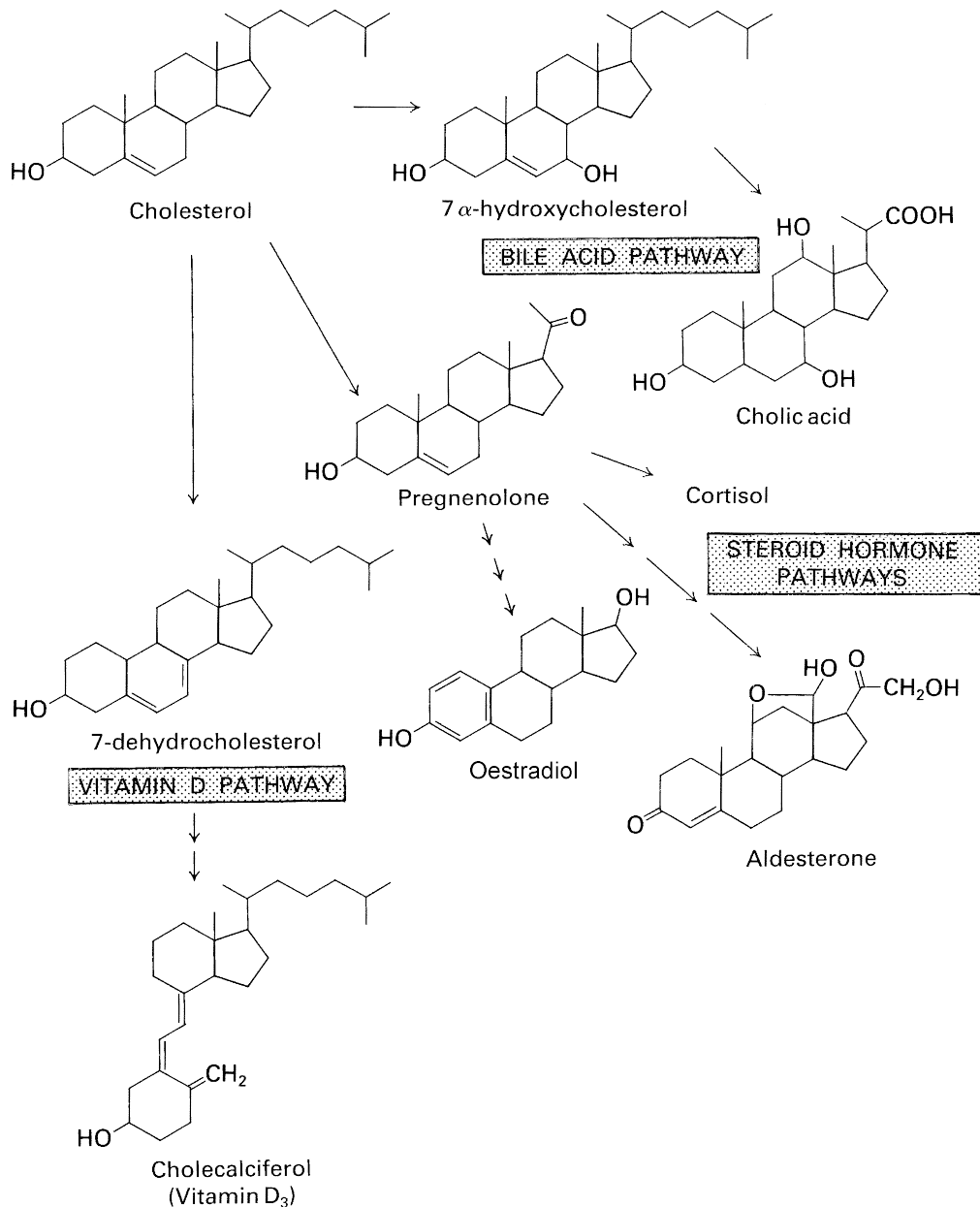


**ANSC/NUTR 618**  
**LIPIDS & LIPID METABOLISM**  
**Cholesterol Synthesis**

**I. Physiological requirements for cholesterol**

- A. Cholesterol is an integral part of membranes.
- B. Cholesterol is converted to vitamin D, estradiol, testosterone, and aldosterone in specific target tissues.
- C. Cholesterol is oxidized to form bile acids, essential in fat digestion.



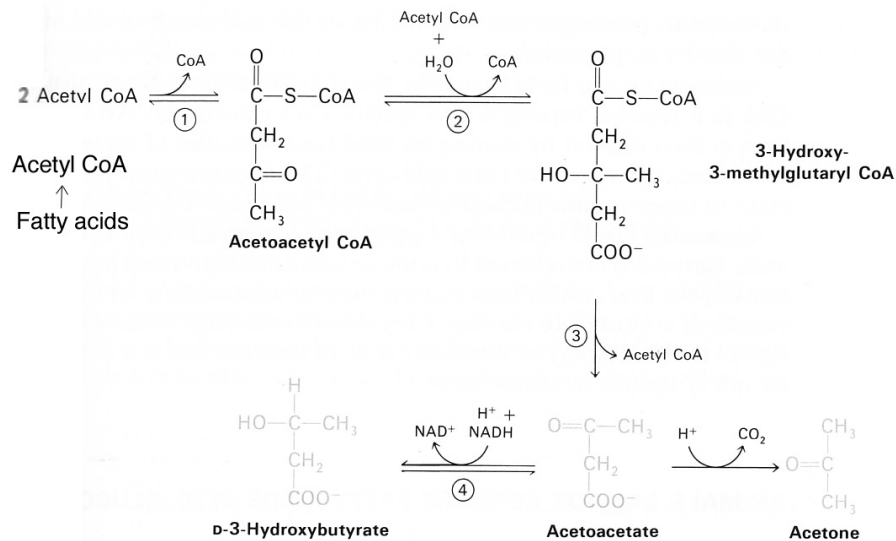
## II. Ketone body synthesis

A. Oversupply of fatty acids in the liver → ketone body synthesis

1. Liver mitochondria do not have enough oxaloacetate (OAA) to oxidize all of the acetyl-CoA produced from fatty acid oxidation.

2. Acetyl-CoA is used to produce ketone bodies *in the mitochondria*.

B. Pathway for ketone body synthesis

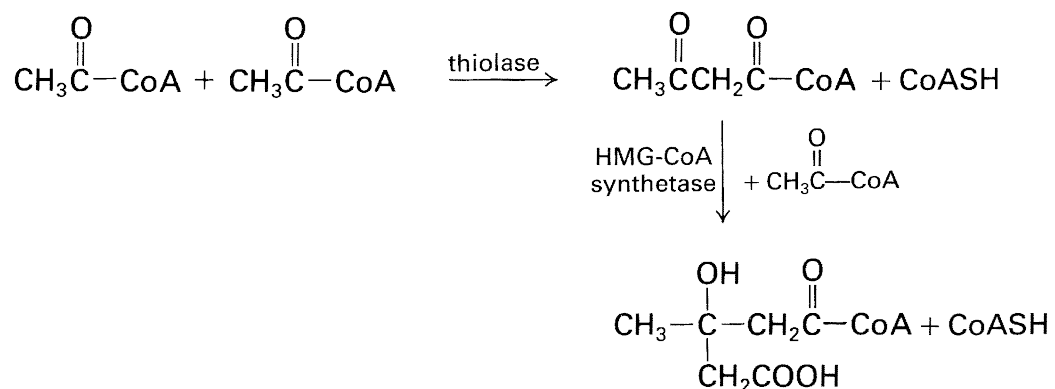


## III. Cholesterol synthesis

A. Formation of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA).

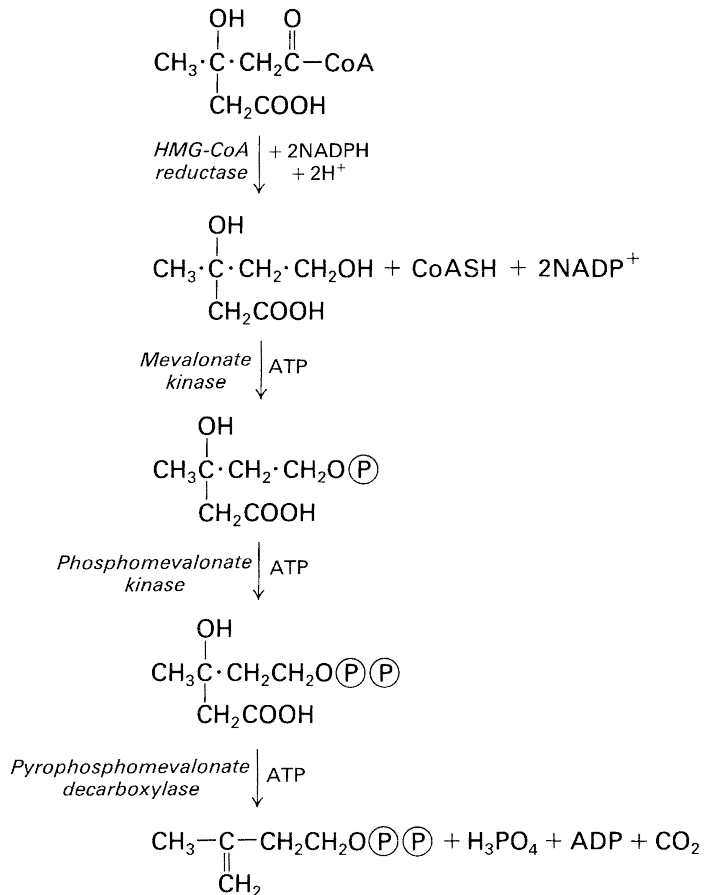
1. Condensation of acetyl-CoAs takes place in the *cytoplasm*.

2. HMG-CoA synthase is a *microsomal* enzyme.



## B. HMG-CoA reductase

1. Rate-limiting and regulatory enzyme for cholesterol synthesis
2. *Microsomal* enzyme that converts HMG-CoA to mevalonate

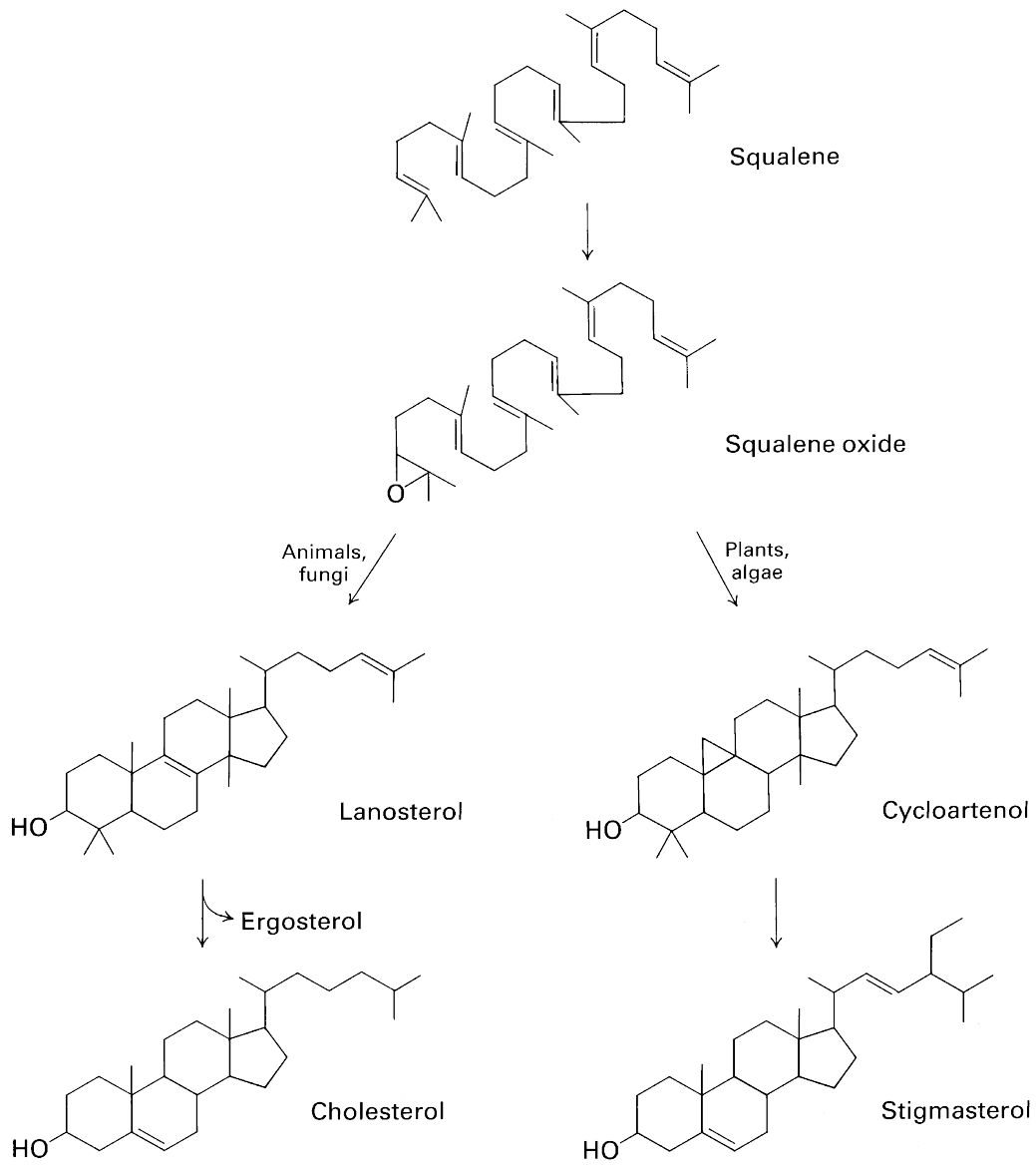


## C. Formation of isopentenyl bisphosphate and squalene from mevalonate

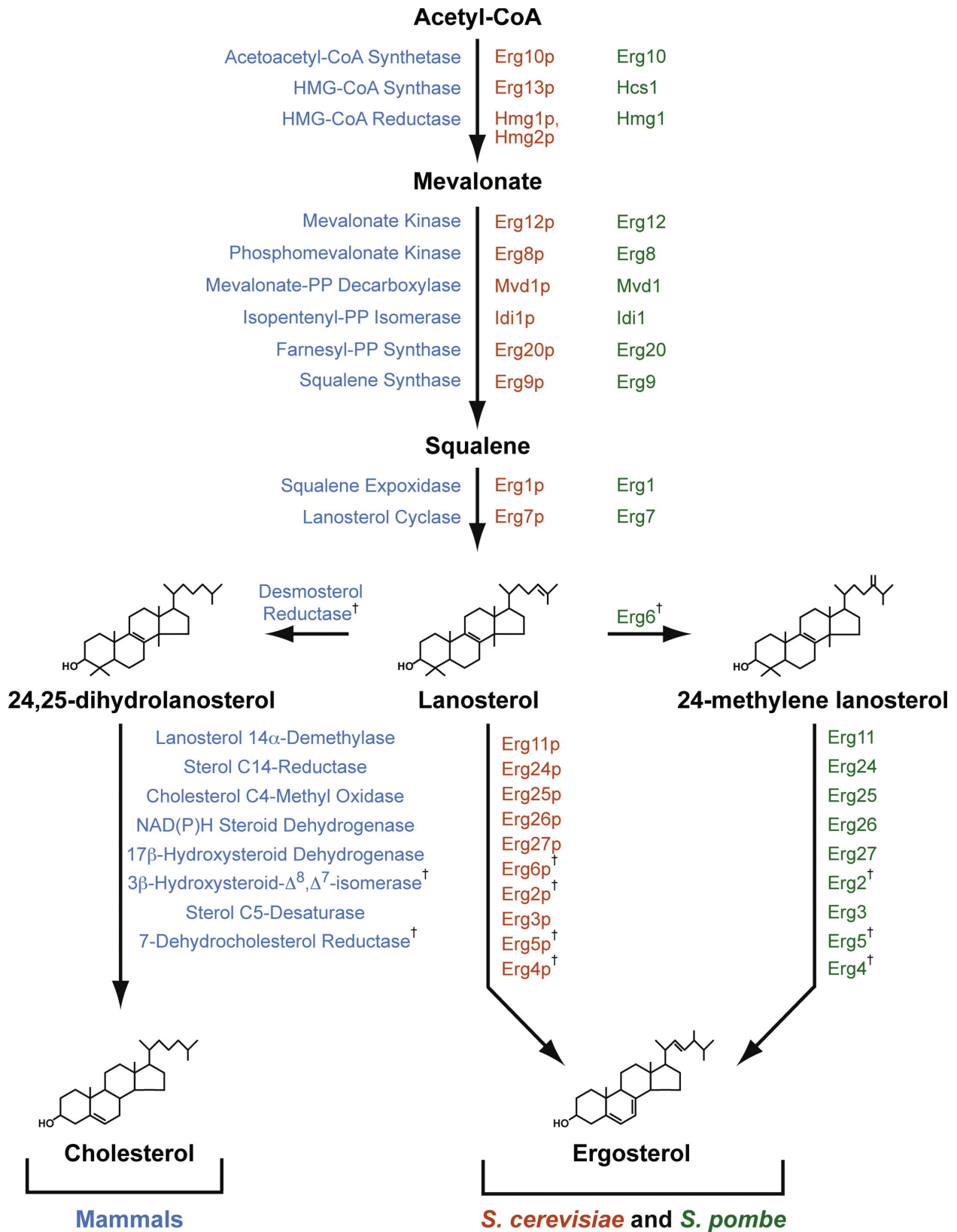
1. 6-carbon mevalonate subunits are phosphorylated and decarboxylated to form 5-carbon isopentenyl bisphosphate subunits
2. Three of the isopentenyl bisphosphate subunits condense to form farnesyl phosphate.
3. Two farnesyl phosphate subunits condense to form squalene (30 carbons)

## D. Synthesis of cholesterol from squalene

1. Squalene is cyclized to form the 5- and 6-sided rings of cholesterol.
2. Three carbons are lost in the process (27 carbons)



Conversion of squalene to into sterols in animals (left) and plants (right).



Overall pathway of cholesterol synthesis, including enzymes and their respective genes

#### IV. Regulation of cholesterol synthesis

A. Phosphorylation of HMG-CoA reductase reduces its activity

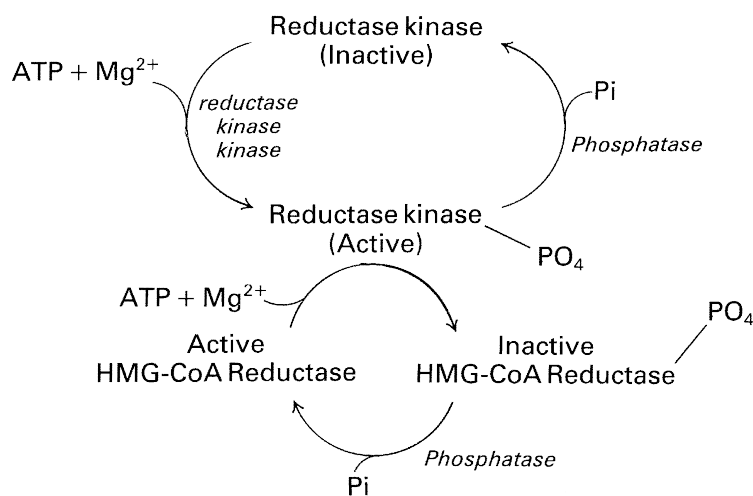
B. Phosphorylation is catalyzed by AMP-dependent protein kinase

1. AMP-dependent protein kinase also phosphorylates and inactivates acetyl-CoA carboxylase.

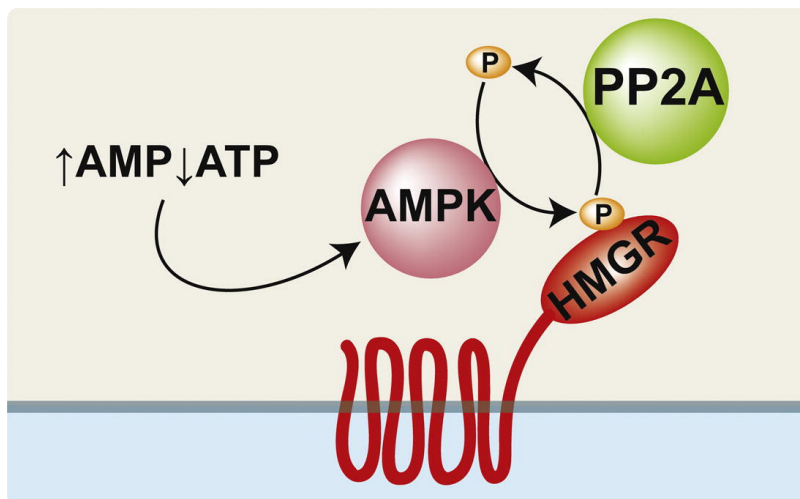
a. AMP increases with low energy supply.

b. Lipid synthetic reactions are decreased during low energy supply.

2. *Fatty acid synthesis and cholesterol are coordinately regulated.*



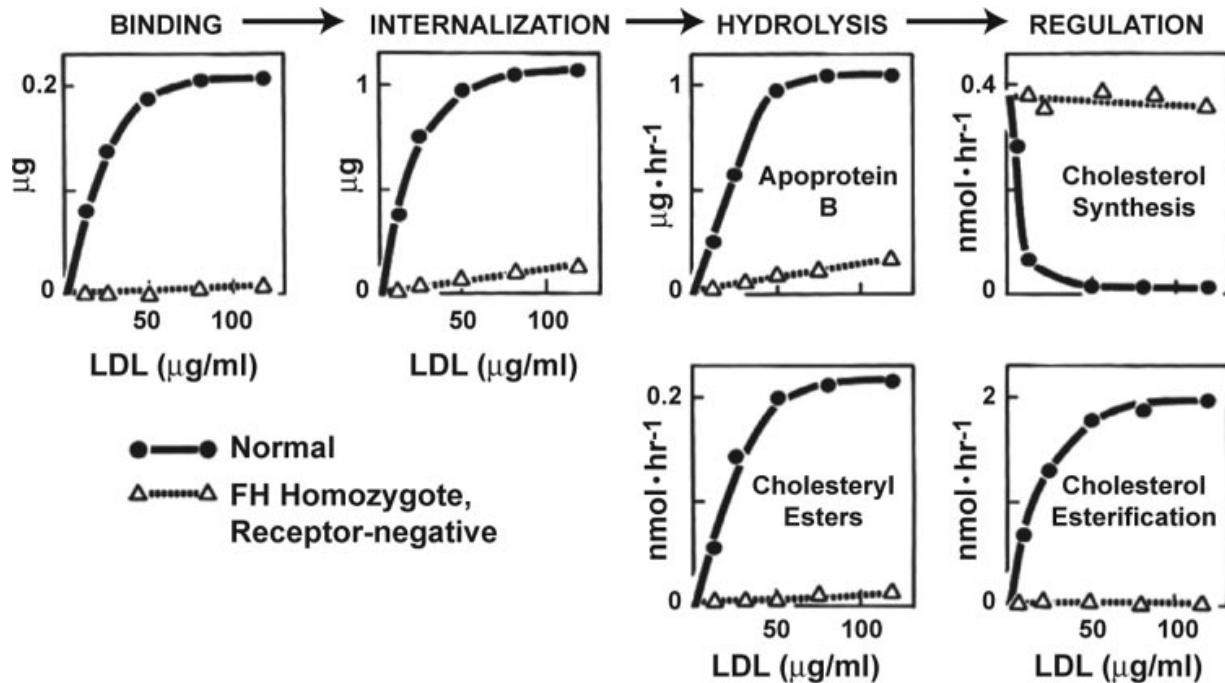
Regulation of HMG-CoA reductase activity by phosphorylation-dephosphorylation



Phosphorylation of HMG-CoA reductase is catalyzed by the AMP-dependent protein kinase.

## V. Regulation of cholesterol synthesis by intracellular cholesterol

- A. Binding, uptake, and breakdown (hydrolysis) of LDL increases intracellular cholesterol.  
 B. Intracellular cholesterol (*especially 25-OH-cholesterol*) increases the formation of cholesterol esters via ACAT (see below for details).



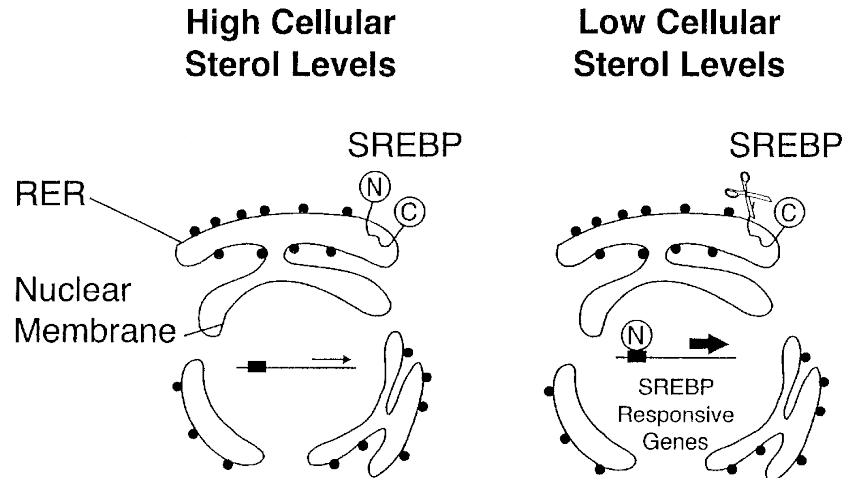
Actions attributable to the LDL receptor in fibroblasts from a normal subject and from a homozygote with the receptor-negative form of FH. Cells were incubated with varying concentrations of  $^{125}\text{I}$ -LDL or unlabeled LDL at  $37^\circ\text{C}$  for 5 hours and assayed. All data are normalized to 1 mg of total cell protein. The units for each assay are as follows: *Binding*,  $\mu\text{g}$  of  $^{125}\text{I}$ -LDL bound to cell surface; *Internalization*,  $\mu\text{g}$  of  $^{125}\text{I}$ -LDL contained within the cell; *Hydrolysis of apoprotein B-100*,  $\mu\text{g}$  of  $^{125}\text{I}$ -LDL degraded to  $^{125}\text{I}$ -moniodotyrosine per hour; *Hydrolysis of cholesteryl esters*, nmol of  $^3\text{H}$ cholesterol formed per hour from the hydrolysis of LDL labeled with  $^3\text{H}$ cholesteryl linoleate; *Cholesterol synthesis*, nmol of  $^{14}\text{C}$ acetate incorporated into  $^{14}\text{C}$ cholesterol per hour by intact cells; *Cholesterol esterification*, nmol of  $^{14}\text{C}$ oleate incorporated into cholesteryl  $^{14}\text{C}$ oleate per hour by intact cells.

### C. Regulation of LDL-receptor synthesis by cholesterol

1. At high intracellular cholesterol concentrations, the endoplasmic reticulum is enriched with cholesterol.
  - a. This stabilizes precursor sterol regulatory element binding protein (SREBP).
  - b. Genes encoding cholesterol synthesis and the LDL receptor are “off”.
2. At low intracellular cholesterol concentrations, the N-terminus of SREBP is cleaved,

travels to the nucleus, and turns on transcription of genes encoding cholesterol synthesis and LDL receptor.

**3. Free cholesterol regulates its own synthesis and synthesis of the LDL receptor.**



The Sterol Regulatory Element Binding Protein (SREBP) system. The precursor protein (125 kDa) is associated with the rough endoplasmic reticulum (RER) (left). When cell cholesterol content is low, a protease (scissors) cleaves this to release the transcriptionally active 68 kDa N-terminal portion, which binds to specific promoter sequences in target genes (e.g. LDL-receptor, enzymes of cholesterol synthesis). When cell cholesterol content is high, this pathway does not operate and transcription of the target genes is low.

## VI. Regulation of intracellular free cholesterol by ACAT

- A. Binding, uptake, and breakdown (hydrolysis) of LDL increases intracellular cholesterol.
- B. 25-OH-cholesterol increases the formation of cholesterol esters by activating ACAT.
  1. 25-OH-cholesterol (formed spontaneously from cholesterol) strongly stimulates ACAT activity.
  2. Increased ACAT activity leads to a decrease in intracellular free cholesterol.
- C. In animal fed saturated or unsaturated fatty acids, oleic and linoleic acid increased LDL receptor activity, whereas lauric, myristic, and palmitic acid decreased LDL receptor activity.
  1. All effects only are evident if cholesterol also is present in the diet.
  2. These effects indicate that unsaturated fatty acids would decrease LDL cholesterol.



### Roles of ACAT *in vivo*

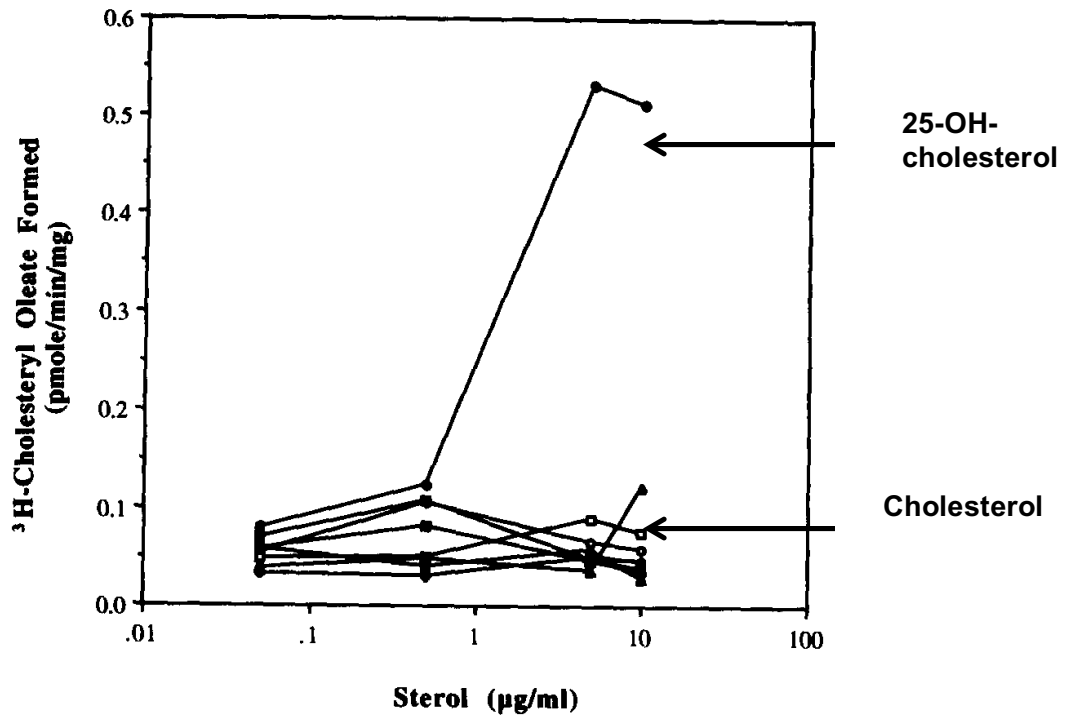
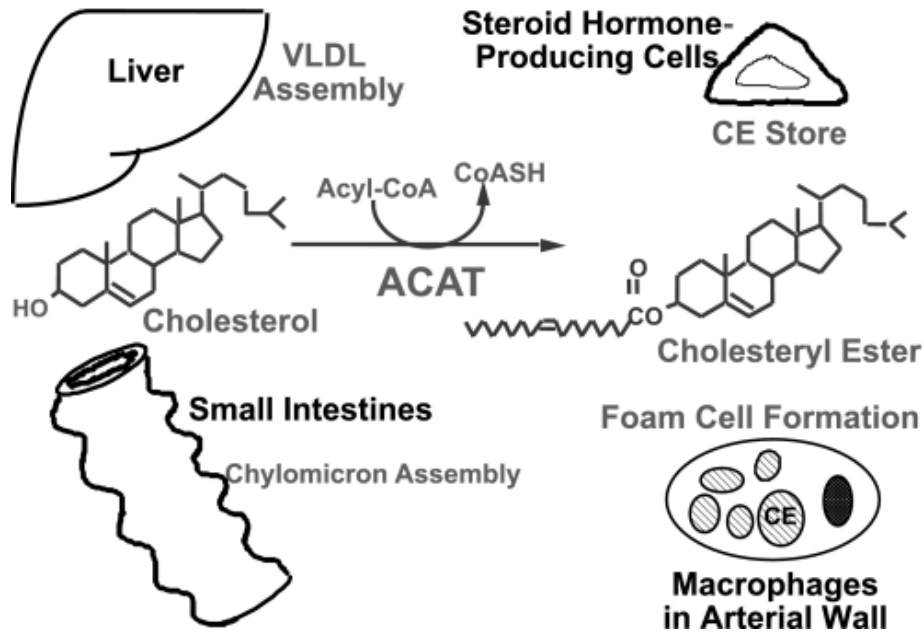
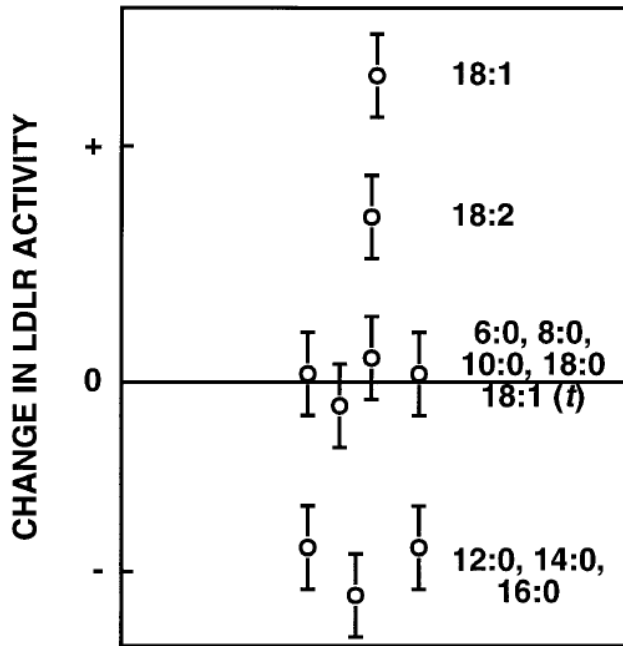


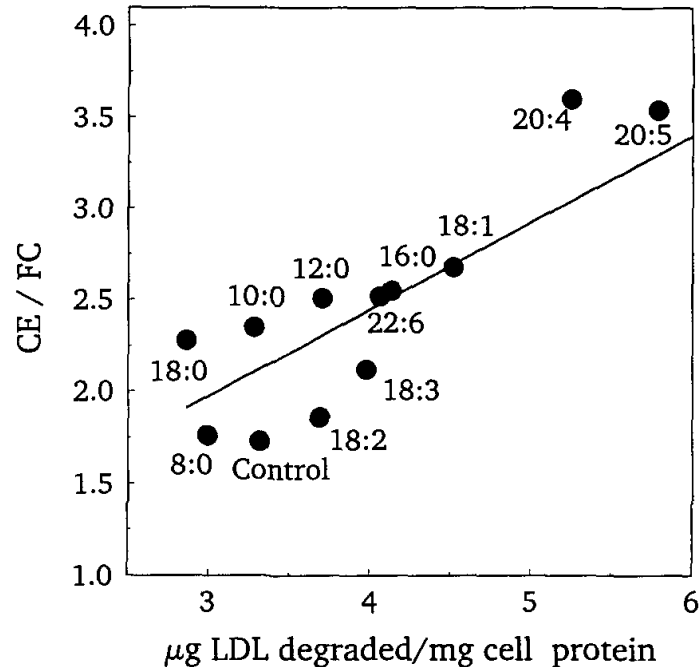
FIG. 5. Sterol specificity in activating the cholesterol esterification rate in intact Sf9 cells expressing ACAT. The experiment was performed as described in Fig. 4. The symbols were: ●, 25-hydroxycholesterol; ○, cholesterol; ■, 7-ketocholesterol; □, 6-ketocholestanol; ◻, β-sitosterol; ◇, 7α-hydroxycholesterol; ▲, cholate; △, deoxycholate.



The relative change in hepatic LDL receptor (LDLR) activity in hamsters fed single, specific fatty acids. These data are derived from a variety of studies in which the intake of cholesterol was kept constant under circumstances in which the animals were fed identical amounts of single, specific fatty acids. Oleic and linoleic acid increased (+) LDLR activity while lauric, myristic, and palmitic acid suppressed (-) the level of LDLR activity. Many fatty acids, including stearic and *trans*-vaccenic acid, decreased LDL receptor activity. (LDL receptor activity was estimated as the uptake and degradation of LDL particles.)

D. Macrophages were exposed to different media fatty acids and the degradation of LDL (i.e., uptake of LDL by the LDL receptor) was measured.

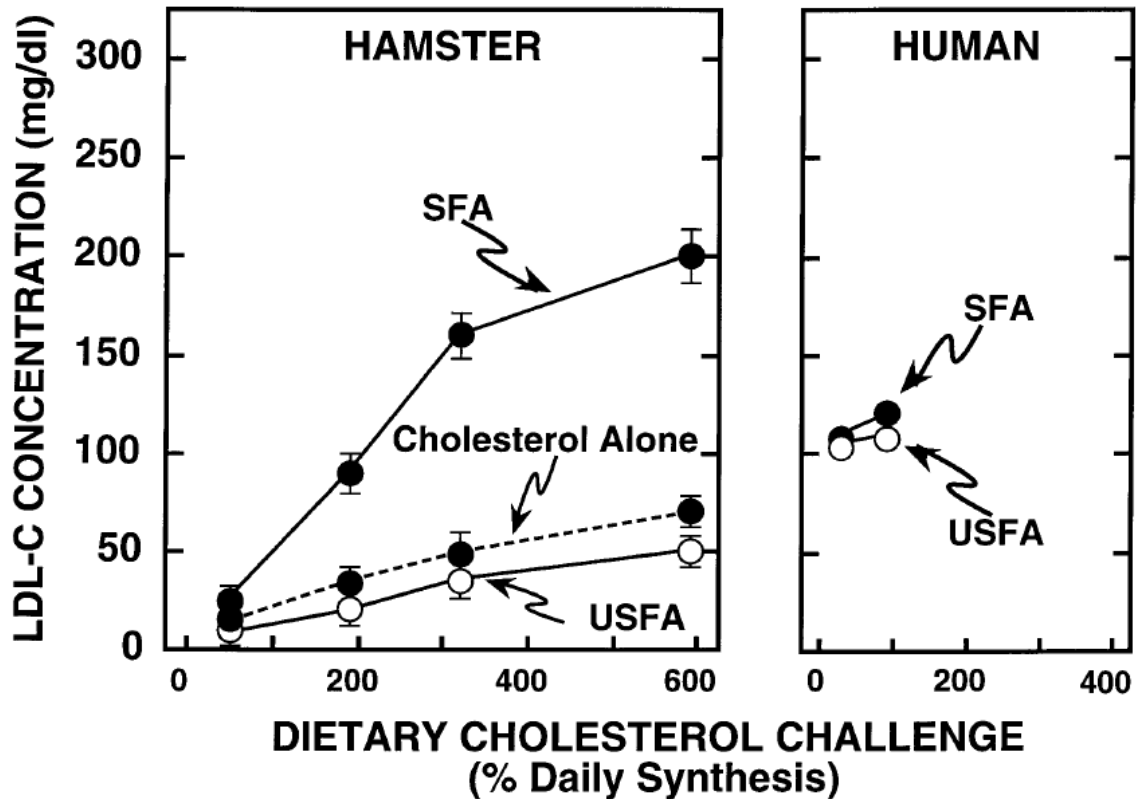
1. There was a linear relationship between the cholesterol ester:free cholesterol ratio and amount of LDL degradation.
  - a. As LDL degradation increases, total cholesterol in the macrophages increases.
  - b. This would specifically increase ACAT activity (via 25-OH-cholesterol).
2. The highest CE:FC ratio and highest LDL degradation occurred in macrophages incubated with arachidonic and eicosapentaenoic acid.



**FIG. 6. Effect of different FFA on LDL degradation and CE/FC mass ratio in J774 macrophages.** In parallel experiments, J774 macrophages were incubated with DMEM, 1% BSA with different fatty acids (FFA:BSA molar ratio 2:1) for 18 h in the presence of either 100  $\mu\text{g/ml}$  non-radiolabeled LDL to determine cellular free and total cholesterol mass accumulation or 100  $\mu\text{g/ml}$   $^{125}\text{I}$ -LDL to determine cellular degraded LDL. Cholesterol mass was determined by extracting cellular lipids into hexane:isopropyl alcohol (3:2 v/v). The samples were split into two aliquots, and total and free cholesterol were measured using gas liquid chromatography as described under "Experimental Procedures." Cholesteryl ester was calculated as  $((\text{TC} - \text{FC}) \times 1.67)$ . Degraded LDL represent the differences between the amount of non-iodine, non-trichloroacetic acid-precipitable radioactivity remaining in the media and the amount due to spontaneous LDL degradation in the absence of cells, after 18 h of incubation ( $r^2 = 0.78$ ).

## VII. Regulation of cholesterol synthesis by dietary fat and cholesterol

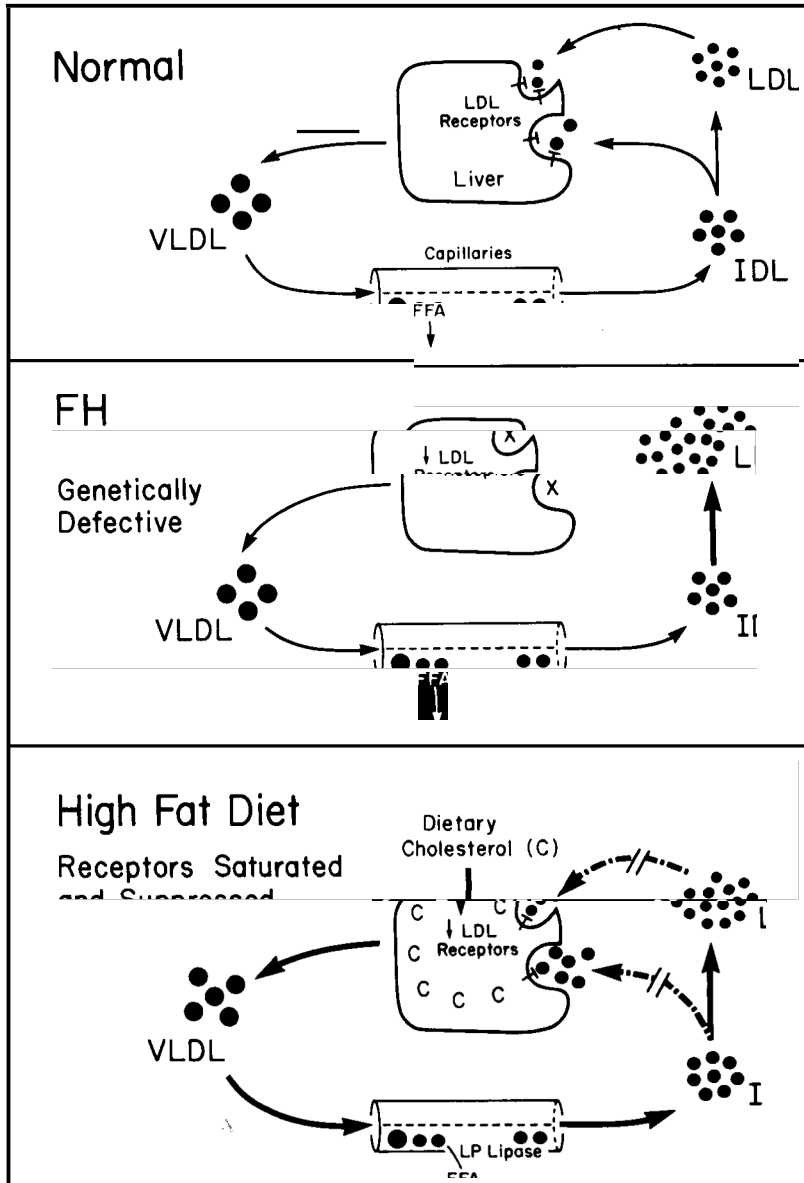
- A. In the presence of dietary cholesterol, saturated fatty acids (SFA) strongly increase LDL cholesterol in hamsters.
1. Unsaturated fatty acids (USFA) have much less effect on LDL cholesterol.
  2. Cholesterol alone causes a significant increase in LDL cholesterol.
- B. In humans, the effect of SFA on LDL cholesterol is much less apparent.
1. Baseline LDL cholesterol is much higher in humans than in hamsters.
  2. There is only a small response to SFA in humans.



The dependency of fatty acid effects on LDL-C concentration on the level of dietary cholesterol intake. This diagram shows the absolute concentration of LDL-C achieved in hamsters or humans fed predominantly saturated fatty acids (SFA) or unsaturated fatty acids (USFA) under circumstances in which the amount of cholesterol in the diet was varied. The dietary cholesterol intake is presented as a percentage of the amount of cholesterol synthesized in the two species each day. This figure is constructed using data from two sources (Fielding et al. 1995, Spady and Dietschy 1988).

### C. High-fat diets can suppress LDL receptor binding.

1. In normal individuals with modest fat and cholesterol intake, LDL receptors adequately clear LDL cholesterol.
2. In FH individuals, LDL receptor number is down, and intracellular cholesterol is low.
  - a. The N-terminus of SREBP is cleaved, travels to the nucleus, and turns on cholesterol synthesis (but not the LDL receptor).
  - b. There is no free cholesterol to make 26-OH-cholesterol, so ACAT activity is nil.
3. In individuals fed a high-fat, high-cholesterol diet, so much VLDL (and hence LDL) is synthesized that the LDL receptors become saturated, leading to increased plasma LDL cholesterol.



Schematic model of the mechanism by which LDL receptors in the liver control both the production and catabolism of plasma LDL in normal human subjects (*Top panel*), in individuals with FH (*Middle panel*), and in individuals consuming a diet rich in saturated fats and cholesterol (*Bottom panel*). VLDL denotes very low density lipoprotein; IDL denotes intermediate density lipoprotein.