NFS 484/1484—ASSIGNMENT #1
Due: September 30, 2010

OVERALL BACKGROUND

The prevalence of type-2 diabetes (T2DM) is increasing steadily throughout the world, and has reached epidemic proportions with prevalence estimated to double within the next 25 years. This epidemic has serious implications for global health because T2DM is associated with a number of secondary complications, and is strongly associated with cardiovascular disease which is a major source of diabetic mortality. The magnitude of increase in blood glucose following a carbohydrate-containing meal, or postprandial glycemia (PPG), is associated with the incidence of T2DM, diabetic complications, and comorbid cardiovascular disease. Therefore, strategies to control PPG in both diabetic and non-diabetic individuals are a growing area of nutritional research. One strategy relies on modulating the action of "incretins" which are hormones secreted from the small intestine following a meal. Incretin hormones influence many processes including postprandial insulin secretion and the rate of gastric emptying. Two important incretins are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP).

STUDY 1

The incretin response to glucose ingestion may be modulated by co-ingestion of fat or protein. In this study, oral glucose was fed to fasted mice with or without the addition of oleic acid (OA) or whey protein (WP). Blood glucose and insulin concentrations were measured serially over the following two hours, and the area under the curve (AUC) was calculated in order to quantify the overall magnitude of glucose and insulin responses throughout the observation period. The GLP-1, GIP, and early insulin responses (EIR) were measured as blood concentrations at 15-minutes after feeding that reflect concentrations of these hormones at only that single point in time. The overall rate of gastric emptying during the two hour observation period was determined using paracetamol technique in which a lower AUC value represents a slower rate of gastric emptying. The small intestine was removed immediately after the mice were killed, and the activity of dipeptidyl peptidase-IV (DPP-4) was measured. DPP-4 rapidly inactivates hormones, like GLP-1 and GIP, such that total circulating GLP-1 and GIP consists of both inactive and bioactive/intact forms. Short protein fragments, like those resulting from partial protein digestion, can decrease DPP-4 activity by binding to the active site on the enzyme and preventing the binding of hormone substrates. Usually, studies only measure the total circulating concentration of incretins that mostly reflects incretin secretion from the intestine. This study is unique because it measures both total and intact/active incretin concentrations.

Questions:

1. Describe the results from Table 1. (2/50 marks)

2. Describe the results displayed in Figure 1. (2/50 marks)

3. Describe the results displayed in Figure 2. (1/50 marks)

4. Using Table 1, identify what measure is the most important modulator of postprandial glycemia. Be sure to defend your answers based on the data in the table, and argue why you are selecting one measure over another. (3/50 marks)

5. Based on the background, Figure 1, and Figure 2 how does the co-ingestion of carbohydrate with fat or protein influence the postprandial incretin response? What role, if any, does DPP-4 play in this response? (4/50 marks)

6. Based on the background information and the data in Study 1, explain how fat and protein influence PPG. Be specific and ensure you explain any differences between the influence of fat and protein on PPG. (6/50 marks)

Table 1: AUC for insulin and glucose, the early insulin response (EIR), and rate of gastric emptying after feeding glucose alone or together with WP or OA to mice

	$AUC_{insulin}$ (nmol x min/L)	EIR (nmol/L)	$AUC_{glucose}$ (mmol x min/L)	Gastric emptying (mmol x min/L)
Glucose	49.0 ± 3.9	1.65 ± 0.12	770.4 ± 51.9	8.4 ± 0.6
Glucose + OA	78.8 ± 12.0[a]	2.24 ± 0.25	696.8 ± 36.7	6.0 ± 0.6[a]
Glucose + WP	144.6 ± 18.8[b]	4.71 ± 0.70[b]	415.6 ± 42.0[b]	4.0 ± 0.9[b]

Means ± SEM are shown. Superscript letters indicate significant difference versus glucose alone within each column: [a] $P<0.05$; [b] $P<0.01$.

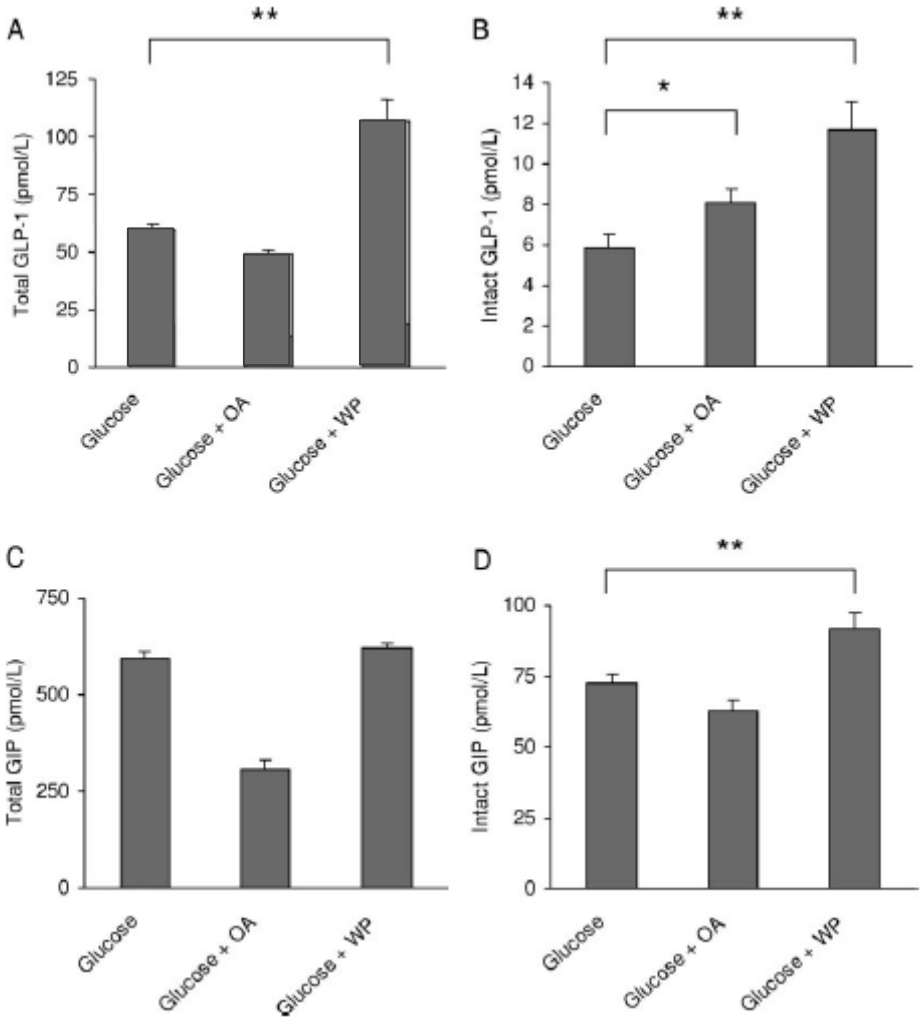

Figure 1: Plasma concentrations of total GLP-1 (A), intact/active GLP-1 (B), total GIP (C), and intact/active GIP (D) after feeding of glucose alone or together with WP or OA. Data are expressed as means ± SEM. Asterisks indicated significant differences between groups: *, P<0.05; **, P<0.01.

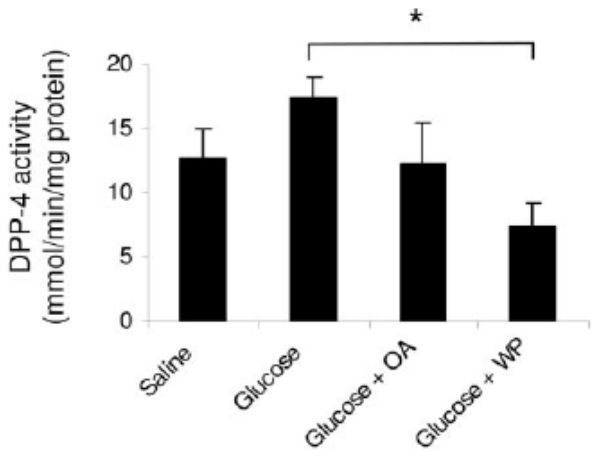

Figure 2: DPP-4 activity in the small intestine after feeding of saline or glucose alone or together with WP or OA. Data are expressed as means ± SEM. Asterisks indicate significant differences between groups: *, P<0.05.

STUDY 2

Ingestion of whey protein increases blood concentrations of the amino acids leucine, isoleucine, valine, lysine and threonine in proportion to their content in whey. These amino acids may promote extraction of insulin from blood by the liver resulting in a lower blood insulin concentration. In this study, healthy volunteers were served drinks consisting of pure glucose (reference drink) or glucose supplemented with the previously mentioned group of five free amino acids (AA5) or intact whey protein. The proportion and amounts of amino acids composing the AA5 drink were similar to their content in the whey protein drink. The subjects arrived at the test facility following an overnight fast, and the drinks were provided as breakfasts in random order on different days under standardized conditions. A peripheral catheter inserted into a vein was used to sample blood from fasting to 120 minutes post-drink consumption. These blood samples were analyzed for the total incretin, insulin, and glucose responses which were expressed in AUC units over the 120 minute period.

Table 2: Insulin, glucose, and total incretin responses (AUC) in healthy subjects after consumption of reference glucose drinks and glucose drinks to which whey protein or free amino acids were added

	$AUC_{insulin}$ (nmol x min/L)	$AUC_{glucose}$ (mmol x min/L)	AUC_{GIP} (pmol x min/L)	$AUC_{GLP\text{-}1}$ (pmol x min/L)
Reference/glucose	10.6 ± 1.3^{a}	103.4 ± 21.0^{a}	1733 ± 204^{a}	472.3 ± 76.2^{a}
Whey protein	17.0 ± 2.0^{b}	45.8 ± 10.8^{b}	1756 ± 328^{a}	734.2 ± 73.6^{b}
AA5	13.9 ± 1.6^{c}	74.2 ± 12.3^{c}	1779 ± 226^{a}	498.6 ± 77.4^{a}

All values are mean ± SEM; AA5 = leucine, isoleucine, valine, lysine, and threonine mixture. Values in the same column with different superscript letters are significantly different, $P<0.05$ (ANOVA followed by Tukey's test).

Questions:

7. Describe the results from Table 2. (3/50 marks)

8. How does the incretin response to whey protein in Study 2 differ from that of the free amino acids? Based on what you know from Study 1, why might this difference exist (Hint: DPP-4 is inhibited only by short protein fragments)? (4/50 marks)

9. Based on the information presented to this point, what is the best explanation for why the effects of whey protein and its constituent amino acids on postprandial glycemia are different? Make sure to defend your answer using the data from this question and information that you know from the previous question. (6/50 marks)

STUDY 3

The influence of different amounts of fat and protein on PPG is unknown. In this study, healthy volunteers consumed glucose drinks with 0-30 gram doses of oleic acid or whey protein on separate occasions after fasting overnight (See Table 3). Blood was sampled from fasting to 120 minutes postprandial, and analyzed to produce AUC values for glucose, insulin, and total GLP-1. An index was calculated based on blood analysis representing the degree of hepatic insulin extraction (HIE) for which higher HIE-values indicated higher postprandial removal of insulin from the blood by the liver compared to lower values.

Questions:

10. Describe the statistically significant findings displayed in Figure 3. (3/50 marks)

11. What do the results of Study 3 indicate about the impact of fat and protein quantity on PPG? What inferences can be made about the physiological mechanisms underlying these impacts? Considering all the information presented in this assignment, would you add any additional measurements to Study 3 in order to help you make these inferences? Why? (8/50 marks)

12. If you knew that total incretin secretion was influenced by the energy content of the ingested nutrients, would this change your overall interpretation of Study 3? Why? (2/50 marks)

13. Based on all of the information presented in this assignment, what specific advice would you give a friend who frequently consumes sugary food and wishes to reduce their risk of developing type-2 diabetes by altering her diet? Defend your advice by summarizing the conclusions of each study. (6/50 marks)

Table 3: Test drinks total energy contents

Test drink	Energy content (kcal)
50 g glucose (control)	200
50 g glucose + 5 g protein	220
50 g glucose + 30 g protein	320
50 g glucose + 5 g fat	245
50 g glucose + 30 g fat	470

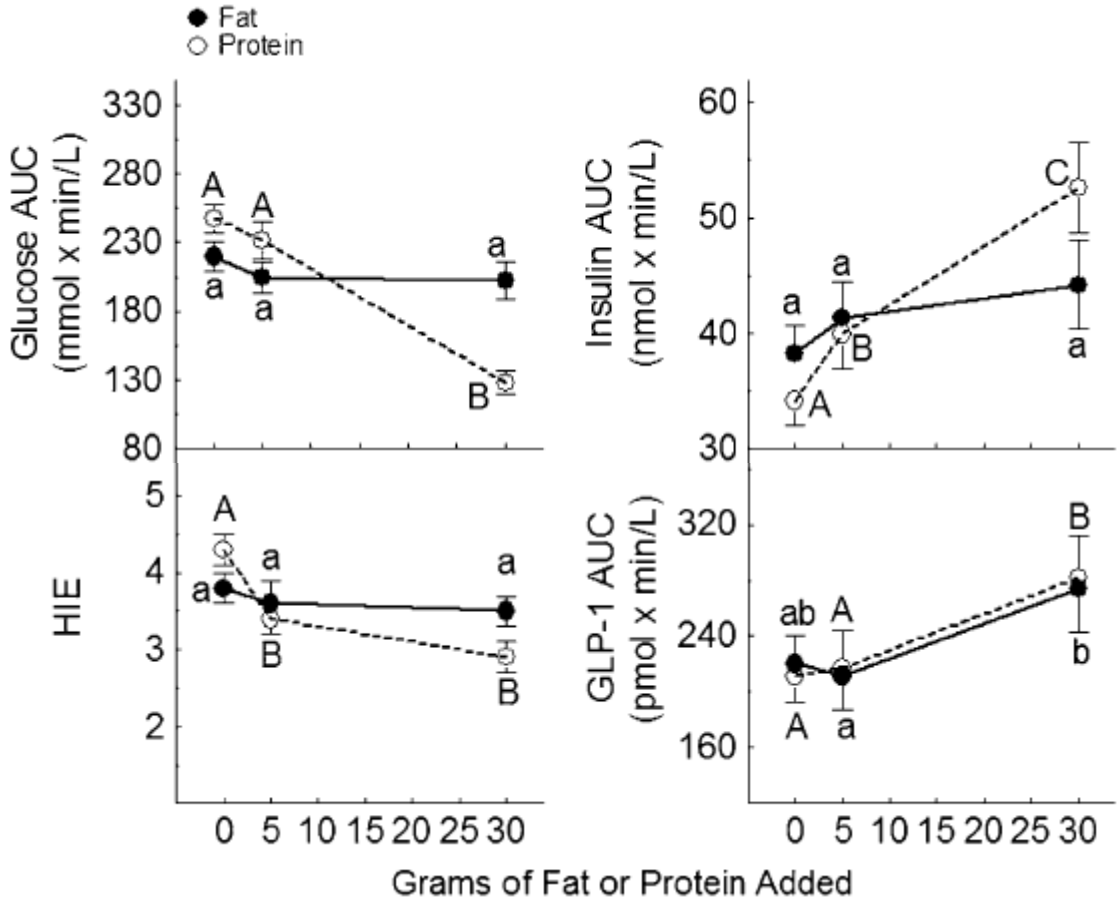

Figure 3: Mean (±SEM) glucose, insulin, and total GLP-1 responses expressed as area under the curve (AUC) and hepatic insulin extraction (HIE) in healthy humans after consuming glucose drinks combined with 0-30 grams of fat and protein. Means not sharing a common letter (a and b are for the comparison of different amounts of fat, and A and B are for the comparison of different amounts of protein) are significantly different ($P<0.05$). The error bars are not shown if they overlap or are smaller than the symbol.