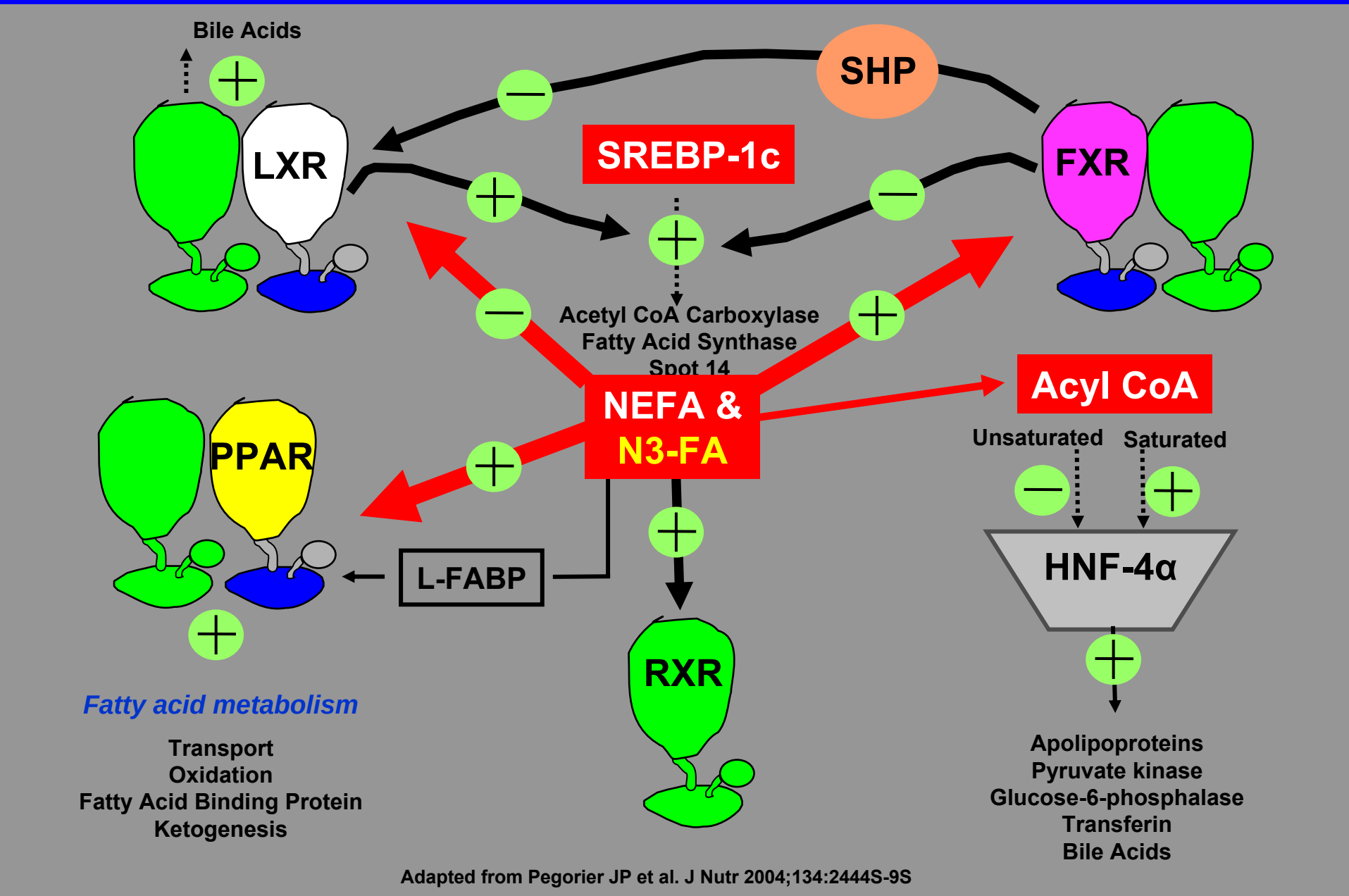
N-3 FA Direct NEFA away from TG storage to oxidation

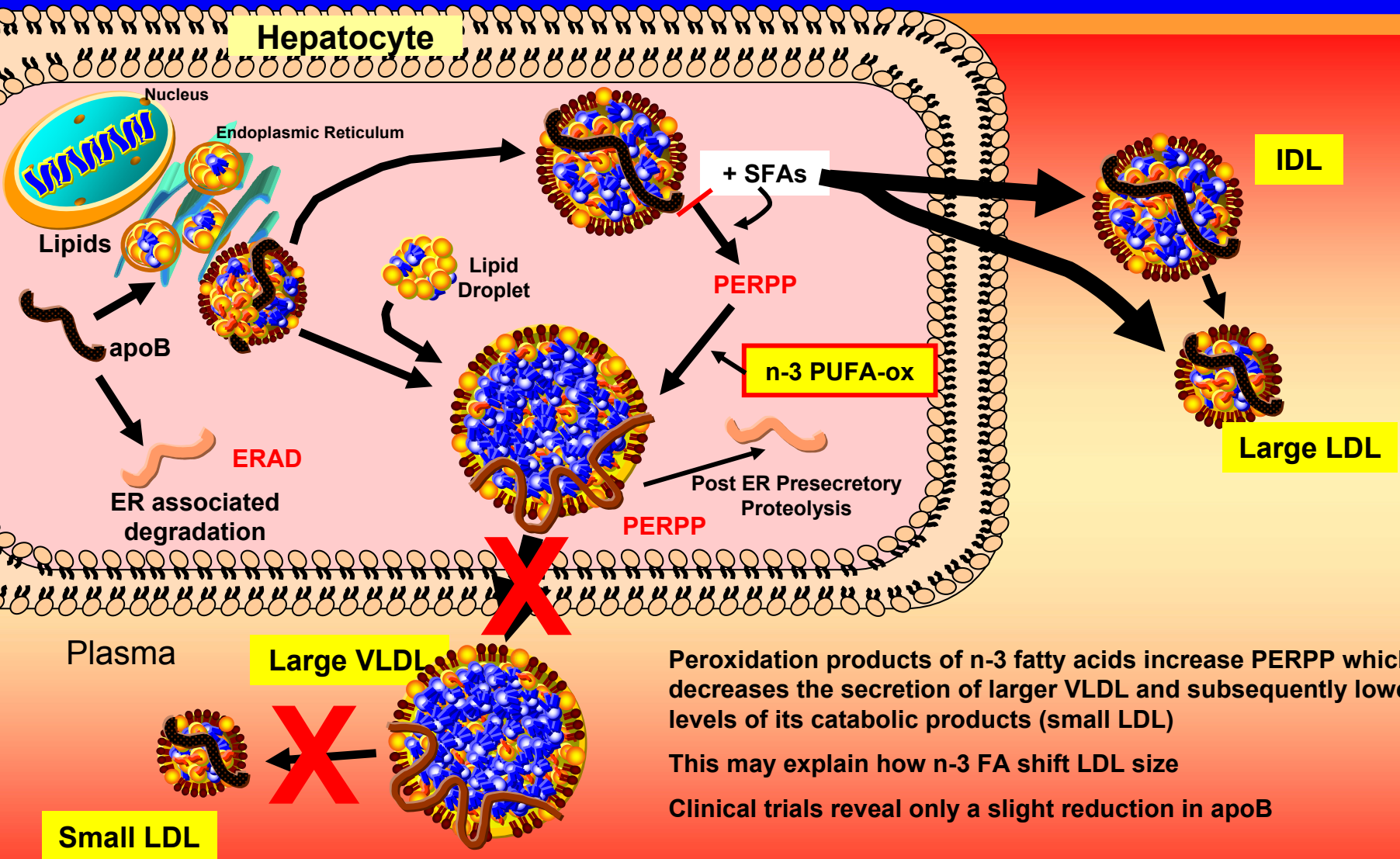


Adapted from Pegorier JP et al. J Nutr 2004;134:2444S-9S

N-3 Fatty Acids on Nuclear Receptors Involved with Lipogenesis

	Effects on Gene Regulation	Expected Changes		
		Triglycerides	HDL	LDL
PPAR-alpha	Increase	↓↓	↑	↓
LXR	Decrease	↓↓	↓	↓
FXR	Increase	↓↓	↑	↑
HNF-4-alpha	Decrease	↓↓	↓	–
Net Effects		↓↓↓↓	Neutral	Neutral

Effects of N-3 FA on Apo B



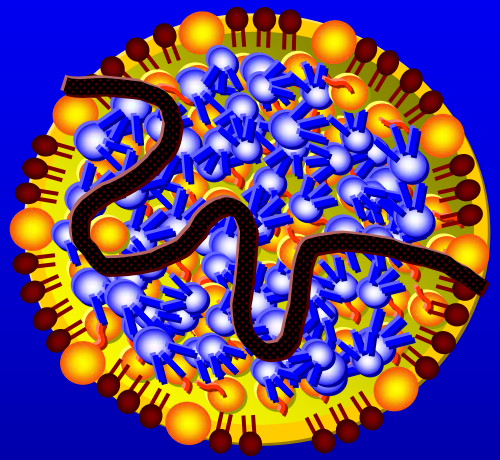
Peroxidation products of n-3 fatty acids increase PERPP which decreases the secretion of larger VLDL and subsequently lowers levels of its catabolic products (small LDL)

This may explain how n-3 FA shift LDL size

Clinical trials reveal only a slight reduction in apoB

Adapted from Krauss RM. J Clin Invest 2004;113:1253-55

N-3 Fatty Acids and Chylomicrons

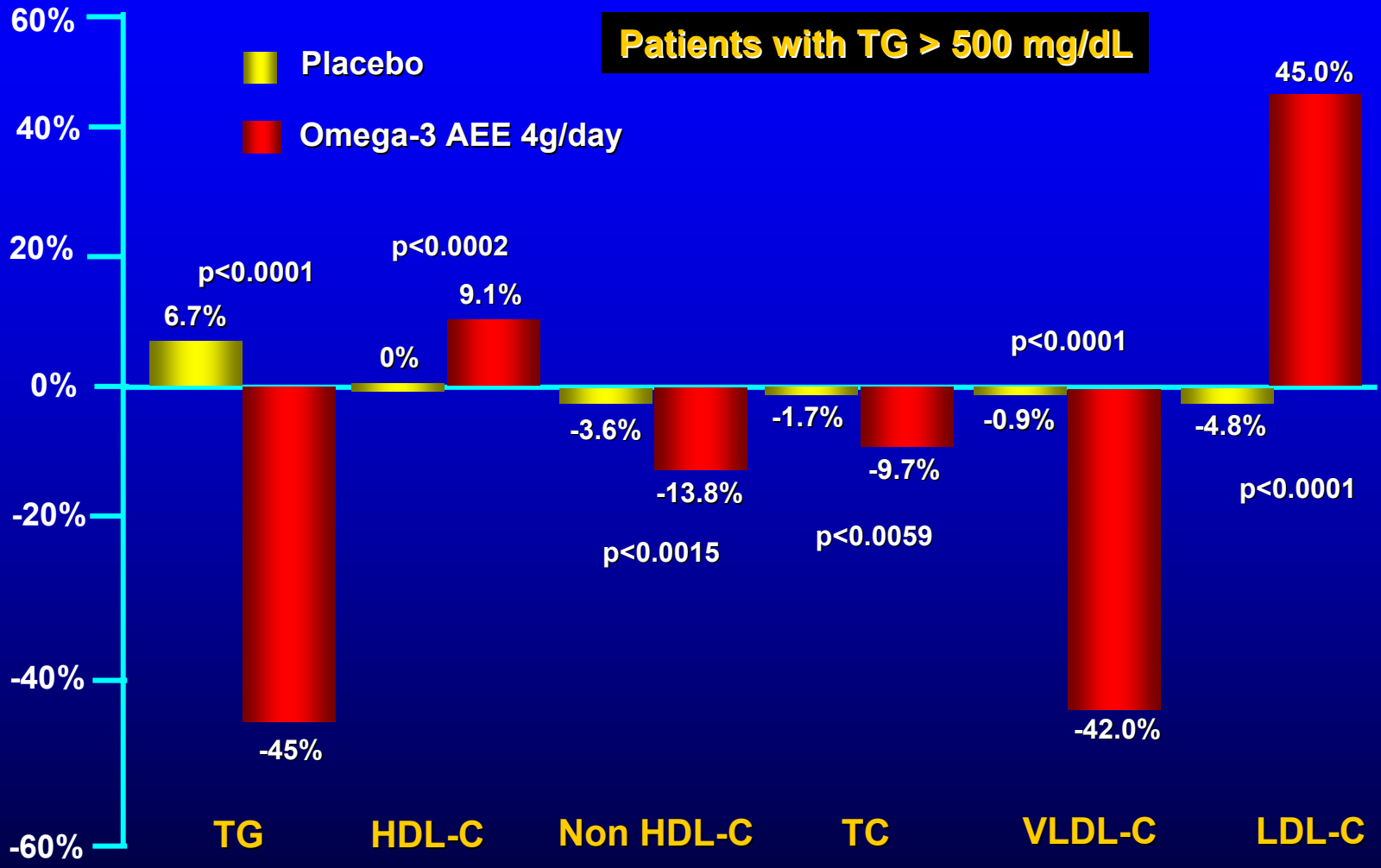


- n-3 FA decrease VLDL secretion which presents less competition for chylomicron lipolysis
- n-3 FA supplementation does decrease chylomicron particle size thereby improving clearance

- n-3 FA increase pre-heparin lipoprotein lipase activity in the fed state but had no effect on post-heparin lipase activity
- These combined effects support the use of n-3 FA as a valuable clinical tool for then treatment of hypertriglyceridemia

Park Y. et al. J Lipid res 2003;44:455-463

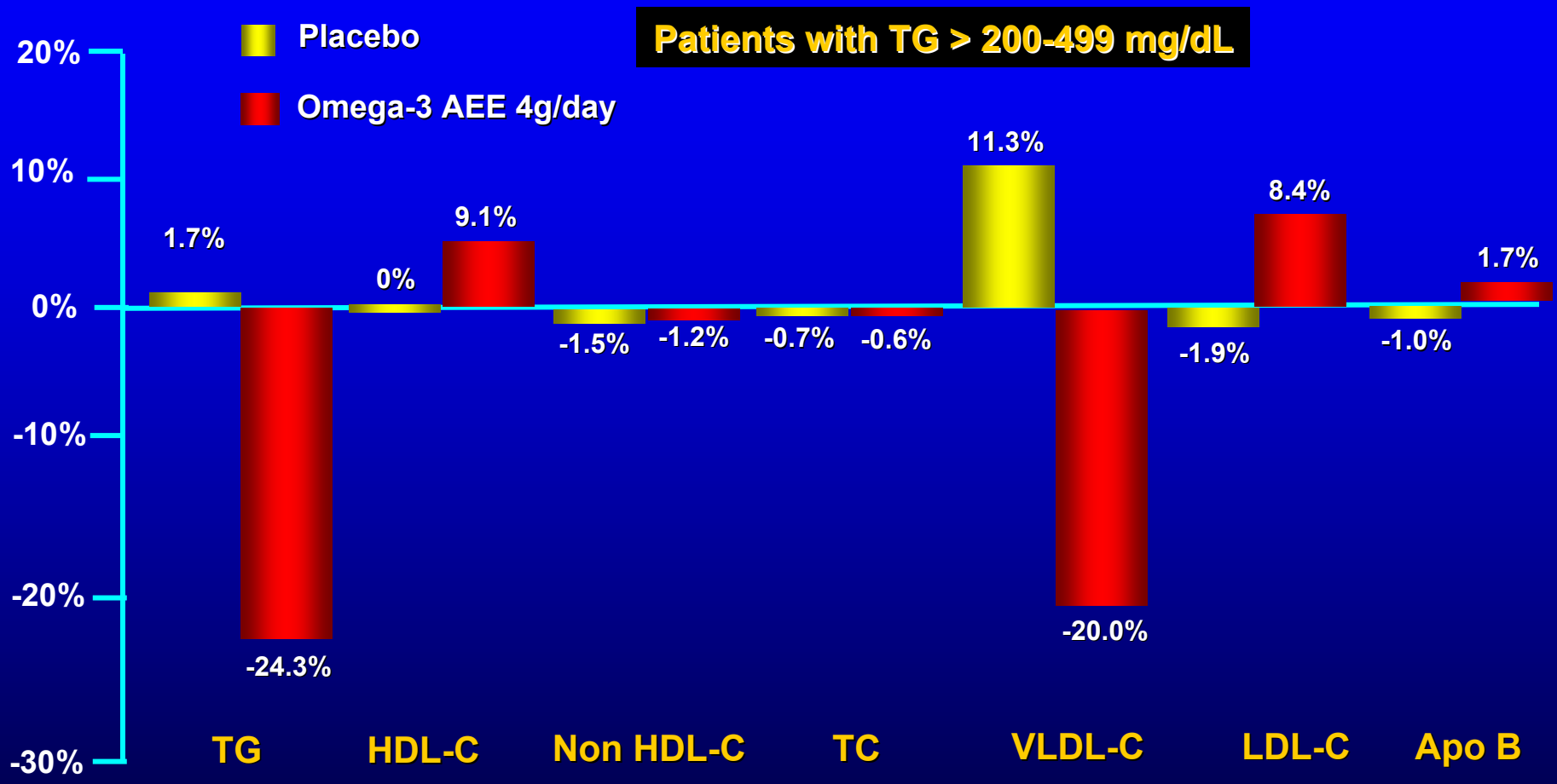
Omega-3-Ethyl Esters and Lipids



Baseline Lipids	816 mg/dL	22 mg/dL	2711 mg/dL	296 mg/dL	175 mg/dL	89 mg/dL

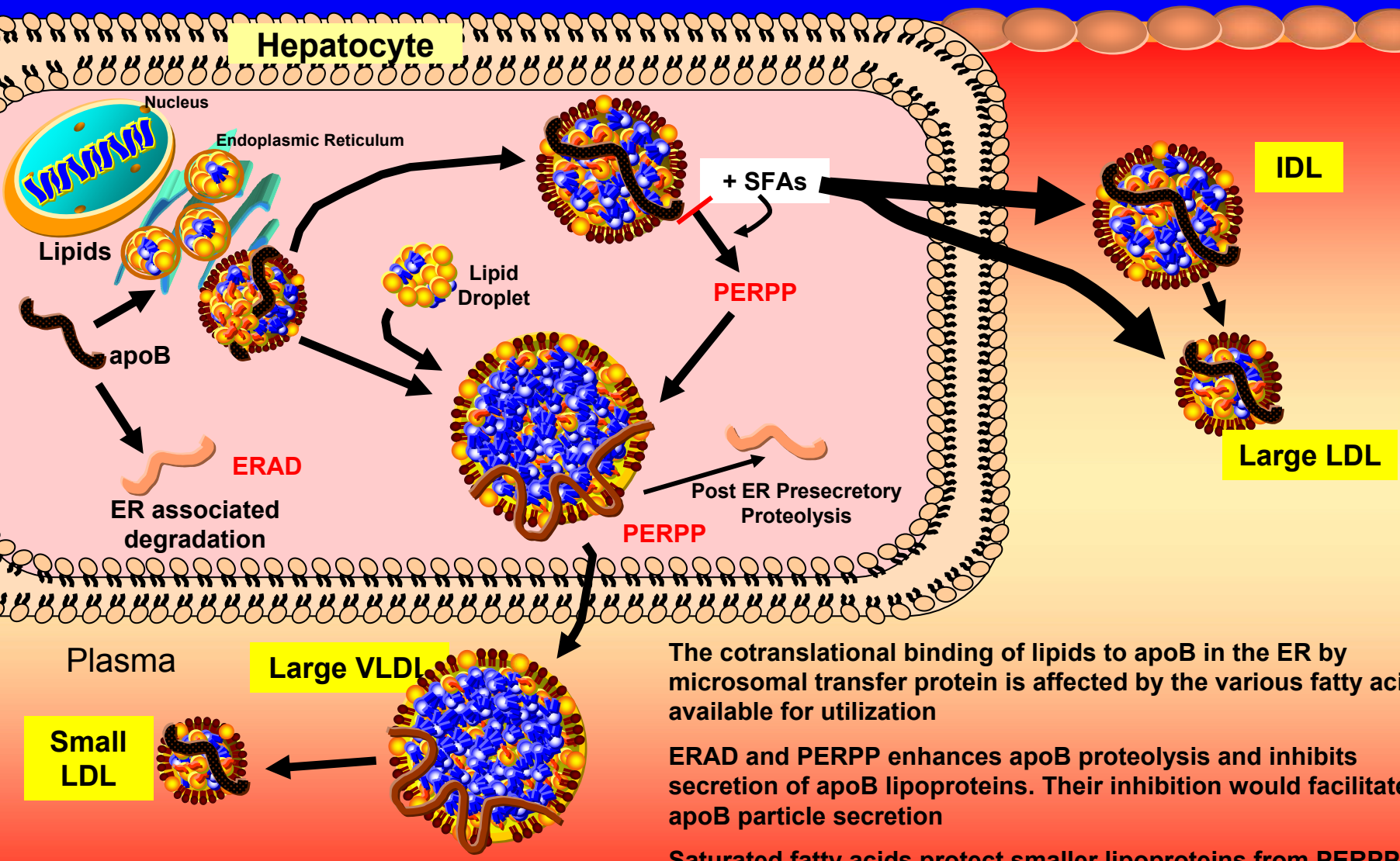
Pownall JH. et al. Atherosclerosis 1999;143:285-297

Omega-3-Ethyl Esters and Lipids



Dara on file, Reliant Pharmaceuticals

Intracellular Regulation of Apo B



The cotranslational binding of lipids to apoB in the ER by microsomal transfer protein is affected by the various fatty acids available for utilization

ERAD and PERPP enhances apoB proteolysis and inhibits secretion of apoB lipoproteins. Their inhibition would facilitate apoB particle secretion

Saturated fatty acids protect smaller lipoproteins from PERPP leading to increased secretion of small VLDL and IDL

Adapted from Krauss RM. J Clin Invest 2004;113:1253-55