NFS 484/1484 Fall 2009: ASSIGNMENT #1 MARKING SCHEME

Question 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)

- On the chow and high fat diets, transgenic mice had significantly lower fasting plasma HDL concentration, and significantly greater HDL particle size, than the wildtype mice ($P<0.05$).
- On a high fat diet, transgenic mice had significantly greater fasting plasma VLDL/LDL and total cholesterol concentrations than the wildtype mice ($P<0.05$).
- On a high fat diet, both transgenic and wildtype mice had significantly greater fasting plasma VLDL/LDL and total cholesterol concentrations compared to mice consuming the chow diet ($P<0.05$).

Question 2

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

- As PLTP concentration increases, the percentage of HDL particles remaining in isolated plasma samples decreases such that at 800 minutes the HDL content of the plasma incubated with 0 mM, 0.5 mM, and 1.0 mM of PLTP are significantly different ($P<0.05$).

Question 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)

- PLTP expression in transgenic mice significantly decreased fasting HDL concentration and increased HDL particle size compared to wildtype mice regardless of which diet was consumed (Table 1).
    - From the background and Figure 1, it can be inferred that the action of PLTP is to transfer lipids from other plasma sources to HDL particles leading to enlargement and destruction of the HDL particles in a dose-dependent manner.
- Consuming a high fat diet increases total cholesterol and VLDL/LDL concentrations compared to a chow diet regardless of PLTP expression (i.e. in both wildtype and transgenic mice). However, this effect is even greater in the transgenic compared to widltype mice.
- Collectively, these findings indicate that HDL concentrations are independently modulated by PLTP expression while the independent effect of diet on total cholesterol and LDL/VLDL concentrations is modified by PLTP expression.
- From these results, it is hypothesized that a high fat diet may promote development of atherosclerotic lesions compared to a chow diet because it raises VLDL/LDL concentrations which may lead to increased delivery of lipids to arterial walls (background). PLTP expression may also promote lesion development because it lowers HDL concentrations which may lead to decreased clearance of lipids from arterial walls. A high fat diet combined with high PLTP expression may promote lesion development to a greater exent than either factor independently because greater delivery (high VLDL/LDL) will be combined with less clearance of lipids to arterial walls.

Question 4

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

- The fasting plasma HDL concentration of indPLTP mice was significantly lower than control mice at 11A and 17B. HDL concentrations of indPLTP mice at 11A and 17B were significantly lower than at any other point in the experiment ($P<0.05$).
- At 9 weeks, fasting plasma VLDL/LDL concentration significantly greater/increased compared to 0 weeks in both control and indPLTP mice. VLDL/LDL concentrations of control mice at 9 weeks was significantly greater than at any other point in the experiment ($P<0.05$).
- indPLTP mice exhibited significantly greater VLDL/LDL concentrations than control mice at 11A and 17B. VLDL/LDL concentrations of indPLTP mice at 9 weeks was significantly greater than at any other point in the experiment. VLDL/LDL concentrations of indPLTP mice at 11A and 17B were significantly lower than at 9 weeks, but significantly higher than at 0 or 11B ($P<0.05$).

Question 5

Describe how plasma PLTP activity changed over the course of the experiment in Figure 3. (2/50 marks)

- indPLTP mice at 11A exhibited significantly greater plasma PLTP activity than control mice, and significantly greater activity than indPLTP mice at 9 weeks ($P<0.05$).
- indPLTP mice at 17B exhibited significantly greater plasma PLTP activity than control mice, and significantly greater activity than indPLTP mice at 11B ($P<0.05$).

Question 6

Describe the effects of PLTP expression on atherosclerotic lesion size in Figure 4. (2/50 marks)

- Induced PLTP expression at 11A coincided with significantly greater lesion size in indPLTP mice compared to all other mice at all other timepoints except 17B ($P<0.05$).
- Induced PLTP expression at 17B coincided with significantly greater lesion size in indPLTP mice compared to all other mice at all other timepoints except 11A ($P<0.05$).

Question 7

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

- Induction of PLTP promotes lesion development (Figure), and loss of HDL particles, regardless/independent of any change in diet.
- An acute dietary change to chow has no effect on lesion area despite significant reductions in VLDL/LDL concentration for control mice.
- Acute dietary change to chow results in lower VLDL/LDL concentrations in indPLTP mice than on a high fat diet, but still elevated compared to baseline indicating that both a high fat diet and PLTP can contribute to increased VLDL/LDL concentrations.
- Elevated VLDL/LDL concentrations and depressed HDL concentrations due to high PLTP expression coincide with much greater lesion size compared to the effects of a high fat diet alone. An acute or chronic dietary change from a high fat diet cannot overcome the deleterious effects of high PLTP activity on pre-existing lesions, VLDL/LDL, and HDL.
- A chronic/long-term dietary change to chow from a high fat diet improves lesion size, but does not completely reduce lesions to baseline levels indicating that pre-existing lesions can be

improved, but not reversed, by a dietary change when PLTP activity is low despite normalized plasma cholesterol levels.

Question 8

Drawing on all of the information presented in the 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)

- Consumption of a high fat diet promoted lesion development and increased VLDL/LDL concentrations over nine weeks as it led to greater VLDL/LDL concentrations (Study 2). HDL concentrations were unaffected.
    - This diet promote delivery of lipids to arterial walls compared to baseline (background) in the face of constant level of clearance.
- An acute change to chow decreased dietary supply, and therefore, depressed VLDL/LDL concentrations to baseline levels in control mice
    - The dietary change resulted in reduced delivery of lipid to arterial wall compared to a high fat diet; more closely aligning clearance of lipid by HDL with delivery.
- A long-term/chronic change to chow diet eventually allows for regression of lesion size, as HDL slowly clears lipid from lesions.
- Switching to a chow diet did not result in the same improvements in lesion size in indPLTP mice because high PLTP activity greatly limits loss of VLDL/LDL concentrations, and greatly decreases HDL concentrations (Study 2).
    - PLTP compromises HDL-mediated clearance of lipid from arteries by destabilizing the HDL particle leading to its destruction, and converting the remaining particles to a less anti-atherogenic form (Study 1 and background).
    - Therefore, elevated delivery of lipid is combined with reduced clearance leading to greatly increased lesion size when PLTP activity is high.
- If control mice were maintained on a chow diet throughout the experiment, they would maintain baseline lesion sizes as there would be no changes in delivery or clearance of lipid to arterial walls.
- If indPLTP mice were maintained on a chow diet throughout the experiment, they would likely maintain baseline lesion size from 0-9 weeks followed by greater lesion size compared to baseline upon induction of the PLTP gene.
    - PLTP activity independently promotes lesion development due to its adverse effects on HDL particles and the subsequent loss of lipid clearance from arterial walls.
    - Lesion sizes following induction of the PLTP gene with chow consumption may not be as large as those observed with a high fat diet because delivery of lipid by VLDL/LDL would not be elevated compared to baseline (Study 1)—alteration of VLDL/LDL by PLTP is conditional on exposure to a high fat diet.

Question 9

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

- As quartile of plasma PLTP activity increased the cumulative survival of individuals decreased, such that as quartile of activity increased survival was significantly lower at 400 weeks ($P<0.05$). (Required indication that each quartile is significantly different from the others; or an inverse relationship)

Question 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)

- It is known that PLTP acts to destroy and convert HDL particles to less anti-atherogenic forms, thereby, limiting the clearance of lipid from vessel walls, and promoting the development of atherosclerotic lesions.
- High plasma PLTP activity increases the ability of a high fat diet to raise VLDL/LDL concentrations (Study 1), and leads to even greater promotion of lesion development (Study 2) than a high-fat diet alone as increased delivery of lipid to arterial walls is combined with decreased clearance resulting from loss of functional HDL.
    - Individuals in Study 3 had pre-existing lesions like the mice in Study 2. Therefore, they may consume a high fat diet which contributes to lesion development. However, consumption of a high fat diet is not needed to promote lesion development, as the adverse effects of PLTP on HDL are independent of diet. [minor point]
- In Study 3, it is evident that there is a dose-response relationship between plasma PLTP activity and cardiac death.
    - Therefore, it is reasonable to assume that and individual's PLTP activity determines the degree to which functional HDL is lost
- Genetic variability/individual differences in plasma PLTP activity result in varying levels of functional HDL, and lipid clearance from arterial walls
- As lesions grow, they obstruct blood flow to the heart resulting in a myocardial infarction and death.
    - Therefore, individuals with relatively greater PLTP activity exhibit greater lesion size, obstruction of blood flow, infarction rates, and death compared to those with less PLTP activity.

Question 11

If two individuals asked your advice on how to decrease their chances 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 lesions? Draw only on all of the information presented in the assignment, and clearly explain the reasons for your advice. (14/50 marks)

- Not knowing any other fact about these individuals, it is reasonable to recommend that they avoid a high-fat diet and consume a low-fat, high-fibre diet. Because:
    - In Study 2 it was observed that a high-fat diet increased lesion size.
    - Furthermore, the lipid profile that promotes atherosclerosis (increased VLDL/LDL) is promoted by a high-fat diet and isn't induced by a chow/low-fat, high-fibre diet (Study 1 and Study 2)
    - Preventing the development of lesions, should prevent obstruction of blood flow to the heart, subsequent MI and death.

- If the plasma PLTP activity of these individual's was known, the dietary advice would not change **OR** an even more fat restricted diet should be followed. It would be relatively more important for the individual with high PLTP activity to follow this advice.

- An individual with high PLTP activity exhibits an increased risk of MI even if they consume a low-fat, high-fibre diet due to loss of functional HDL (Study 1)
- A high-fat diet compounds this increased risk by causing greater non-HDL cholesterol concentrations compared to someone with lower PLTP activity
    - Both individuals undergo increases in non-HDL cholesterol, but the individual with high PLTP activity exhibits as significantly greater concentration under high-fat conditions
- In addition, the individual with high PLTP activity also exhibits smaller concentration of HDL and larger HDL-particle size
    - High PLTP individuals always exhibits altered HDL concentration and particle size
- Lesion size will grow in both individuals due to increased delivery of lipid to vessel wall. Lesions will be larger in the individual with high PLTP activity as she will exhibit even greater delivery combined with reduced clearance.

- If these individuals possessed pre-existing lesions, my advice would not change from when I knew that they had different PLTP activities. However, the chances of having an MI would likely be greater in both individuals, but the low-PLTP individual would be of lower risk.
    - Based on Study 2, allow it is likely that individuals developed lesions due to a high-fat diet.
    - The low-PLTP individual must undergo a chronic change to a low-fat, high-fibre diet. In Study 2 this diet did reduce lesion size, but not to baseline levels. Thus, they are still at increased risk of MI.
        - Decreased lesion size resulted from corrected non-HDL cholesterol concentrations.
        - An acute change was not sufficient to reduce lesions size.
    - Lesions size in the high-PLTP individual will not respond to an acute or chronic dietary change to a low-fat, high-fibre diet.
        - However, non-HDL cholesterol levels will be lower compared to when on a high-fat diet. Therefore, this less atherogenic lipid profile (background) should be encouraged.

It was also acceptable to argue that based on this assignment the high-PLTP individual cannot reduce MI risk because lesion size is not altered even with a partially improved lipid profile. However, the student must justify why possible improvements in VLDL/LDL resulting from a switch to a low fat, high fibre diet should be discounted