**UNIVERSITY OF TORONTO**

**Department of Nutritional Sciences**

**Mid-Term Examination 2013**

**November 7, 2013**

**Advanced Nutrition**

**NFS 484H1 F/ 1484H1 F**

**Duration – 2 hours**

**General Instructions**

1. This is an open book examination; therefore students may use any aids that have been brought into the examination room.
2. The examination is divided into 2 parts (2 studies) worth 15 marks and 25 marks respectively, for a total of 40 marks. The midterm examination will comprise 30% of your final grade.
3. Students must answer all questions in all parts of the examination. Please be certain to allocate your time appropriately to ensure that you are able to complete all parts of the examination.
4. All answers should be clearly provided in the answer booklets provided. Please provide your answer on the right-hand side of the page only. It will be assumed that the left-hand side of the page is used for note making purposes only and material appearing on this side of the page will not be read or graded.
5. PLEASE DO NOT WRITE IN PENCIL. Feel free to cross out anything that you want. Pencil smears and is often difficult to read. Thanks for your consideration in this request.

**Background**

Dietary fatty acid (FA) intake influences risk for diabetes, with saturated fatty acid (SFA) and n-3 fatty acid intake associated with an increased and decreased risk, respectively. Transition into type 2 diabetes (T2DM) occurs when the pancreatic, insulin secreting beta-cells fail to adequately respond to elevated blood glucose levels in the face of reduced insulin sensitivity. Saturated fatty acids may mediate their influence on diabetes risk through impairing beta-cell function (possibly via increased fat deposition in the cells, impairing cellular activity), while a variety of different mechanisms have been explored to uncover the protective effect of the n-3 fatty acids. Likely candidates for SFA and n-3 fatty acids are palmitic acid (PA) and eicosapentaenoic acid (EPA), respectively.

There are a number of pathways that could be influenced by dietary FA. Glucose-stimulated insulin secretion (GSIS) occurs when glucose enters the pancreatic beta-cell, goes through the glycolytic pathway, and enters the Kreb Cycle to produce ATP. The accumulation of intracellular ATP closes beta-cell K+ channels, resulting in depolarization of the cell membrane and ultimately insulin release into the circulation.

**Study 1 (Value 15/40 marks)**

A randomized controlled trial was conducted to determine the influence of EPA and docosahexaenoic acid (DHA) on glucose control. Forty participants at-risk for diabetes were put into a control and an experimental group. Both groups were instructed to replace their breakfast meal with a prescribed liquid diet while maintaining their typical diet at remaining meals and snacks for 3 months. The control and experimental liquid meals were isocaloric and had the same macronutrient composition, except the experimental meal contained EPA and DHA, while the control meal was devoid of these two fatty acids. Fasting plasma glucose (FPG), hemoglobin A1c (HbA1c; a measure of long term blood glucose), and C-peptide (C-pep; a protein co-released with insulin and used as a measure of total insulin secretion) were measured in a fasted state in the morning at baseline and after the three month treatment (Table 1). Subjects were given a 75g oral glucose tolerance load and blood was drawn over a 2 hour period to monitor glucose and insulin levels. These data were used to determine the insulin sensitivity index (ISI; a measure of peripheral (muscle) insulin sensitivity, with higher values indicating better insulin sensitivity), and glucose and insulin area-under-the-curve (gAUC and iAUC, respectively) (Table 2). There were no significant changes in body weight between groups.

**Questions:**

1. Describe the results from Table 1. (2/40 marks)
   * FPG and HbA1c were significantly decreased, and C-pep was significantly increased, following the n-3 FA treatment, relative to baseline.
   * There were no significant differences seen in FPG, HbA1c, or C-pep in the control diet.
2. Based on the data in Table 1, taken under fasting conditions, discuss a potential mechanism whereby n-3 fatty acids may decrease the risk of T2DM. (4/40 marks)
   * n-3 FA intake is associated with a decrease in FPG, a decrease in HbA1c (a long term indicator of the level of blood glucose), and an increase in C-peptide (which is an indication of insulin secretion as it is co-secreted) (Table 1).
   * The data suggests that n-3 FA increase the ability of the pancreas to release insulin (increased C-pep) and control blood glucose (decreased FPG), likely inducing long term improvements in glycemic control as seen in the reduced HbA1c.
   * Because of the better blood glucose control, the risk for T2DM may decrease.
3. Describe the results in Table 2. (2/40 marks)
   * gAUC was significantly decreased and iAUC was significantly increased following the n-3 FA treatment, relative to baseline; while there was no significant difference in ISI following the treatment.
   * There were no significant differences in gAUC, iAUC or ISI in the control condition.
4. What was the value of adding a glucose load, compared to just using fasting measures, to this experiment and what additional information does it provide, in terms of mechanisms, as it relates to the impact of the long chain n-3 fatty acids on diabetes risk? (7/40 marks)
   * It provides information such as quantity of insulin secreted (iAUC) following an elevation in blood glucose and provides information on muscle insulin sensitivity, to determine how n-3 FA influence insulin sensitivity.
   * Allows for the differentiation of pancreas *versus* muscle in mediating the effects of n-3 FA and shows that the muscle does not lower its insulin sensitivity in the fact of the higher insulin levels secreted by the pancreas.
   * Adding a glucose load allows the investigation into how n-3 FA affect GSIS and whole-body insulin sensitivity post-prandially, as both of these are important factors in the development of T2DM (Background).
   * Based on the data from Table 2, n-3 FA do not significantly influence insulin sensitivity, but do increase insulin secretion. As such, n-3 FA seem to influence GSIS/pancreatic insulin release; and do not exert their effect by altering muscle.

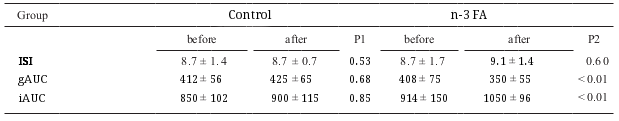


Table 1: Randomized controlled trial of n-n-3 FA and control liquid meals. Columns represent values at baseline and after intervention. FPG = fasting plasma glucose, HbA1c = hemoglobin A1c , and C-pep = C-peptide . P1 and P2 are the probabilities calculated between before and after treatment for control and n-3 group, respectively.

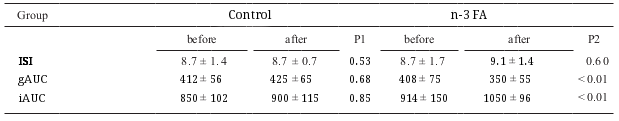


Table 2: Randomized controlled trial of n-3 FA and control liquid meals. Columns represent values at baseline and after intervention. ISI = Muscle insulin sensitivity index , gAUC and iAUC = glucose and insulin area-under-the-curve. P1 and P2 are the probabilities calculated between before and after treatment for control and n-3 group, respectively.

**Study 2 (Value 25/40 marks)**

SFA are proposed to have a lipotoxic effect on pancreatic islets, however, it is unknown how this occurs. A study, using rats, was conducted to determine the effect of PA or EPA on the pancreas and mechanisms underlying glucose stimulated insulin secretion (GSIS). Rats were fed control, PA, EPA, or EPA-PA diets for 3 weeks, after which they were sacrificed to obtain the pancreas. Pancreatic islets were then measured for level of insulin production, triglyceride (TG) content, SREBP-1c (transcription factor which initiates lipogenesis), and UCP-2 (uncoupling protein-2; disrupts ATP production in the mitochondria).

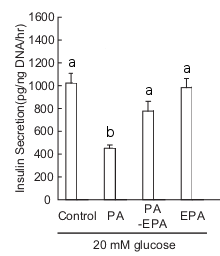


Figure 1: Insulin secretion in control, PA, EPA, and PA-EPA feeding groups. Groups that share a superscript are not significantly different from each other at p<0.05.

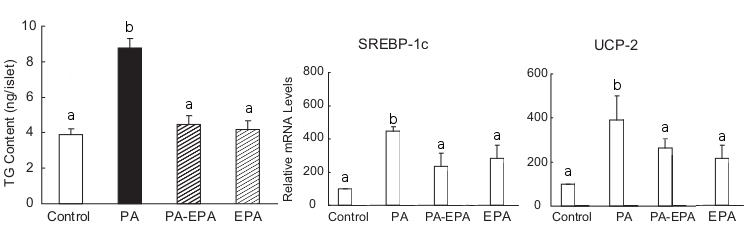


Figure 2: Pancreatic islet triglyceride (TG) content and mRNA levels of SREBP-1 (lipogenic enzyme transcription factor) and UCP-2 (uncoupling protein) in control, PA, EPA, and PA-EPA feeding groups. Groups that share a superscript are not significantly different from each other at p<0.05.

**Questions:**

1. Describe the results presented in Figure 1. (2/40 marks)
   * There was a significant decrease in insulin secretion in the PA group compared to all other groups.
   * No other significant differences were seen in the other groups.
2. Describe the results shown in Figure 2. (3/40 marks)
   * Pancreatic TG content, SREBP-1c, and UCP-2 were significantly higher in the PA condition, compared to the other 3 conditions. The other groups were not significantly different from each other.
3. Using information from the background and Study 2, propose mechanism(s) on how PA and EPA may contribute to regulation of insulin secretion occurring both within the mitochondria and cytosol. (6/40 marks)
   * PA significantly increased TG content, SREBP-1c, and UCP-2 (Figure 2) and significantly decreased insulin secretion (Figure 1).
   * Since SREBP-1c increases lipogenesis (Study 2 Background), this explains the increase in TG content within the beta-cell. PA seems to influence this transcription factor to increase, thus increasing TG production, leading to an accumulation of cellular TG that will impair cellular activity (Background). Impaired beta-cell cytosolic activity may reduce insulin release.
   * Since UCP-2 disrupts ATP production in the mitochondria (Study 2 Background), and since ATP is involved in initiating insulin release (Background), this suggests that PA impairs GSIS via increased UCP-2 that leads to a reduced ability of the storage vesicles to release insulin into circulation.
   * While EPA itself does not affect UCP-2 or SREBP-1c, it inhibits PA effect.
4. Given the data in Study 2, give 3 examples of factors/measures that the investigators could have measured in study 1 to get a better understanding of the role of n-3 fatty acids in diabetes risk. Provide a one to two sentence justification of these factors. (6/40 marks)
   * Could have measured PA in the blood or in the diet.
   * Or had another treatment group with equal parts EPA and PA.
   * Could have measured EPA within the blood to get a better understanding between EPA intake, EPA in serum, and pancreatic islet function.
   * Could have measured markers of lipogenesis.
5. Think back to the course assignment regarding the Sandy Lake population, where some individuals had a single nucleotide polymorphism (SNP) at amino acid 319 (GS) in the *HNF1A* gene which caused a decrease in pancreatic insulin production. Given the increasing consumption of Western style foods that are high in SFA in Sandy Lake, discuss 1) how the increase in SFA intake could contribute to T2DM risk and 2) whether the return to a traditional diet, including increased consumption of fatty fish (which are high in EPA), would impact risk for diabetes . Be certain to compare and contrast the impact of dietary FA in both the GG (wild type) and SS (homozygote for the SNP) groups (you do not have to consider the GS (heterozygote) group in your answer). Defend your answer by drawing on data provided in this exam. (8/40 marks)
   * Because the SNP on the *HNF1A* gene affects insulin production, and because a high SFA (i.e. PA) diet reduces the ability of insulin storage vesicles to release the insulin that has been produced, the First Nations individuals may be at much greater risk for developing diabetes/hyperglycemia if they consume a high SFA diet (reduce insulin release) and have the SNP (reduced insulin production).
   * However, if an individual did have the SNP, consuming a diet high in EPA may increase the amount of released insulin that has been produced. This may potentially offset the harm of the SNP by reducing the risk for diabetes.
   * But, an individual who consumes EPA and doesn't have the SNP is the least likely to develop diabetes because both insulin production and insulin release are functioning optimally.