NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Statins Stat!

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Part I – Cholesterol Metabolism

Naomi, who had just turned 50, decided it was high time to get a physical. At a preliminary visit, she exchanged brief pleasantries with her physician, Dr. Hernandez, and continued with the following conversation.

Dr. Hernandez: As you get older, there are some issues you need to think about. Tell me about your eating habits.

Naomi: I try to eat healthy.

Dr. Hernandez: What kinds of food do you eat?

Naomi: I try to eat fresh fruits and vegetables, I avoid refined flour and sugar, and I eat mostly chicken and fish, very little red meat.

Dr. Hernandez: And what about exercise?

Naomi: I try to exercise a few times a week. I like to walk and I go swimming when I can.

Dr. Hernandez: You're at a good weight for your height, so no concerns there. Tell me about your family—your grandparents, parents and siblings. Have they had any health issues? Cancer, diabetes, heart disease?

Naomi: We're mostly pretty healthy, though my father did have a heart attack a few years ago.

Dr. Hernandez: How old was he then?

Naomi: I think he was 77. He's 79 now and doing well.

As a result of this conversation, Dr. Hernandez ordered some blood work, which included measurement of Naomi's fasting glucose, high-density lipoprotein, low-density lipoprotein, and triglyceride levels.

Background

Non-communicable diseases (NCDs) are on the rise around the world. In developed countries like the United States, heart disease tops the list of major causes of death (Table 1) [Ref. 1].

According to the CDC about 32% of American adults have high levels of low-density lipoprotein (LDL), a risk factor for heart disease and stroke [Ref. 2].

Of these, only about one in three have their LDL numbers under control; about ½ are undergoing some kind of treatment [Ref. 3].

Table 1. Leading causes of death in U.S. Data from Centers for Disease Control and Prevention. An asterisk (*) indicates an NCD.

Cause of death	*Deaths per year*
* Heart disease	633,842
* Cancer	595,930
* Chronic lower respiratory diseases	155,041
Accidents (unintentional injuries)	146,571
* Stroke (cerebrovascular diseases)	140,323
* Alzheimer's disease	110,561
* Diabetes	79,535
Influenza and Pneumonia	57,062
* Nephritis, nephrotic syndrome, and nephrosis (kidney disease)	49,959
Intentional self-harm (suicide)	44,193

Circulating Lipoproteins

While there are many types of lipoprotein complexes that circulate in the bloodstream, LDL is the so-called "bad cholesterol." High LDL levels are associated with arterial plaques that occlude arteries (Figure 1).

However, the situation is complicated because these risks are modulated by high-density lipoprotein (HDL, "good cholesterol") levels and circulating triglyceride (TG) levels. High HDL levels are thought to be protective, while high circulating TG levels exacerbate the risk. Additional risk factors for heart disease include age, gender, and family history, as well as high blood pressure and tobacco use.

So what are lipoproteins? As the name suggests, these are complexes of lipids and proteins. LDL particles contain a protein called ApoB-100. As shown in the figure below (Figure 2), the complex also contains free cholesterol (Figure 3), cholesteryl esters, and a phospholipid monolayer.

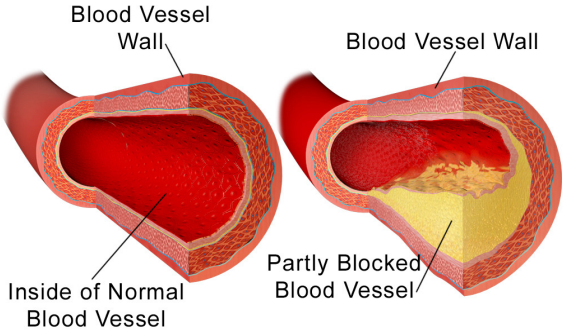

Figure 1: Normal (left) and partially occluded (right) arteries. *Credit:* BruceBlaus, CC BY 3.0 <https://commons.wikimedia.org/wiki/File:Blausen_0052_Artery_NormalvPartiallyBlockedVessel.png>.

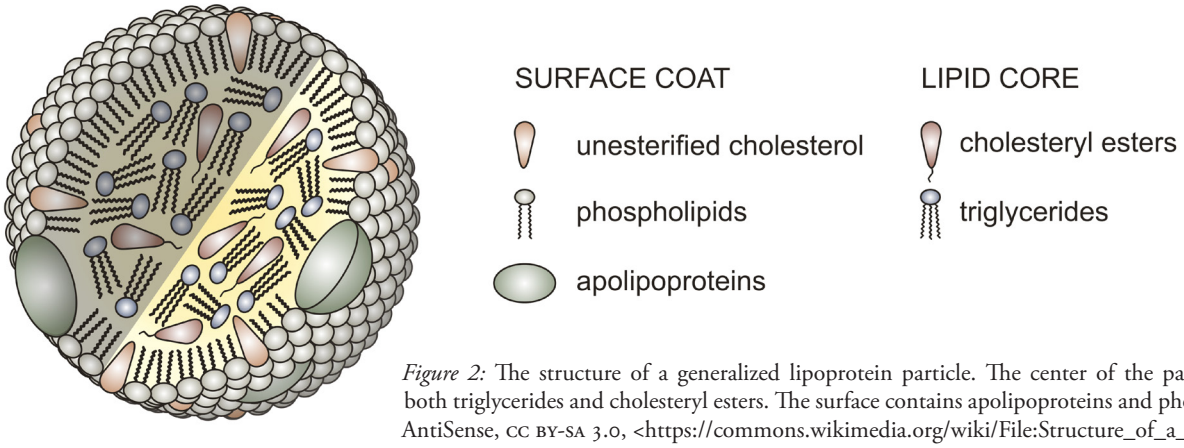

Figure 2: The structure of a generalized lipoprotein particle. The center of the particle is filled with both triglycerides and cholesteryl esters. The surface contains apolipoproteins and phospholipids. *Credit:* AntiSense, CC BY-SA 3.0, <https://commons.wikimedia.org/wiki/File:Structure_of_a_Lipoprotein.png>.

Questions

1. What is the role of the phospholipid monolayer at the outer surface of the particle?

2. Why are cholesterol, cholesteryl esters, and triglycerides on the inside of the particle?

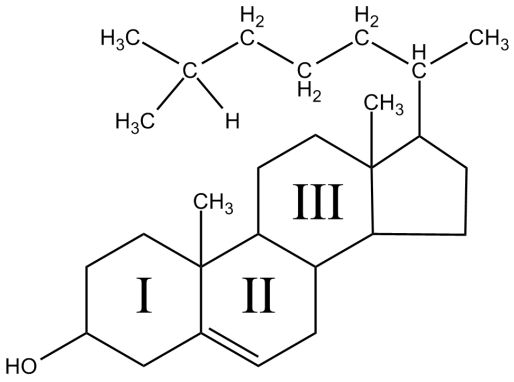

Figure 3: Chemical structure of choloesterol. *Credit:* MarcoTolo, CC BY-SA 3.0, <https://commons.wikimedia.org/wiki/File:Cholesterol_01.png>.

LDL, HDL, and many other lipoprotein particles mediate cholesterol traffic through your body. Moreover, there are two sources of cholesterol: dietary cholesterol from the food you eat and the cholesterol your body makes *de novo* (from scratch). Further, cholesterol can be oxidized, resulting in oxysterols, which may be more closely associated with development of atherosclerosis. Nevertheless, many physicians use LDL levels or the ratio of HDL to LDL as a marker for coronary artery disease risk [Ref. 4]. The pathways are fairly complicated, so we won't investigate them in detail in this case. However, despite its bad reputation, it's important to remember that cholesterol is necessary in your body for several reasons. Cholesterol modulates the fluidity of cellular membranes; it is the precursor for a number of important steroid hormones including estrogen, progesterone, testosterone and corticosteroids; and it is the precursor for bile acids—molecules that help solubilize dietary fat for degradation.

Cholesterol Synthesis

De novo cholesterol synthesis, a complex multi-step enzymatic process, is controlled by a key enzyme called hydroxyl-methyl-glutaryl-Co reductase (HMG-CoA reductase) (Figure 4). This is the *committed step* for cholesterol synthesis.

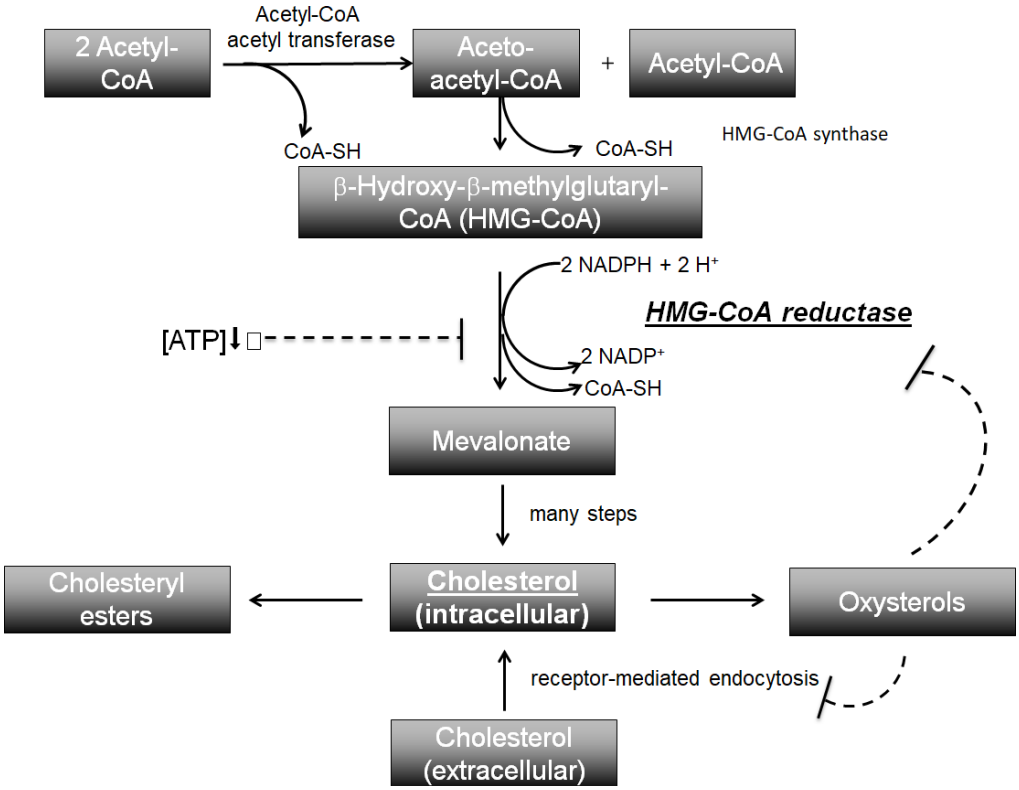

Figure 4: Generalized scheme for *de novo* cholesterol synthesis.

Questions

3. What is a committed step? Why do complex pathways have enzymes that are subject to regulation near the start of the pathway? Contrast committed steps to rate-limiting steps. Are all committed steps rate-limiting steps? Do all rate-limiting steps function as the committed step in a given pathway?

4. Why does it make "metabolic sense" that lower levels of ATP turn down HMG-CoA reductase activity even though ATP is not a direct substrate for the enzyme?

5. If you fed cells radioactive acetate (labeled with ^{14}C), would you expect to make radioactively labeled mevalonate? (*Hint:* Look back at Figure 4.)

Inhibition of Cholesterol Synthesis

Naomi again met with Dr. Hernandez, who said, "Your blood work generally looks good—most of your values are within the normal ranges, including your fasting blood glucose levels. That's important because it shows that at this point you're not in danger of developing diabetes. However, one set of numbers is cause for concern." Dr. Hernandez then went on to explain that Naomi's lipid numbers were outside the normal range. Shown below are Naomi's results.

Table 2. Naomi's Results.

Test	*Naomi's Values*	*Normal Values*
Total Cholesterol	240 mg/dL	< 200 mg/dL
HDL Cholesterol	48 mg/dL	> 40 mg/dL
LDL Cholesterol	150 mg/dL	< 100 mg/dL
Triglycerides	175 mg/dL	< 150 mg/dL

Based on Naomi's lifestyle choices (remember she eats a healthy diet and engages in moderate exercise several times a week) and family history (also remember her father had a moderately severe heart attack), Dr. Hernandez decided to prescribe her a cholesterol-lowering drug, a member of the statin class of drugs. Dr. Hernandez recommended trying the drug for six months. After that time period Naoimi should get her blood work done again, and based on the results they would see if the drug had been effective. Naomi was warned that many statins have side effects; although the side effects were very rare, she needed to keep on the lookout for muscle pain or mental fuzziness. If she experienced either of these, she was to notify Dr. Hernandez as soon as possible [Ref. 5].

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One means of controlling circulating LDL levels is to inhibit *de novo* cholesterol synthesis. In the mid-1970s, 8000 strains of microorganisms were screened for their ability to inhibit sterol synthesis. The fungi *Penicillium citrinum* and *P. brevicompactum* were shown to contain the same compound, compactin (now known as mevastatin, see Figure 5). Such statin drugs are now in wide use as cholesterol-reducing agents. A large variety is now available; differences stem from the different R groups (for some examples, see Figure 5).

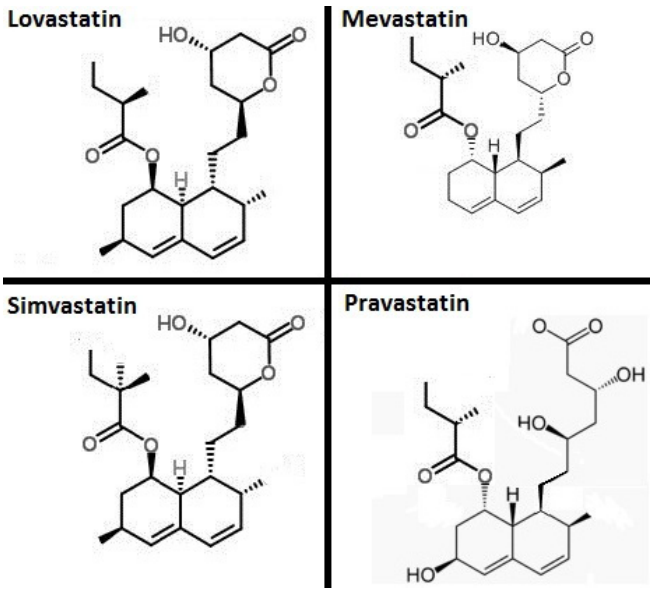

Figure 5: Different statin drugs. *Credit:* Jatlas2, CC BY-SA 3.0, <https://commons.wikimedia.org/wiki/File:Statin.structures.jpg>.

We'll explore some of the first data that was published about these drugs to examine the effects on cholesterol synthesis following the work of Endo, Kuroda, and Tanzawa, 1976 [Ref. 6] (Table 3).

Table 3. Effect of mevastatin on incorporation of radiolabeled carbon into sterols.

Substrate	*Mevastatin (nM)*	*Incorporation into Sterols (cpm/mg protein)*	*% of no drug control*
^{14}C-acetate	0	13770	(100)
	5	10080	73
	50	4120	30
^{14}C-acetyl-CoA	0	8270	(100)
	5	6020	73
	50	2410	29
^{14}C-HMG-CoA	0	1050	(100)
	5	570	54
	50	270	26
^{14}C-Mevalonate	0	35870	(100)
	5	34940	97
	50	34180	95

For this set of experiments, the radiolabeled precursor listed was incubated with rat liver lysates (broken cells). The reaction mixture in each case contained 1 mM ATP, 10 mM glucose-1-phosphate, 6 mM glutathione, 6 mM $MgCl_2$, 40 μM CoA, 0.25 mM NAD, 0.25 mM $NADP^+$, 100 mM potassium phosphate buffer pH 7.4, and 1.65 mg rat liver proteins. Reactions were incubated for 60 min at 37°C, then the reaction was terminated by the addition of KOH. Lipids were extracted from the mixture and subjected to scintillation counting (measured in cpm [counts per minute]) to determine the amount of radiolabel incorporated into cholesterol. Adapted from Endo, Kuroda, and Tazawa [Ref. 6].

Question

6. The data in Table 3 allowed the authors to zero in on which enzyme was the drug target. Looking back at Figure 4 (the reaction pathway from acetyl-CoA), which enzyme is likely to be the target of mevastatin?

Part II – Enzymatic Effects of Statins

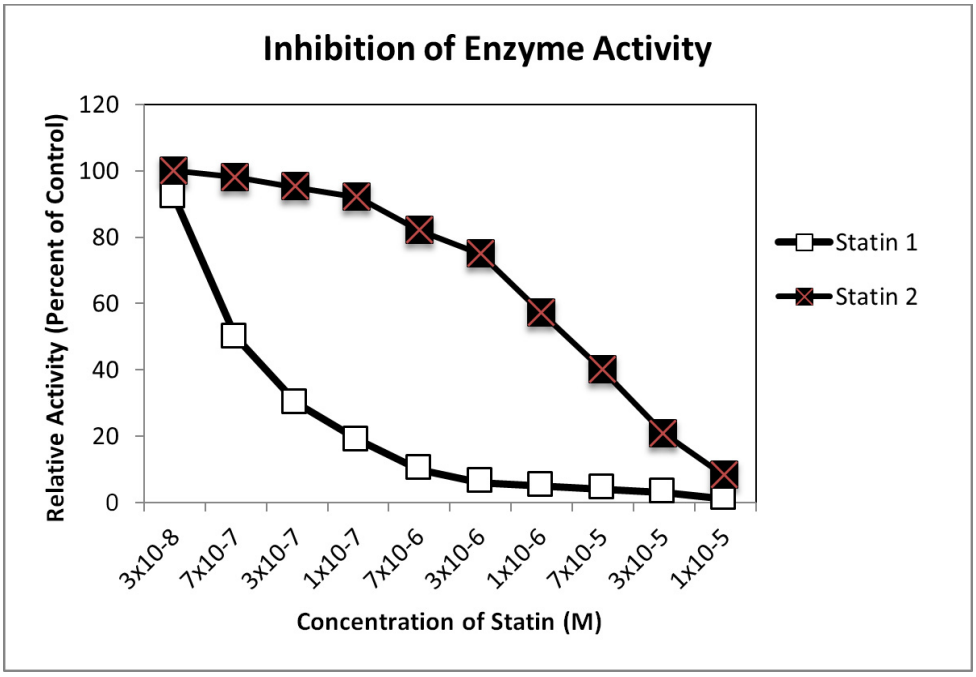

Figure 6: Activity of two different statins against HMG-CoA reductase. Rat liver microsomes (vesicles derived from the endoplasmic reticulum) were treated with a mild detergent to release the membrane proteins, one of which is HMG-CoA reductase. The reaction mixture contained 100 mM potassium phosphate buffer pH 7.4, 10 mM EDTA, 10 mM dithiothreitol, 5 mM NADPH, 0.11 mM ^{14}C-HMG-CoA and 1-2 µg membrane protein. The reaction was incubated for 20 min at 37°C, then terminated by the addition of HCl to a final concentration of 0.6 M.

Questions

7. Estimate the EC_{50} (the effective dose that results in 50% inhibition of enzyme activity) for each of the two statins shown in Figure 6. Which of the two statins is more effective? How did you come to that conclusion? Why is effective drug concentration an important consideration for treating patients?

8. Enzyme kinetics were performed with and without drug. In Figure 7, the drug was evaluated with respect to HMG-CoA as the substrate. A Lineweaver-Burk (double-reciprocal) plot is shown. What kind of inhibitor is mevastatin with respect to HMG-CoA based on this information?

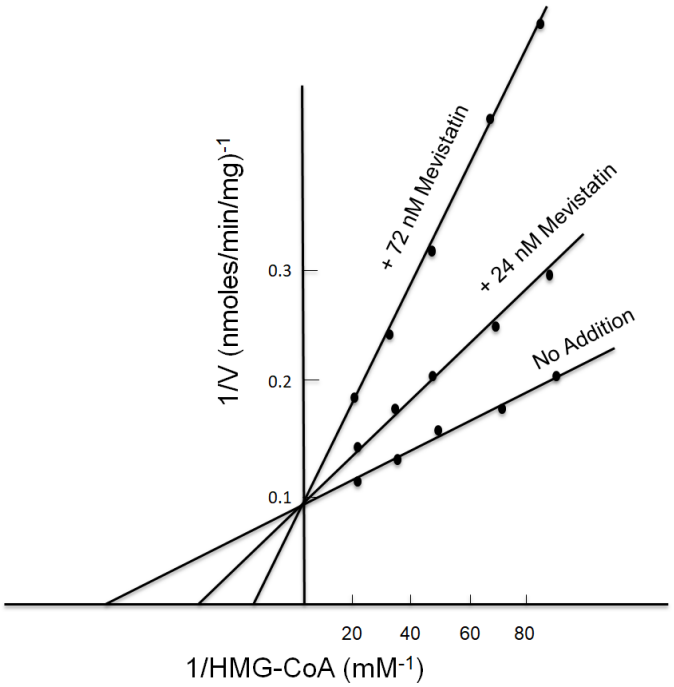

Figure 7: Double reciprocal plot of the inhibition of HMG-CoA reductase by mevistatin with respect to the substrate HMG-CoA. Adapted from Endo, Kuroda, and Tanzawa [Ref. 6].

9. Enzyme kinetics were also performed with respect to NADPH as the substrate (Figure 8). Again, the data are presented as a Lineweaver-Burk plot. What kind of inhibitor is mevastatin with respect to NADPH?

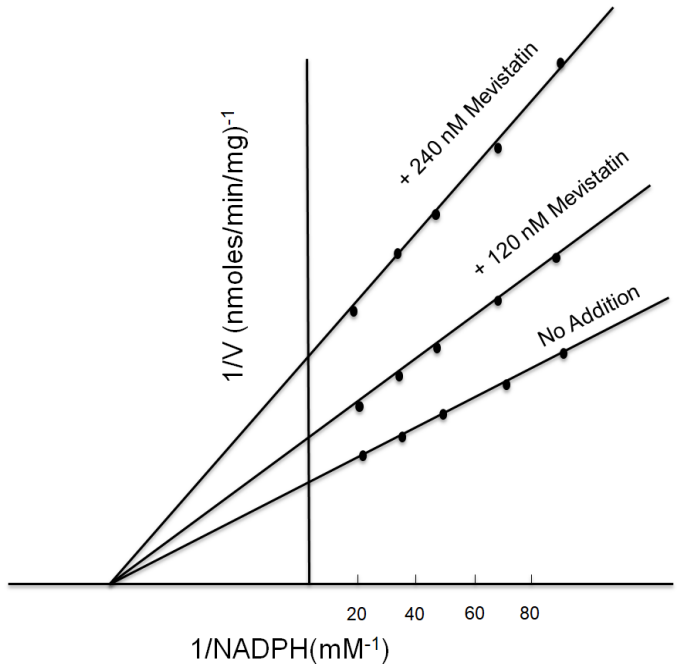

Figure 8: Double reciprocal plot of the inhibition of HMG-CoA reductase by mevistatin with respect to the substrate NADPH. Adapted from Endo, Kuroda, and Tanzawa [Ref. 6].

10. Given the information above, where does mevastatin bind on the enzyme?

Naomi took the statin that Dr. Hernandez prescribed for her for six months, then had her blood work repeated. Her new results are in Table 4.

Table 4. Naomi's new results.

Test	*Naomi's Values*	*Normal Values*
Total Cholesterol	198 mg/dL	< 200 mg/dL
HDL Cholesterol	45 mg/dL	> 40 mg/dL
LDL Cholesterol	109 mg/dL	< 100 mg/dL
Triglycerides	175 mg/dL	< 150 mg/dL

Question

11. Has the statin been effective for Naomi? What might she want to discuss further with Dr. Hernandez?

References

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