（ 様 式 14 ）

最終試験の結果の要旨

報告番号	総研第 432 号		学位申請者	Pulong Wijang Pralampita
審査委員	主査	西尾 善彦	学位	博士（医学・歯学・学術）
	副査	嶽崎 俊郎	副査	古川 龍彦
	副査	橋口 照人	副査	浅川 明弘

主査および副査の5名は、平成29年8月7日、学位申請者 Pulong Wijang Pralampita 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. Why did the control mice with H-diet show similar insulin level in the control mice with L-diet, in Fig. 2?

A1. Because the insulin response may be suppressed by a long fasting time (18 h), and/or the low sensitivity in measurement of the insulin.

Q2. What are the histological changes of kidney in the *db* mice?

A2. The renal histological changes in the *db* mice are the thickness of the glomerular base membrane (Zhang H., et al. 2017. J Diab Res) and the mesangial matrix expansion (Sharma K., et al. 2003. Am J Physiol). To our previous study *db* mice showed higher values in extraglomerular, intraglomerular, and PAS-positive glomerular areas (Arimura E., et al. 2013. Eur J Nutr).

Q3. Do you think the high protein diet affected β-cell?

A3. Yes, we do. Based on the previous study (Arimura E., 2013), the high protein diet decreased the β-cell distribution rate.

Q4. Which organ is affected by the high protein diet in the present study, pancreas or kidney?

A4. Both organs are affected by the high protein diet.

Q5. Why did *db* mice with L-diet show a lower insulin level at 150 min?

A5. The reason and mechanism are not so clear.

Q6. Was the composition of major nutrients in H- or L-diets higher and lower, compare with standard diet in mice?

A6. One of the standard diet for mice is CE2 (CLEA, Japan). The CE2 shows the ratio of carbohydrate, protein, and fat as 58%, 29%, and 13% (energy distribution). Protein composition was lower in both H- or L-diets, compared with CE2 standard diets.

Q7. Why didn't you use the standard diet for mice as a control diet for the experiments?

A7. We did not include the standard diet for mice in this study design. Because the standard diet in mice contains different composition from human diet. We focused on the evaluation of diet based on human diet.

Q8. The high protein diet affected for the advancement of the diabetic condition. Does the high protein diet affect the development of diabetes, too?

A8. Yes, it does. Based on our results, control mice with H-diet showed higher urinary C-peptide than L-diet. This increment of insulin secretion after high protein intake suggested a potential risk for the development of diabetes, too.

Q9. There are many kinds of risk factors for diabetes in human studies. How do you think that the increase of protein intake is a risk factor?

A9. Increased protein intake will increase the risk of diabetes. This thought emerges based on our experiments results and several study data. Based on the recent meta-analysis and cohort study, the higher intakes of total and animal protein were both associated with increased risk of diabetes (Shang X., et al. 2016. Am J Clin Nutr).

Q10. How is the total intake of proline different between Asia and western countries, when the total intake amount and frequency was taken into consideration?

A10. The total intake of proline in the Western countries is higher than in Asia. Western countries consumed 3.6 x proline than Japanese, this estimation is based on the diet sample that we made between Western (bread, beef, and cheese) and Japanese

diet (rice, fish: red sea beam, and momen tofu).

Q11. Can you apply the result that increased HbA1c with high protein diet to human cases, as shown in introduction?

A11. Yes, we can.

Q12. Why was the urinary albumin at the 1st week of the experiment higher than that at the 2nd week of the experiment, in Fig. 1?

A12. Maybe, because mice at the 1st week of the experiment (4 weeks of age) were in the developed phase of the renal dysfunction such as reabsorption of albumin.

Q13. Why did *db* mice with L-diet show higher glucose level at 30 min than *db* mice with H-diet?

A13. *Db* mice with L-diet had higher intake of carbohydrate (maltose), so it showed a higher glucose level at 30 min after the mixed nutrient.

Q14. Why did you take the different time points for glucose and insulin measurement?

A14. We performed the measurements with different mice groups. Because more plasma was needed to measure insulin, therefore, the sampling times were fewer and different from glucose measurement.

Q15. Did you examine the pathological examinations of the pancreas?

A15. No, we did not examine it in the present study.

Q16. Why did you measure blood leptin level of mice with leptin receptor deficiency?

A16. We measured the blood leptin level to know the effect of high protein diet on leptin level of control mice.

Q17. Why was there no significant difference of leptin level between control and *db* mice in high protein diet condition?

A17. There was no significant difference of leptin level between control and *db* mice in high protein diet condition because there was a high variance of leptin level in the *db* mice.

Q18. You used 5 weeks-old mice and this condition was the same with the young age of human. The fasting blood glucose of even *db* mice is around 100 mg/dl, meaning a prediabetes condition. Will the glucose level show a different result when you conduct this experiment in the older mice?

A18. Yes, they showed higher level in fasting condition in the previous study. The older *db* mice have the complications such as renal damage, therefore, we used only young *db* mice as prediabetic condition in the present study.

Q19. How did you perform the oral administration experiment, especially on fasting period?

A19. We performed 18 hours fasting time (14:00 – 08:00), which was confirmed to be the depletion of glycogen in even *db* mice. The administration test needs for deleting the contamination of the release of glucose from the glycogen.

Q20. How do you think that the fasting period may affect the insulin levels?

A20. The longer fasting period may make insulin level lower and insulin response lower.

Q21. How did the high protein diet affect kidney weight and albumin excretion?

A21. The mechanism is still unclear, to our knowledge. The possibility that high protein diet induced kidney hypertrophy and albumin hyperexcretion due to the increased glomerular pressure and renal hyperfiltration. In the previous our experiments (Arimura E., 2013), the high protein diet increased expression of angiotensinogen and renin mRNAs. The increased renin-angiotensin system may be related to kidney hypertrophy and albumin hyperexcretion.

Q22. Did *db* mice with H-diet show hyperfiltration?

A22. No, they did not. The data of creatinine clearance of *db* mice with H-diet and L-diet showed similar values.

Q23. Did you measure the blood pressure of the mice?

A23. No, we did not.

Q24. How did you determine the amount of supply in the case of the oral administration experiment?

A24. We determined the amount of supply in the case of oral administration experiments based on the experiment 1 using metabolic cage.

Q25. The amount in energy of the mixtures was too small. Why did you use the small amount?

A25. This is related to the small capacity of the stomach of the mice, as compared to human cases.

Q26. Do you think that insulin will be useful for the treatment of diabetic nephropathy from your study?

A26. I think so.

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。