

## 最終試験の結果の要旨

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主査および副査の5名は、平成27年6月29日、学位申請者 Juliet David 君に面接し、学位申請論文の内容について説明を求めると共に、関連事項について試問を行った。具体的には、以下のような質疑応答がなされ、いずれについても満足すべき回答を得ることができた。

**Q1. What was the exclusion criterion for 18 subjects? How were the ranges of their docosahexaenoic (DHA)/ arachidonic acid (AA) and eicosapentaenoic acid (EPA)/AA?**

A. Because our purpose is to examine the association between mild cognitive impairment and fish consumption/serum DHA level, we excluded the subjects with Kana Pick-out Test (KPT) scores less than the age-specific cut-off points proposed by Dr. Kaneko (1996), suggesting severe cognitive impairment. The DHA/AA and EPA/AA values ranged from 0.15 to 0.89 and 0.24 to 0.67, respectively. The levels were moderate as found in other participants of the present study.

**Q2. Why did you exclude elderly people, over 70 years, in this study?**

A. Because our main interest was the preventive effect of DHA/fish intake on cognitive impairment, we mainly targeted middle-aged people and young old. In addition to that, the completion rate of all examinations was very low among elderly people over 70 (<20%), and thus, we decided to focus on the subjects under 70.

**Q3. Why did you apply KPT for the examination of cognitive function?**

A. We used KPT over other diagnostic tools because 1) KPT has been designed to detect the early onset of dementia, 2) its instruction is easy to understand for examinees, and 3) this is sensitive for middle-aged population.

**Q4. Why didn't you include age distribution in the baseline characteristics, Table 1?**

A. Age is an important factor in determining level of cognitive impairment. The KPT distributions by age-category are shown in Table 2, while Table 1 mainly shows the comparison of each variable between male and female subjects.

**Q5. How do you explain the positive association between hair mercury levels and KPT scores in Table 4?**

A. We measured mercury level as a surrogate marker of fish intake. The positive association between hair mercury level and KPT was observed among age group less than 60 years, and this was relevant to the association between DHA/AA and KPT in this age group. The highest mercury level did not reach the neurotoxic dose which potentially affects cognitive function.

**Q6. What kinds of fish contain high level of mercury and high content of free fatty acids (FFAs)?**

A. Predatory (eat other fish) or big fish are at top of the food chain, and they tend to contain high levels of mercury because of the bioaccumulation. These types of fish includes: king mackerel, swordfish, tilefish, and shark. Mostly, fish containing high level of mercury may have high level of FFAs. On the other hand, small fish like sardines, tuna, sprats, and anchovies also contain high levels of FFAs.

**Q7. Do all fish contain high level of FFAs?**

A. All fish contain some amount of omega-3-FFAs, but its quantity varies among species and size. Sea-fish tend to have more omega-3-FFAs than freshwater fish. Similarly, wild fish contain more omega-3-FFAs than farmed fish because the main source of omega-3-FFAs for fish is algae.

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### Q8. How are DHA and EPA transferred to the brain?

A. Mechanisms of FFA transport to brain are controversial. There are some studies showing that FFAs move via diffusion across the membranes. DHA, EPA, and other FFAs can be readily solubilized in the luminal membrane, flip flopped towards the inner membrane and undergo desorption into the cytoplasm. However, some studies have suggested that membrane proteins, for example, fatty acids binding proteins, CD36, fatty acid transporter protein are involved in the transport into cells.

### Q9. Why did you use KOH for the FFA pre-treatment? Did you check the FFA level without KOH?

A. We used KOH because N-acyl derivatives of long-chain bases are saponified only slowly by alkali. Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by treatment with alkali. We have not checked the FFA level without pretreatment by KOH.

### Q10. Why did you take a ratio of DHA to AA? How was the result when you analyze the data using serum levels of DHA?

A. Dietary intake of n-3 PUFA is known to reduce the amount of AA in phospholipids by diminishing its synthesis and simple physical replacement, which suggests that the DHA/AA is better indicator of serum DHA level (Hossain *et al.*, 1998). The analysis using serum levels of FFA gave similar results.

### Q11. Are there any other metals used as surrogate markers of fish intake?

A. There is no other metal to use as a surrogate maker of fish consumption.

### Q12. Did you examine fasting blood specimens?

A. Yes, the blood was drawn in fasting state because the annual health check-up was held in early morning.

### Q13. Can recent diet affect on the serum FFA levels of the subjects?

A. Yes. We assume that FFA levels measured in this study are reflected recent regular FFA levels, since the serum was taken in the fasting state. The examination using erythrocytes is more preferable to determine the exposure to DHA/EPA, sine n-3 PUFA persists for months and serum/plasma FFA level reflects the dietary intake of past few hours.

### Q14. EPA is important for muscle function. Did you examine this association?

A. No, we did not examine EPA in relation to muscle dysfunction.

### Q15. DHA and EPA may affect mood or depression. Did you examine these associations?

A. No, we did not examine DHA/EPA in relation to mood or depression in this study.

### Q16. Did you validate the hair MeHg as a surrogate marker of fish intake? Are there any studies showing fish intake and mercury levels?

A. There are several studies reporting the association between fish intake and MeHg level. (Domingo *et al.* 2007; Virtanen *et al.* 2007; Elhamri *et al.* 2007; Carrington and Bolger 2002) Although the correlation between MeHg and fish consumption varies by other factors, moderate/strong correlations were reported.

### Q17. Egg is another important source of DHA in addition to fish. How do you interpret your results?

A. Egg is a rich source of omega-3 FFAs, and nowadays, omega-3-FFA-rich eggs (fortified) are also produced. Although DHA contents of regular and DHA-rich eggs are approximately tenth and fourth part of fish, respectively, egg is not negligible as a source of DHA, and this may explain the weak correlation between fish consumption and DHA in the present study. Although we did not examine the egg consumption, the lack of this information does not affect the association between KPT and DHA/AA which were directly measured in the serum.

### Q18. Why did you collect the information on education level?

A. Previous studies have suggested that education level is a strong indicator of cognitive function and that was true in our study. Furthermore, education level is also related to the awareness level of the general public, and more educated people might be aware of the benefits of eating fish.

### Q19. Is KPT influenced by mild visual impairment like as aged eye?

A. The prevalence of aged eye increased with age, like as dementia. We conducted KPT under appropriate visual condition using glass.

以上の結果から、5名の審査委員は申請者が大学院博士課程修了者としての学力・識見を有しているものと認め、博士（医学）の学位を与えるに足る資格を有するものと認定した。