ASSIGNMENT #1 2010: REAL ANSWERS FROM PEOPLE IN THE "A" CATEGORY

Question 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)

In my opinion, based on Table 1, EIR is the most important modulator of PPG, which is quantified by the glucose AUC. Following ingestion of glucose + WP, a significantly greater EIR is mirrored by a significantly lower glucose AUC, when compared to glucose given alone. A non-significant EIR following ingestion of glucose + OA is also mirrored by a non-significant glucose AUC, when compared to glucose. However, ingestion of glucose + OA produces significantly greater insulin AUC and a significantly lower gastric emptying when compared to glucose. Although both insulin AUC and gastric emptying were significantly different following ingestion of glucose + OA when compared the glucose, they were both unable to impact glucose AUC values. This suggests that they are not as important modulators of PPG as EIR is. EIR appears to be driving the significance, or non-significance, in glucose AUC.

Question 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)

It is known that incretin hormones (GLP-1 and GIP) are released from the small intestine in response to glucose ingestion but may be modulated differently by co-ingestion of protein or fat (Overall background).

Protein	- Protein resulted in higher concentration of total GLP-1 but not total GIP (Fig 1). - Total circulating concentration of incretins mostly reflects incretin secretion from the intestine (Study 1 background). - Thus, protein resulted in higher secretion of GLP-1.	- Protein resulted in higher concentration of active GLP-1 and active GIP (Fig 1). - In the small intestine, the enzyme DPP-4 rapidly inactivates GLP-1 and GIP, such that total circulating hormones consist of both inactive and active forms (Study 1 background). - Protein resulted in lower DPP-4 activity (Fig 2). - In this case, short protein fragments 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 (Study 1 background). - Thus, protein resulted in lower inactivation of GLP-1 and GIP (because of lower DPP-4 activity).
	The co-ingestion of carbohydrate and protein leads to higher incretin response (due to higher secretion of GLP-1 as well as lower inactivation of GLP-1 and GIP from lower DPP-4 activity).	
Fat	- Fat had no effect on total GLP-1 and total GIP concentrations (Fig 1). - Total circulating concentration of incretins mostly reflects incretin secretion from the intestine (Study 1 background). - Thus, fat does not affect the incretin secretion.	- Fat resulted in higher concentration of active GLP-1 but not active GIP (Fig 1). - Fat had no effect on DPP-4 activity (Fig 2) - Thus, fat resulted in higher concentration of active GLP-1 not by regulating the effect of DPP-4.
	The co-ingestion of carbohydrate and fat leads to higher incretin response (seen as higher concentration of active GLP-1).	

NOTE: Organizing answers/points into a table can be useful for organizing your thoughts. If you choose to present your answers like this in an assignment remember to link the ideas together for full marks. On these questions the real marks come from interpretation of the results—not additional description.

Question 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)

FAT: The co-ingestion of fat chiefly affects the concentration of intact GLP-1 as supported by a significantly higher intact GLP-1 level when compared to the glucose diet alone ($p<0.05$) (figure 1B). This higher level of GLP-1 activity significantly slows the rate of gastric emptying and also results in a significantly higher $AUC_{insulin}$ ($p<0.05$) (table 1). The slowed rate of gastric emptying suggests that there is less glucose entering the blood system at any one time, thus it can be more effectively taken up by tissue over the processing period. Although there was a significantly higher $AUC_{insulin}$ as previously mentioned, the co-ingestion of fat still did not significantly affect the $AUC_{glucose}$ despite the slowed gastric emptying rate. This suggests that any increase in insulin secretion that is induced by GLP-1 will be significantly reflected in the $AUC_{insulin}$ but has a negligible effect on the PPG possibly due to a delayed onset of insulin secretion which has little effect on glucose levels if blood glucose has already returned to fasting levels. Furthermore, since the co-ingestion of fat was observed to have no significant effect on total GIP or intact GIP levels (figure 1C & 1D) it does not elicit any significant changes in EIR. Consequently, it does not strongly influence PPG as evidenced by no significant deviation in its $AUC_{glucose}$ when compared to glucose ingestion alone (table 1).

PROTEIN: The protein influence over PPG can be explained through its effects on the incretin response. Not only did protein particularly enhance the intestinal secretion of GLP-1 which increased the total GLP-1 levels (figure 1A), but the concentration of intact and active GLP-1 was also higher with protein co-ingestion due to the ability of protein to inhibit DPP-4 activity (figure 1B). Collectively, these effects on GLP-1 significantly slowed the rate of gastric emptying when compared to the glucose diet alone ($p<0.01$) (table 1). This data suggested that GLP-1 also increased the insulin secretion perhaps as a lesser secondary function, which would explain why there was a significantly higher $AUC_{insulin}$ for the glucose + WP diet compared to the glucose diet ($p<0.01$) but why this significant difference did not also correspond to a similar significant change in $AUC_{glucose}$ (table 1). In regards to GIP, protein co-ingestion increased levels of intact GIP via DPP-4 inhibition ($p<0.01$) (figure 1D). This resulted in a significantly greater EIR as compared to glucose ingestion alone ($p<0.01$) which also contributed to the significant change in $AUC_{insulin}$; the ensuing higher circulating concentration of insulin will then act on the lowered blood glucose brought about by the slowed rate of gastric emptying. This two-fold promotion for the uptake of glucose resulted in a significant reduction in PPG in comparison to a glucose diet alone ($p<0.01$) (table 1).

In regards to the PPG, the major difference that exists between the influence of fat and protein is that fat does not significantly impact the PPG whereas protein does. Although fat and protein both act through GLP-1 activity to significantly increase the $AUC_{insulin}$ and slow the rate of gastric emptying as compared to the glucose diet alone ($p<0.05$ & $p<0.01$ respectively), they enhance the activity of GLP-1 via different pathways. Fat is assumed to have a pathway that directly activates the GLP-1 because it cannot competitively inhibit the DPP-4 enzyme like protein does. Regardless, the effects on blood insulin and

gastric emptying rates observed in the fat + glucose diet were not sufficient enough to elicit any significant change in the $AUC_{glucose}$/ PPG. However, the protein + glucose diet exhibited a more pronounced increase in $AUC_{insulin}$ and a slower gastric emptying rate through other incretin reponses, such as GIP that amplify the blood insulin response. This difference as well as other intrinsic properties of the protein accounts for a greater insulin response and lower blood glucose level which results in a notable effect on PPG as evidenced by a significantly lower $AUC_{glucose}$ ($p<0.01$) (table 1).

NOTE: The response directly answers the question. It addresses how protein and fat may separately influence (or do not influence) PPG, and then attempts to explain the differences.

Question 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)

Total GIP AUC was not statistically different between the WP and the AA5 treatments. This result for WP is consistent with what is observed in Study 1, Figure 1C. **Total GLP-1 AUC** was significantly greater after ingestion of WP than after AA5. This result cannot fully be explained by changes in the activity of DPP-4, although glucose + WP would be expected to lead to a decrease in DPP-4 activity (Figure 2). Total circulating concentrations of incretin hormones mostly reflect secretion (study 1 background), thus one can postulate that the increase seen in total GLP-1 AUC is the result of an increase in secretion due to whey protein's effects in the intestine. However, DPP-4 activity may still possibly influence total GLP-1 AUC. If that were so, then we would also expect to see an increase in incretin response following WP when compared to AA5. DPP-4 activity is inhibited by short protein fragments created through the digestion of WP (background). Amino acids, being the individual building blocks of proteins, and not protein fragments, would not be able to inhibit DPP-4. Thus we would expect to see less activity of DPP-4 after WP than after AA5 ingestion, and therefore a greater increase in GLP-1. This is consistent with what is observed in study 1, where WP significantly increased total GLP-1 compared to glucose, although this measurement was only made at 15 minutes.

Question 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)

Whey Protein: The co-ingestion of protein significantly enhanced GLP-1 intestinal secretion when compared to the AA5 and control groups ($p<0.05$) (table 2) and as observed from study 1, protein co-ingestion also significantly increases GLP-1 activity ($p<0.01$) (figure 1B). This in turn significantly slowed gastric emptying rates as well as significantly increased the $AUC_{insulin}$ as compared to the AA5 and control groups($p<0.05$) (table 2). Also, a significantly higher concentration of intact and active GIP was observed in study 1 with the co-ingestion of protein which corresponded to a significantly higher EIR when compared to control groups ($p<0.01$) (figure 1D and table 1 respectively). The supposed increase in EIR would have further increased the $AUC_{insulin}$. Furthermore, the five constituent amino acids of the whey protein suppressed hepatic insulin extraction which further increased blood insulin concentration.

Collectively, the GLP-1, GIP, and the AA5 within the protein explain the significantly higher $AUC_{insulin}$ compared to the AA5 and reference drinks ($p<0.05$) (table 2). This would conclude that whey protein co-ingestion has more pronounced effects on PPG than the AA5 drink alone because of the combined effects of GLP-1, GIP in conjunction with the AA5 as evidenced by the significantly lower AUCglucose as compared to the AA5 and reference treatments ($p<0.05$) (table 2).

AA5: Any significant differences in both $AUC_{insulin}$ and $AUC_{glucose}$ observed when comparing the AA5 group to the reference group and to the WP group respectively suggest that these differences were due solely to the amino acid effect on hepatic insulin extraction ($p<0.05$) (table 2). The magnitude to which these five free amino acids suppressed insulin extraction significantly increased the $AUC_{insulin}$ as compared to the reference group ($p<0.05$). This illustrated that the change in blood insulin was sufficient enough to significantly lower the $AUC_{glucose}$ observed in the AA5 group as compared to the reference group ($p<0.05$) (table2). Also since the free AA5 lacked the certain properties that the whole WP or the derivates of partially digested WP may have, these free amino acids could not influence GLP-1 and GIP activity in order to elicit further amplifications of blood insulin and could not slow the rates of gastric emptying as well. Thus AA5 can significantly influence PPG but not to the extent that the whey protein would.

Question 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)

Study 3 indicates that an increase in fat quantity has no impact on glucose AUC, as there are no significant differences at 0, 5, or 30 grams of fat. Increasing quantity of protein, however, leads to a significant decrease in glucose AUC at 30 grams compared to 0 and 5 grams, thus there may be a dose-response relationship between protein quantity and glucose AUC. One can infer physiological mechanisms impacting PPG are specific to protein only. Drawing from Figure 2 we observe that protein produced a significantly lower activity of DPP-4 compared to glucose, while fat did not. This is due to the fact that small protein fragments produced during WP digestion bind to DPP-4's active site inhibiting the enzyme. We can speculate that the more protein ingested, the more protein fragments will be available, and thus the more DPP-4 will be inhibited. Inhibiting more DPP-4 would lead to a greater incretin response, and thus to a greater EIR, insulin AUC and subsequently to a lower glucose AUC. This would also explain why differing fat quantity did not produce any changes in glucose AUC, as fat is not known to inhibit DPP-4. We can also infer that increasing WP quantity would increase its constituent amino acids in the blood in proportion to their content in the WP (study 2 background), and this explains the lower HIE values seen with increasing content, as the amino acids in WP may suppress removal of insulin. What is interesting is the fact that increasing both fat and protein quantity to 30 grams appears to be increasing total GLP-1 AUC (Figure 3), which cannot be explained, at least by fat, by differing DPP-4 activity, as fat does not alter it (Figure 2). However, total GLP-1 does not directly reflect the active amount of the hormone.

Additional measurements I would add to Study 3 include EIR, as well as active GLP-1, GIP and DPP-activity if it can be measured in human subjects. If there is a dose response relationship between protein level and DPP-4 activity as I have speculated, then one would expect to see this firstly reflected in the measurement of DPP-4 activity. However, if this measurement were unable to be performed in human subjects, then EIR and active GLP-1 and GIP can be measured to infer the activity of DPP-4. A lower activity would result in a greater proportion of active incretin hormones with increasing dose. As well, as EIR is the most important modulator of glucose AUC presented thus far, we would expect to see greater EIR as well with increasing doses of WP. From this data we can infer lower activity of DPP-4 present with larger quantity of WP.

NOTE: From this answer I can easily understand the way that the student's thought process was. Although it could be a bit more explicit and directly distinguish what was known from what was speculated/inferred, it does link its recommendations for additional measurements to the inferences. This question was difficult, and required some reflection and critical appraisal. For full marks students needed to acknowledge both incretins and HIE as being possibly responsible for an augmented insulin response and reduced PPG. There was no way to definitively separate the two mechanisms, thus, further study was required using the suggested measurements.

Question 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)

- No, my interpretation of Study 3 results would not be different.
- Adding 30 g protein to the glucose drink resulted in higher total GLP-1 response compared to adding 5 g protein to the glucose drink and glucose drink alone (Fig 3).
- If total incretin secretion was influenced by the energy content, higher protein dose (30 g) having higher calories may have resulted in higher concentration of total GLP-1 compared to lower protein dose (5 g) and glucose drink alone, both having lower calories.
- If this is the case, even greater incretin response is expected with different doses of fat since the calories are higher for a given gram of fat than for a given gram of protein (Table 3).
- With this logic, high dose (30 g) of fat, which has even higher calories than high dose of protein, would result in even higher concentration of total GLP-1 compared to low dose (5 g) of fat, which again has higher calories than low dose of protein, and glucose drink alone.
- However different doses of fat doses had no effect on total GLP-1 response (Fig 3).
- Therefore, my overall interpretation that protein quantity (rather than the energy content) has an impact on total incretin secretion and PPG, where higher dose of protein results in higher incretin response and lower PPG, remains intact.

NOTE: This answer starts with a strong opinion, which is essential to success in this type of question, and then wraps up by again piecing together the information. It is probably longer than it needs to be for 2 marks, but does get its point across. This student realized that the GLP-1 AUC for 30 grams of fat did not significantly differ from the response to 0 grams of fat in Figure 3—something that would not be anticipated if incretin secretion was exclusively related to energy content.

<u>Question 13:</u> 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)

From the results of Study 1, it can be concluded that while the consumption of fat has the ability to slow down the rate of gastric emptying and increase the postprandial insulin response, the magnitude of glucose response was not significantly lowered (Table 1). In contrast, whey protein significantly increased the activity of total incretin response, via the inhibition of DPP-4 activity (Figure 1 and 2), thereby slowing down the rate of gastric emptying and increasing the early insulin response (Table 1). As a result, whey protein significantly reduced the postprandial glycemic response, in comparison to glucose only.

Study 2 reveals that more complex proteins are more effective in increasing the GLP-1 and insulin responses, and in turn, decreasing PPG (Table 2). Free amino acids are less effective in lowering blood glucose after a meal, partly due to the inability to bind to DPP-4, thereby leaving less competition for DPP-4 to bind to and inactivate incretins.

Study 3 demonstrated that different quantities of protein have different effects on the ability to reduce PPG. Only at 30g addition of protein was the glucose response significantly lowered (Figure 3). The quantity of fat did not have a significant effect on glucose response, despite significantly greater GLP-1 at 30g of fat.

In order to reduce the risk of developing type-2 diabetes, it is strongly recommended to consume foods that are high in protein to accompany a carbohydrate-containing meal. Recommendations for the exact quantity of protein for minimal increase in blood glucose cannot be made, but based on Study 3, a minimum of 30g will suffice. Since type-2 diabetes is associated with a large increase in blood glucose after a meal, the ability of protein to lower postprandial glycemia by increasing incretin activity and decreasing the rate of gastric emptying would be a method to reduce the risk of type-2 diabetes. Sources of whey protein would be in cow's milk, white meat, lean beef, or via the use of whey protein supplements. Consumption of sugary foods should also be minimized, as it contributes to the magnitude of postprandial glucose response.

NOTE: This is a good answer because it succinctly and accurately summarizes each study, and then uses that information to drive the advice. It could have provide a stronger recommendation to not consume the free amino acids, and directly stated that fat was not recommended.