UNIVERSITY OF TORONTO

Department of Nutritional Sciences

Mid-Term Examination 2013

November 7, 2013

Advanced Nutrition
NFS484H1 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 type 2 diabetes (T2DM), with saturated fatty acid (SFA) and n-3 FA intake associated with an increased and decreased risk, respectively. SFA may mediate their influence on T2DM risk through impairing beta-cell function (possibly via increased cytosolic fat deposition in the beta-cells, leading to impaired cytosolic activity and functioning). A variety of different mechanisms have been explored to uncover protective effects of the n-3 FA, with several still under investigation. Likely candidates for the effects of SFA and n-3 FA 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 results in depolarization of the cell membrane and ultimately releasing insulin into the circulation.

Study 1 (Value 15/40 marks)

A randomized controlled trial was conducted to determine the influence of EPA on glucose control. Forty participants at-risk for T2DM were put into a control and an experimental group. Both groups were instructed to replace the dietary fats they typically use with either butter (45% SFA, does not contain EPA) or butter supplemented with EPA, both of which were provided by the researchers, while maintaining their usual diet for 3 months. 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 glucose 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)
    - Control: FPG, HbA1c, C-pep before=after
    - EPA: FPG, HbA1c before > after; C-pep before < after (or before=after)
    - Marks off if between group comparison was made (there was no tests for between group), if no direction was indicated, if non-significant findings were not mentioned, or if other variables were not mentioned
2. Based on the data in Table 1, taken under fasting conditions, discuss a potential mechanism whereby EPA may decrease the risk of T2DM. (4/40 marks)
    - Many possible answers, but marks were given at most to some mention of T2DM risk, relating to glucose control, arguing that insulin secretion was involved (or not involved) using C-pep, and potentially arguing that there was a problem with the pancreas (or with IR)

- Potential answers include:
  - EPA decreases FPG and HbA1c, increases (or no change) C-peptide (Table 1).
  - The data suggests that EPA increases (or doesn't) the ability of the pancreas to release insulin (C-pep) and control fasting blood glucose (FPG), inducing long term improvements in glycemic control (HbA1c).
  - Better blood glucose control = lower risk for T2DM.
  - Or: May be insulin resistance because of no C-pep change, may be affecting GLUT4 translocation or insulin receptor
  - Or: Competes with SFA to inhibit the effect of increased fat deposition in the cytosol (Background)
  - Or: EPA increases membrane fluidity in the beta-cell for better entry of glucose (or in the muscle cells)

3. Describe the results in Table 2. (2/40 marks)

   - Control: gAUC, iAUC, ISI before = after
   - EPA: ISI before = after, gAUC before > after, iAUC before < after
   - Marks off as described in Q1.

4. What was the value of adding a glucose load, compared to just using fasting measures, to this experiment? What additional information does the glucose/insulin response to the glucose load provide, in terms of mechanisms, as it relates to the impact of EPA on T2DM risk? (7/40 marks)

   - Marks were given for comparing fed vs fasted conditions (glucose load vs fasting), stating that peripheral insulin sensitivity was **not** involved and that EPA has a role in insulin secretion, and lastly relating that to T2DM risk
   - Potential answer:
     - Glucose load provides information on insulin sensitivity (i.e. muscle) and on GSIS (pancreas), while fasting does not
     - Given increased iAUC, lower gAUC, and no change in ISI (Table 2), EPA influences GSIS (potentially via lower cytosolic fat deposition, problems with glucose entry into glyocolysis, or with ATP buildup (Background)) but does not influence insulin sensitivity

Group	Control			EPA		
	Before	After	P1	Before	After	P2
FPG (mg/dL)	104.5 (24.5)	103.6 (29.5)	0.62	105 (40)	94.5 (27.4)	< 0.01
C-pep (ug/dL)	1.5 (1.1)	1.7 (1.1)	0.17	1.1 (1.1)	1.5 (1.1)	0.05
HbA1c (%)	6.7 (1.1)	6.7 (0.7)	0.16	6.6 (0.7)	6.0 (0.9)	< 0.01

Table 1: Randomized controlled trial of EPA and control groups. Columns represent values as means and the standard errors in brackets at baseline (before) and after intervention. FPG = fasting plasma glucose, HbA1c = hemoglobin A1c, C-pep = C-peptide. P1 reflects comparisons within the control group and P2 reflects comparisons within the EPA group. Significance is denoted at $P<0.05$.

Group	Control			EPA		
	Before	After	P1	Before	After	P2
ISI	8.7 (1.4)	8.7 (0.7)	0.53	8.7 (1.7)	9.1 (1.4)	0.60
gAUC	412 (56)	425 (65)	0.68	408 (75)	350 (55)	< 0.01
iAUC	850 (102)	900 (114)	0.85	914 (150)	1050 (96)	< 0.01

Table 2: Randomized controlled trial of EPA and control liquid meals. Columns represent values as means and the standard errors in brackets at baseline (before) and after intervention. ISI = Muscle insulin sensitivity index, gAUC and iAUC = glucose and insulin area-under-the-curve. P1 reflects comparisons within the control group and P2 reflects comparisons within the EPA group. Significance is denoted at $P<0.05$.

Study 2 (Value 25/40 marks)

SFA are proposed to have a lipotoxic effect (which includes impairment to cytosolic metabolism caused by an accumulation of intracellular FA) on pancreatic islets, however, it is unknown how this occurs. An experimental study using rats was conducted to determine the effect of PA or EPA on the pancreas and mechanisms underlying glucose-stimulate insulin secretion (GSIS). Rats were fed standard chow (control; contains no PA or EPA), or chow supplemented with PA, EPA, or PA and EPA (denoted as PA-EPA in the figures) diets for 3 weeks, after which they were sacrificed to obtain the pancreas. Pancreatic islets were then measured for the level of GSIS (Figure 1), triglyceride (TG), SREBP-1c (transcription factor that initiates lipogenesis), and UCP-2 (uncoupling protein-2; disrupts ATP production in the mitochondria) content (Figure 2).

Questions:

5. Describe the results presented in Figure 1. (2/40 marks)

    - Insulin secretion: Control = EPA = PA-EPA > PA
    - Marks off as per Q1.

6. Describe the results shown in Figure 2. (3/40 marks)

    - TG, SREBP1c, UCP-2: Control = EPA = PA-EPA < PA
    - Marks off as per Q1.

7. Using information from the background and Study 2, propose mechanism(s) on how PA and EPA may contribute to the regulation of insulin secretion within the mitochondria and the cytosol. (6/40 marks)

    - Marks were given based on clear separation of mitochondria and cytosol, which were linked to UCP and SREBP/TG, how these individual molecules contributed to GSIS, using the information in the background, and that the PA-EPA finding was discussed, stating that EPA alone does **not** contribute to GSIS, but inhibits/competes with PA (which does affect GSIS)
    - Potential answer:
        - PA increased TG, SREBP, UCP, decreased insulin secretion (Figures 1 and 2). Addition of EPA attentuated the levels to normal, but EPA alone had no effect
        - PA increased lipogenesis, which increased TG content, which affects cytosolic metabolism (Background), potentially impair glucose entry into the beta cell or its passage through glycolysis to create ATP (Background), which would reduce GSIS and increase the risk for T2DM
        - PA increased UCP, which disrupts production of ATP and preventing the accumulation of ATP, which is needed for the depolarization of the beta cell membrane in order to release insulin (Background), therefore reducing GSIS and increasing the risk for T2DM
        - EPA alone has no influence on GSIS, but inhibits the action of PA

8. Given the data in Study 2, give 3 examples of additional factors/measures the investigators could have considered in Study 1 to get a better understanding of the role of n-3 FA in T2DM risk. Provide a one or two sentence justification for each of these factors/measures. (6/40 marks)

    - Could have many different types of answers.
    - Marks were taken off if more than 3 examples were given, if the measure or the location of the measure was ambigious, if the measure was entirely unrealistic in humans, if there was more than 2 sentences, and if the exact same measures as in Study 2 were suggested (we aren't looking for regurgitation)
    - Potential (brief) answers:
        - Exercise
        - Diet
        - Serum fatty acids
        - C-pep during the glucose load
        - Use a control diet
        - Include a PA group
        - Measure serum PA
        - Do comparisons between groups
        - Measure markers of lipogenesis (e.g. fatty acid synthase) in serum
        - Include a EPA only diet
        - Measure lipoproteins

9. 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 the risk for T2DM. Be certain to compare and contrast the impact of dietary fat in both the GG (wild type) and SS (homozygote for the SNP) groups [you do not need to consider the GS heterzygote group in your answer]. Defend your answer by drawing on data provided in this exam. (8/40 marks)

    - Marks were given for distinguishing between insulin production (genotype) and insulin secretion (diet/FA type), contrasting the different impacts of the SFA vs EPA on the two genotypes (GG vs SS), distinguishing between the fact that EPA alone does not contribute to reducing risk for T2DM, but that it inhibits effect of SFA, and any additional pieces that may have come from the assignment or from general knowledge
    - Potential (brief) answer:
        - SS have reduced insulin production compared to GG and higher risk for T2DM (regardless of environment), and in fact all SS individuals got diabetes (Assignment)

- SFA reduce insulin secretion (Study 1 and 2) and thus an increased risk of T2DM
- Dietary SFA would increase risk of T2DM in both GG and SS, but more so in the SS group because of defect in insulin production AND reduced insulin secretion because of SFA
- Returning to a traditional diet (with EPA) would reduce the risk of developing T2DM in GG and potentially in SS. However, traditional diet would probably only delay the development of T2DM in SS individuals as they still have defect in insulin production.
- EPA alone does not improve GSIS, however, it competes/inhibits the effect of SFA and will reduce the risk for T2DM.

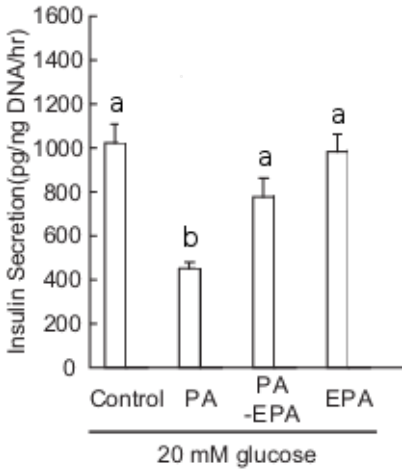

Figure 1: Pancreatic islet GSIS in control, PA, EPA, and PA and EPA (PA-EPA) feeding groups. Groups that share a superscript are not significantly different from each other at $p<0.05$.

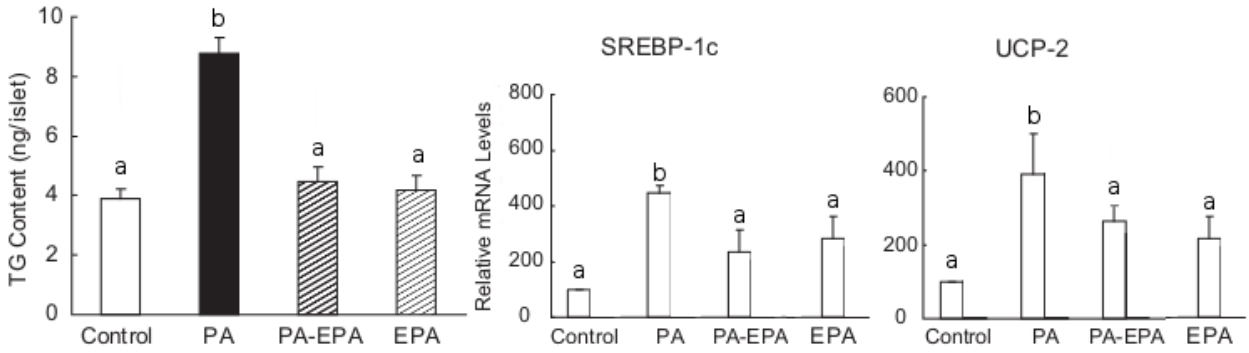

Figure 2: Pancreatic islet triglyceride (TG) content and mRNA levels of SREBP-1 (lipogenic enzyme transcription factor) and UCP-2 (uncoupling protein) in control, PA, EPA, and PA and EPA (PA-EPA) feeding groups. Groups that share a superscript are not significantly different from each other at $p<0.05$.