NFS 484/1484—ASSIGNMENT #1
Due: September 24, 2009

BACKGROUND INFORMATION

Cardiovascular disease, the leading cause of death in North America, is a multifactorial condition influenced by metabolic and dietary differences between individuals—particularly differences that modulate plasma cholesterol levels. Cardiovascular disease (CVD) is characterized by the development of atherosclerotic lesions in blood vessels resulting from the deposition of cholesterol and lipids from plasma into arterial walls. These lesions compromise the function of arteries and cause obstructions that reduce blood flow to important organs such as the heart—eventually resulting in myocardial infarction and possibly death. Plasma cholesterol is carried by various lipoprotein particles. For instance, elevated, fasting plasma concentrations of cholesterol carried by very-low density lipoprotein (VLDL) and low-density lipoprotein (LDL) particles are related to an increased risk of CVD as they deliver cholesterol and lipids of dietary origin to arterial walls after being secreted by the liver. Conversely, an elevated high-density lipoprotein (HDL) cholesterol concentration is related to a reduced risk of CVD as it removes cholesterol and lipids from plasma and arterial walls. Cholesterol carried by HDL is eventually absorbed by the liver, and then excreted.

Lipoproteins frequently donate or accept lipids from each other. For instance, HDL particles can be modified in circulation by various enzymes that influence the amount of lipid and cholesterol they carry. Such changes can destabilize the HDL particle resulting in its destruction, or can sometimes convert the HDL into larger or smaller particles. As HDL particles increase in size they become much less anti-atherogenic.

STUDY 1

Phospholipid transfer protein (PLTP) is secreted into circulation where it binds HDL particles, and influences the ability of HDL to accept lipids and cholesterol from VLDL particles. It is unknown what effects PLTP has on plasma cholesterol and HDL metabolism. Using transgenic technology, the human PLTP gene was inserted into the genome of mice. Groups of transgenic and wildtype (i.e. not expressing human PLTP) mice were fed normal rodent chow (a low-fat, high-fibre diet) or a high-fat diet for 10 weeks. Fasting plasma cholesterol concentrations were then determined. The size of the HDL particles present in the fasting samples were also measured. Lastly, an *in vitro* experiment was conducted in which the plasma of wildtype mice fed rodent chow was incubated with three different concentrations of purified human PLTP. The percentage of original HDL particles remaining in the plasma were monitored over 800 minutes.

Questions:

1. Describe the effects of human PLTP expression on total, HDL, and VLDL/LDL cholesterol concentrations and HDL particle size under chow and high-fat diet conditions in Table 1. (3/50 marks)

2. Describe the effect that PLTP concentration has on the HDL content of serum samples *in vitro* (Figure 1). (1/50 marks)

3. Using all of the information presented in Study 1 and the background, discuss how PLTP and diet influence plasma cholesterol concentrations. In your answer speculate as to how these effects may influence risk of developing atherosclerotic lesions. (5/50 marks)

Table 1: Fasting plasma cholesterol concentrations and HDL particle size of human PLTP transgenic mice and wildtype littermates maintained on either on chow or high-fat diet

	Total Cholesterol	HDL	HDL size (nm)	VLDL/LDL
Chow Diet				
Transgenic	2.77 ± 0.49^a	0.55 ± 0.04^a	10.38 ± 0.44^a	3.09 ± 0.41^a
Wildtype	2.54 ± 0.44^a	1.84 ± 0.15^b	8.12 ± 0.23^b	2.92 ± 0.10^a
High-fat Diet				
Transgenic	13.47 ± 3.74^b	0.30 ± 0.11^a	10.49 ± 0.40^a	11.24 ± 3.81^b
Wildtype	9.38 ± 2.70^c	1.09 ± 0.57^b	8.09 ± 0.21^b	7.50 ± 2.90^c

Values (mean ± standard deviation) not sharing the same superscript are significantly different within each column ($P<0.05$).
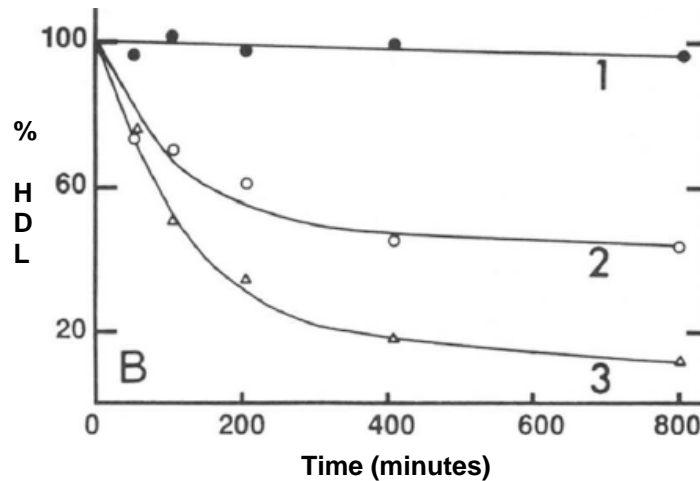

Figure 1: Percentage of HDL particles remaining in plasma after incubation with 0 mM (●), 0.5 mM (○), or 1.0 mM (Δ) of purified human PLTP over 800 minutes. Curves 1, 2, and 3 are significantly different at 800 minutes ($P<0.05$).

STUDY 2

Dietary recommendations to reduce fat consumption are frequently made to patients with suspected CVD, or pre-existing atherosclerotic lesions. The primary objective of this study was to examine the influence of PLTP activity on pre-existing disease. Transgenic mice were developed that carried an inducible form of the human PLTP gene. The gene was designed to only be expressed, or "induced", in the presence of a drug called doxycycline which does not independently influence plasma cholesterol. Littermates not carrying this gene were also obtained to act as controls. Control and inducible (indPLTP) animals were placed on a high-fat diet for 9 weeks, after which all animals were placed on a chow diet. Groups of both control and indPLTP mice were subjected to either Treatment A or Treatment B. In Treatment A, the mice consumed a chow diet and received doxycycline for two weeks (11A), and were then sacrificed to examine fasting plasma cholesterol concentrations, atherosclerotic lesion size, and PLTP activity (i.e. the capacity of PLTP to perform its transfer function). In Treatment B, animals consumed a chow diet for two weeks in the absence of doxycycline (11B), and then received chow and doxycycline for an additional 6 weeks before being sacrificed (17B).
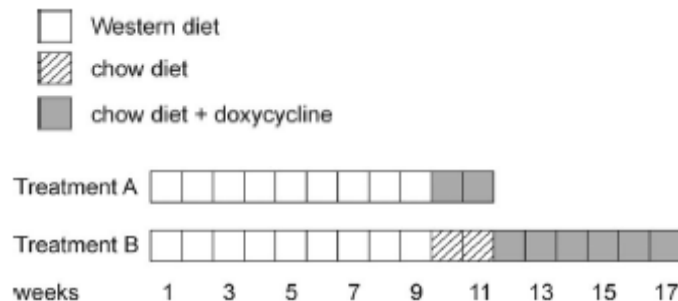

Figure 2: Experimental design

Questions:

4. Describe the statistically significant results presented in Table 2. (3/50 marks)

5. Describe how plasma PLTP activity changed over the course of the experiment. (2/50 marks)

6. Describe the effects of PLTP expression on atherosclerotic lesion size in Figure 3. (2/50 marks)

7. Discuss how PLTP activity in Study 2 influenced plasma cholesterol concentrations and atherosclerotic lesion size in response to dietary change. (5/50 marks)

8. Drawing on all of the information presented in this assignment up to this point, propose a comprehensive mechanism explaining differences in lesion size observed in Study 2. Would you expect the same results if animals in Study 2 were maintained on a chow diet throughout the entire study? (6/50 marks)

Table 2: Fasting plasma cholesterol concentrations over 17 weeks of study

	0	9	11A	11B	17B
HDL					
Control	1.9 ± 0.4	2.0 ± 0.5	1.7 ± 0.2^a	1.8 ± 0.4	1.7 ± 0.4^a
indPLTP	1.9 ± 0.6	2.1 ± 0.5	$0.3 \pm 0.1*^b$	1.8 ± 0.3	$0.2 \pm 0.1*^b$
VLDL/LDL					
Control	4.7 ± 0.7	$24.7 \pm 8.0*$	5.9 ± 0.2^a	5.8 ± 0.3	5.7 ± 1.3^a
indPLTP	4.6 ± 0.8	$27.5 \pm 5.1*$	$15.4 \pm 5.2**^b$	5.8 ± 1.5	$12.6 \pm 3.2**^b$

Values (mean ± S.D.; mmol/L) not sharing the same superscript letter are significantly different within each column and lipoprotein particle grouping (P<0.05). Values not sharing the same number of asterix symbols are significantly different within each row (P<0.05). Measurements taken at baseline ('0') and after 9 weeks of high-fat diet ('9'). After stopping high-fat diet, mice were fed either chow+doxycycline for 2 weeks ('11A') or chow without doxycycline for 2 weeks ('11B'), followed by doxycycline administration for 6 weeks ('17B').
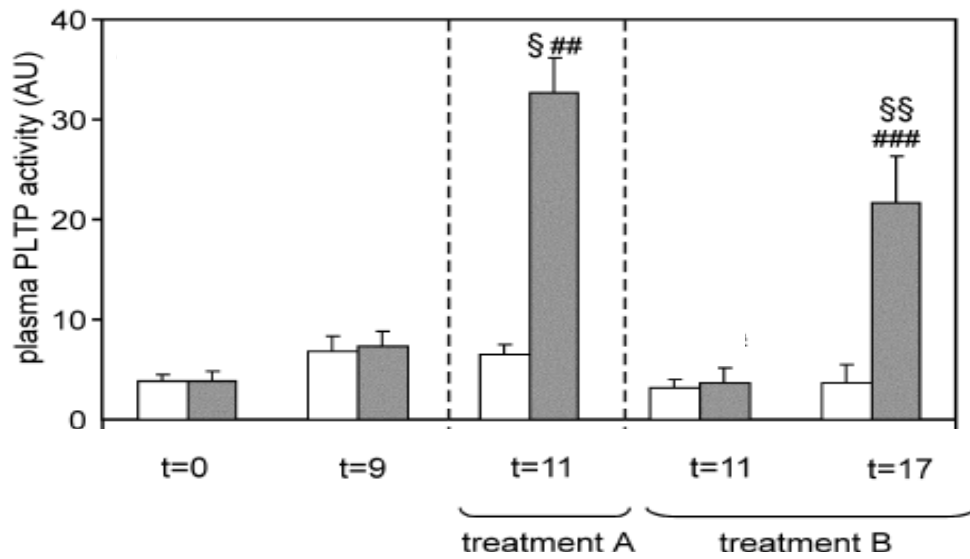

Figure 3: Plasma PLTP activity at various time periods measured in weeks. White bars, control mice; gray bars, indPLTP mice. (§ , P<0.05 compared to control at t=11; ## , P<0.05 compared to indPLTP at 9 weeks; §§ , P<0.05 compared to control at 17 weeks; ### , P<0.05 compared to indPLTP at 11 weeks)
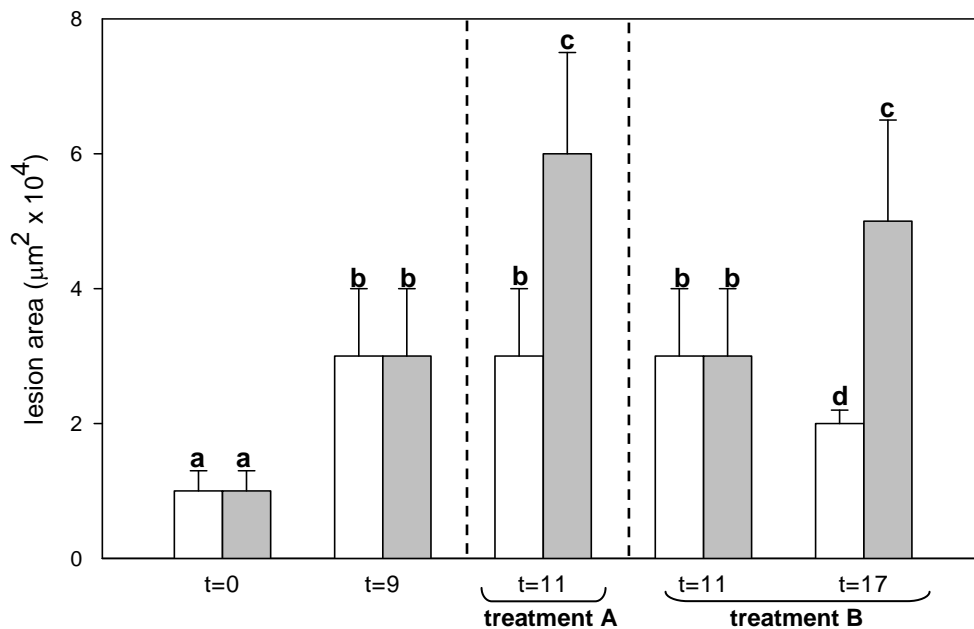

Figure 4: Atherosclerotic lesion size in sections of the aortic root at various time periods measured in weeks. White bars, control; gray bars, indPLTP. Bars with different letters are significantly different (P<0.05).

STUDY 3

Amongst CVD patients, large differences in plasma PLTP activity exist between individuals presumably due to genetic variability. In order to examine how such variability may influence CVD outcome, 5000 individuals with confirmed atherosclerotic lesions of similar size and position were continually monitored for the incidence of death from myocardial infarction over almost 8 years of follow-up. The proportion of patients surviving after 400 weeks was examined by quartile grouping of PLTP activity. Quartile groupings (Q1-4) were compiled by sorting subjects into four groups based on their plasma PLTP activity. Q1 contains those individuals with the lowest PLTP activity and looks at their survival over time. Q4 contains those individuals with the highest PLTP activity, and examines their survival over time. Therefore, as quartile grouping increases so does the plasma PLTP activity of the individuals who are found in the grouping.

Questions:

9. Describe the effect of plasma PLTP activity on cardiac survival (1/50).

10. Using all of the information presented in this assignment, explain how differences in plasma PLTP activity may influence cardiac survival in individuals with pre-existing atherosclerotic lesions. (7/50 marks)

11. If two individuals asked your advice on how to decrease their chance of dying from a myocardial infarction, what dietary advice would you give? What advice would you give if you knew that they exhibited different plasma PLTP activity (high vs. low)? Would your advice change if you knew that they possessed pre-existing atherosclerotic lesions? Draw only on all of the information presented in the assignment, and clearly explain the reasons for your advice. (14/50 marks)
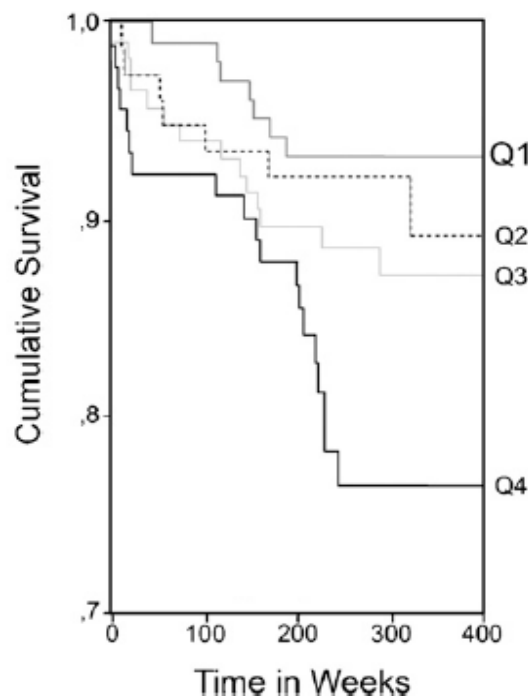

Figure 5: Proportion of patients with pre-existing atherosclerotic lesions activity that survived over 400 weeks of follow-up sorted by quartile of plasma PLTP activity. Lesions were of similar size and position. The results are controlled for sex, bodyweight, smoking, blood pressure, and other relevant demographic variables. (Each curve is significantly different from each other at 400 weeks of follow-up; $P<0.05$)