**Background:**

Greater systemic inflammation can disrupt metabolic processes throughout the body. Greater inflammation has also been implicated in the development of plaque buildup in blood vessels, potentially leading to cardiovascular disease (CVD). The most common type of CVD is coronary artery disease (CAD), which can increase the risk for heart attacks.

The n-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are precursors to potent anti-inflammatory molecules. While EPA and DHA can be obtained from the diet, we can also synthesize them from the fatty acid (FA) alpha-linolenic acid (ALA; also an n-3). Conversely, the n-6 long chain polyunsaturated fatty acid (n-6 LC-PUFA) arachidonic acid (ARA) is used in signaling pro-inflammatory processes. Similar to the n-3 LC-PUFA, ARA can be obtained from the diet as well as synthesized from linoleic acid (LA; also is an n-6). However, both ALA and LA are essential FA and can *only* be obtained from the diet. ALA and LA are converted into their longer chain equivalents (EPA+DHA and ARA, respectively) by the same delta-6 desaturase (D6D) enzyme and therefore compete for its activity.

**Study 1 (Value 10/50):**

To observe the role of fatty acids on the risk for CAD, a large prospective longitudinal cohort was initiated many years ago in order to record cardiovascular events. Participants were recruited from several cities in Canada. At the baseline visit, participants had their body mass index (BMI) determined and had blood samples taken. Blood samples were analyzed for C-reactive protein (CRP; a protein that rises in response to, as well as contributes to, inflammation), serum FA, and D6D activity. Every year, participants were called to record any cardiovascular events that had occurred over the previous year.

After 15 years, the data collected were analyzed. Relative risks (RR) were calculated on tertiles of serum CRP levels and D6D activity with CAD events. The RR represents the risk as a percent that greater CRP (or D6D) have on CAD events (for example, a RR of 1.30 equals a 30% greater risk). A RR is *not* significant if the range crosses the 1.0 value (for example, a RR of 1.30 with a confidence interval of 0.90 to 1.50 is considered not significant).

Table 1: **Baseline** characteristics of participants who either developed CAD or did not develop CAD (CAD-free) within a 15 year timeframe.

| | CAD-free (n=621) | CAD (n=457) | p-value |
|---|---|---|---|
| BMI | 25.5 | 26.3 | 0.11 |
| Serum LA (g/100g) | 9.77 (1.38) | 9.05 (1.40) | <0.001 |
| Serum ARA (g/100g) | 19.1 (1.45) | 23.2 (1.75) | <0.001 |
| Serum ALA (g/100g) | 0.10 (0.03) | 0.09 (0.04) | 0.24 |
| Serum EPA+DHA (g/100g) | 6.84 (1.47) | 7.03 (1.56) | 0.34 |

*Values are the means and standard deviations.*

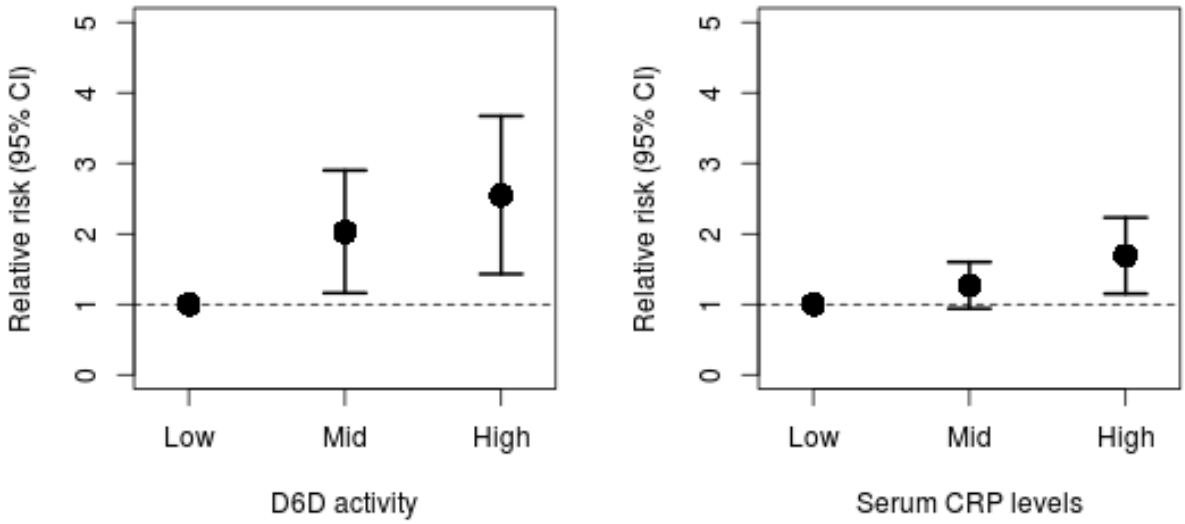


Figure 1: Relative risks of tertiles of D6D and CRP with CAD. Error bars (RR range) that cross the 1.0 line (dashed horizontal line) are **not** considered statistically significant.

**Questions:**

1. Describe the results in Table 1 and Figure 1. (Value 4/50)

    - Table 1:
        - BMI + ALA + EPA+DHA: non-CAD = CAD
        - LA: non-CAD > CAD
        - ARA: non-CAD < CAD
    - Figure 1:

  - Risk, D6D: Low < Mid < High (or Low<Mid=High)
  - Risk, CRP: Low = Mid < High
- Full marks for the above
- Marks off:
  - -0.75 for each non-significant not mentioned, max -2
  - -1 if direction not mentioned, max -3

2. Drawing on information from the Background and the data in Table 1 and Figure 1, discuss a potential mechanism for how dietary PUFA may influence the risk for developing CAD. (Value 6/50)

   - Background:
     - Systemic inflammation disrupt metabolism -> plaque buildup
     - Plaque buildup -> CAD + heart attack
     - EPA+DHA anti-inflammatory (+ ALA)
     - ARA pro-inflammatory (+ LA)
     - ALA + LA only from diet (EPA+DHA+ARA also, but also from ALA+LA)
     - Conversion to LC-PUFA increased by D6D
   - Study 1: CRP -> inflammation
   - Table 1: CAD had > ARA, < LA, same ALA+EPA+DHA+BMI
   - Figure 1: Increasing D6D activity increases risk for CAD and the highest CRP increases risk for CAD
   - Synthesize:
     - Those with CAD had lower LA and higher ARA, suggesting greater D6D activity and conversion of LA to ARA (Table 1)
     - This is confirmed in Figure 1 with the greater risk for CAD with greater D6D
     - Since ALA+EPA+DHA was the same between groups, suggests that the CAD group were consuming more n-6, as both n-3 and n-6 compete for the D6D activity (Background)
     - Since n-6 are pro-inflammatory (Background), and since greater inflammation via CRP increased risk for CAD (Background + Figure 1), this suggests that dietary n-6 -> D6D -> serum n-6 LC-PUFA -> CRP -> CAD
   - Marks off:
     - ~ -1 if info isn't properly cited
     - -2 if somewhere this isn't shown (*diet* n-6 -> D6D -> serum n-6 LC-PUFA -> CRP -> CAD)
     - -2 if synthesis isn't shown or developed (no clear line of thinking shown)
     - No marks off if they don't use everything (question doesn't ask that)
     - -1 if not tie back to diet

**Study 2 (Value 40/50):**

There is some public health concern that the ratio of dietary n-6 to n-3 FA is important for cardiovascular health, particularly in regard to Western style diets. It is generally argued that the North American diet is too high in n-6 relative to n-3 FA. Therefore, a community intervention was conducted over one year in the US to determine the effectiveness of strategies that aim to reduce dietary n-6 PUFA (indicated as the "Low n-6" group). A nearby community with similar characteristics as the intervention community was used as the control group (indicated as the "High n-6" group). BMI, dietary intake, and blood samples were collected from each participant. Blood samples were used to measure serum CRP and FA levels.

The field of nutrigenomics has revealed several candidate genes that may influence FA metabolism. This group of alleles, called the *FADS* gene cluster, has been associated with modulation in D6D activity. Therefore, a cheek swab was taken to extract DNA to quantify the gene cluster in the participants, who were then classified as "Low FADS" if they had <4 *FADS* alleles present and "High FADS" if they had >4 *FADS* alleles present.

There were no significant differences in BMI and dietary intake of n-3 FA between the two groups. However, dietary n-6 did decrease in the intervention group, suggesting good participation in the intervention.

Table 2: Differences between a low number of FADS alleles and a high number of FADS alleles before the intervention.

| | Low FADS alleles | High FADS alleles |
|---|---|---|
| Serum LA (g/100 g) | 12.2 (1.54) | 9.96 (1.10)* |
| Serum ARA (g/100 g) | 18.54 (2.08) | 20.19 (1.98)* |
| Serum ALA (g/100 g) | 0.11 (0.02) | 0.09 (0.01)* |
| Serum EPA+DHA (g/100 g) | 7.33 (1.45) | 7.78 (1.23)* |

* *indicates significantly different (p<0.05) from participants with "Low FADS".*

**Questions:**

3. Describe the results of Table 2. (Value 2/50)

    - Table 2:
        - LA + ALA: Low FADS > High FADS
        - ARA + EPA+DHA: Low FADS < High FADS
    - Marks off:
        - -0.5 if direction not mentioned, max -1.5

4. Using the Background information and the data from Table 2, discuss how the *FADS* gene cluster is influencing D6D activity. Assume that dietary intake of n-6

and n-3 FA did not differ between groups at the baseline measurements. (Value 5/50)

- Background:
  - EPA+DHA comes from diet + ALA conversion
  - ARA comes from diet + LA conversion
  - ALA + LA only from diet
  - Conversion occurs via the D6D activity
  - ALA + LA compete with D6D activity
- Study 2: Concern of n-6 to n-3 ratio, but both groups have the same dietary intake (from Question). FADS genes influence D6D
- Table 2: High FADS < LA, > ARA, < ALA, > EPA+DHA
- Synthesize:
  - Given that both high and low FADS had the same dietary intake of n-6 and n-3 (Background), but serum FA were different (Table 2), suggests FADS is at work.
  - Both ALA + LA are lower while ARA+EPA+DHA are higher in the High FADS group (Table 2), which suggests that more FADS genes increases D6D activity, converting available ALA+LA to their longer chain equivalents.
- Marks off:
  - -1 if info from Background + data is not summarized
  - -1 if info isn't properly cited
  - -2 if somewhere this isn't shown (more FADS genes -> more D6D activity -> less ALA+LA -> more ARA+EPA+DHA)
  - -1 if synthesis isn't shown or developed (no clear line of thinking shown)
  - -1 if strongly state that High FADS are eating more ARA+EPA+DHA, no marks off if they make a suggestion but state very likely because of higher D6D activity.

5. Describe the results of Figure 2. (Value 2/50)

- Figure 2:
  - CRP + ARA to EPA+DHA ratio — LL = LH < HL < HH
- Marks off:
  - -0.5 if non-significant is not mentioned, max -1
  - -0.5 if direction not mentioned, max -1.5

6. Using **all** information and data up to this point, discuss how the *FADS* gene cluster may influence the risk for CAD. (Value 6/50)

- Background:
  - Systemic inflammation disrupt metabolism -> plaque buildup
  - Plaque buildup -> CAD + heart attack
  - EPA+DHA anti-inflammatory (+ ALA)
  - ARA pro-inflammatory (+ LA)

  - EPA+DHA comes from diet + ALA conversion
  - ARA comes from diet + LA conversion
  - ALA + LA only from diet
  - Conversion occurs via the D6D activity
  - ALA + LA compete with D6D activity
- Study 1: CRP -> inflammation
- Study 2: Concern of n-6 to n-3 ratio. FADS genes influence D6D
- Table 1: CAD had > ARA, < LA, same ALA+EPA+DHA+BMI
- Figure 1: Increasing D6D activity increases risk for CAD and the highest CRP increases risk for CAD
- Table 2: High FADS < LA, > ARA, < ALA, > EPA+DHA
- Figure 2: Reducing n-6 reduces CRP + ratio, having higher n-6 + high FADS has greatest CRP + ratio
- Synthesize:
  - In Study 1, those who got CAD likely had greater D6D, leading to more conversion of LA (less) to ARA (more), increasing CRP and increasing plaque build up, leading to increased risk of CAD. This could have been because of greater n-6 intake or because of greater D6D.
  - In Study 2, those who had greater FADS had greater D6D activity and so more conversion of LC-PUFA (both n-3 and n-6). When on the intervention, reducing n-6 had equal effect on both FADS groups in terms of CRP. The high and low FADS group in the intervention had equal conversion of PUFAs into LC-PUFA (same ratio). But high n-6 intake + high FADS had greater conversion of ARA (ratio) as the greater D6D activity combined with the greater n-6 ratio outcompeted the n-3 for the D6D enzyme. Thus they also had the highest CRP because of the greater ARA.
  - Therefore, FADS genes alone do not do anything wrong. But in combination with greater n-6 they convert more n-6 into ARA and thus are at a higher risk for CAD because of the greater CRP and inflammation, which contributes to plaque build-up and disruption to metabolism.
- Marks off:
  - -1 if info from Background + data is not summarized
  - -1 if info isn't properly cited
  - -2 if somewhere this isn't shown (more FADS genes + more n-6 leads to greater CRP -> CAD, more FADS genes + equal n-6 and n-3 => no increase in CRP -> no CAD) D6D activity -> less ALA+LA -> more ARA+EPA+DHA)
  - -2 if synthesis isn't shown or developed (no clear line of thinking shown)
  - -1 if not all information is used as the question states use **all**.

7. Imagine you are clinician and a patient comes in who has a mixed, but predominately African ancestry. Considering that individuals with African ancestry are more likely to have more alleles of the *FADS* gene cluster, using the information and data in this exam and your previous knowledge, how could you reduce his/her risk for CAD disease? Defend your answer by incorporating data from both studies. (Value 7/50)

- Multiple answers, though generally:
  - Heavily reduce n-6 and/or increase n-3 to have more balanced n-6 to n-3 ratio.
- Marks given/off:
  - +2 for clear thinking, logical, flow, rational
  - +2 for defending using evidence (no extra marks for evidence outside of exam as question doesn't ask that)
  - -1 for not citing info/data (from exam)
  - +3 base if they at least somewhere/somehow answer the question with something like the possible answer above

8. Given that all of these studies were conducted in Western countries with a high n-6 to n-3 ratio, discuss how the association between higher D6D activity and CAD risk may differ in countries with a lower dietary n-6 to n-3 ratio (for example, in Inuits consuming a traditional diet with a high dietary intake of fish and seafood, which has large amounts of n-3 FA). Explain any neutral or positive influences the *FADS* alleles may have. Defend your answer using your own knowledge, the Background information and the two studies. (Value 8/50)

   - Multiple answers, though generally:
     - D6D is indiscriminate, if more n-3, more conversion to EPA+DHA, if more n-6, more conversion to ARA.
     - If more n-3, more EPA+DHA, likely less inflammation, likely lower risk.
   - Marks given/off:
     - +3 base if they indicate something like the above answer
     - +2 for clear, logical, rational thinking
     - +3 for defending using evidence
     - -1 for not citing info/data from exam
     - ~ -1 if not tie back to country/question
     - * marks if they discuss how FADS may be disadvantageous in North American context, but not in other contexts

9. A recent large randomized, controlled clinical trial showed no effect of n-3 LC-PUFA supplementation on CAD. Comment on 1) why a clinical trial may not always be able to identify effects with nutrient supplementation in the general population and 2) why targeting only n-3 LC-PUFA may not always be effective. Use your previous knowledge and all the information and data from this exam. (Value 10/50)

   - Multiple answers, though generally:
     - Clinical trial may not catch subsets of the population (ie FADS).
     - The story includes both n-3 AND n-6. Targetting one may not be enough.
   - Marks given/off:
     - +4 base if they indicate at least one problem with clinical trials and at least one reason why n-6 is also important

- +2 for clear, rational thought process
- +2 for defending using info/data
- +1 for defending using outside knowledge
- +1 for being creative
- -1 for not citing info/data from exam
- -1 for not using all data

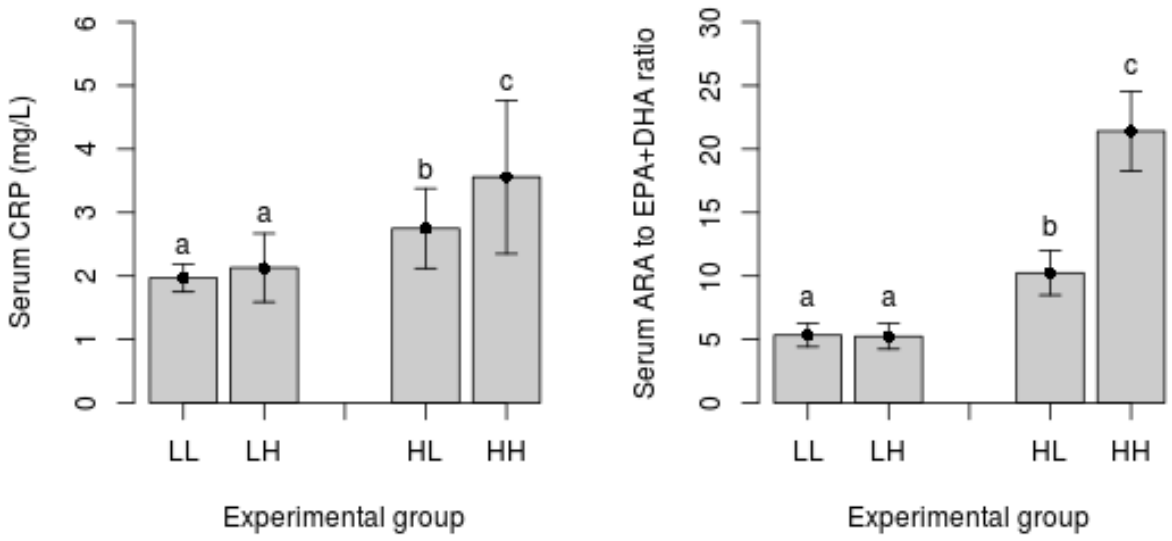


Figure 2: Effect of intervention on participants with either a low or a high number of FADS alleles. LL = low n-6 (intervention) and <4 FADS alleles (low FADS); LH = low n-6 (intervention) and >4 FADS alleles (high FADS); HL = high n-6 (control) and <4 FADS alleles (low FADS); HH = high n-6 (control) and >4 FADS alleles (high FADS).