EFFECTS OF DIETARY ALPHA LINOLENIC ACID ON BIOSYNTHESIS OF N-3 LONG CHAIN POLYUNSATURATED FATTY ACIDS IN ANIMALS

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M. Sci.

A thesis submitted for the Degree of Doctor of Philosophy

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June 2011

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Abstract

Omega-3 (n-3) long chain polyunsaturated fatty acids (LCPUFA), particularly eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), are important for normal health as well as growth and brain development in humans. These fatty acids can be consumed in the diet directly, or synthesised from short chain PUFA consumed in the diet. Fish, particularly in species with a high fat content like salmon, are a major source of these beneficial fatty acids in the human diet.

Fish production from aquaculture continues to expand due to a growing human population and demand for fish. Currently there is a reliance on fish oil and fish meal derived exclusively from wild fish as the primary lipid and protein source in fish feeds. Depleted wild fish stocks have made this source of n-3 LCPUFA unsustainable and alternative sources of n-3 LCPUFA are required to fill the void.

Most animal species can convert the plant derived 18 carbon (C18) n-6 linoleic acid (LA, 18:2 n-6) and n-3 α-linolenic acid (ALA, 18:3 n-3) to 20 and 22 carbon (C20-C22) LCPUFA by using a series of enzymes to extend and alter the saturation level. There are two types of enzymes responsible for desaturating and elongating fatty acids are desaturases and elongases. The genes associated with these processes appear to be regulated by the extremes of dietary PUFA intake but the extent is currently unclear.

This thesis is aimed to examine the effect of dietary PUFA on tissue n-3 LCPUFA levels in animals (rat and fish) after the consumption of diets with increasing levels of ALA, and to investigate whether the expression of desaturases and elongases is involved in the regulation of lipid metabolism and therefore LCPUFA biosynthesis. Furthermore, this thesis also investigated the potential enzyme functions of barramundi Δ6 desaturase and elongase using a yeast heterologous system.

Experiments showed that while high ALA diets consistently produced higher levels of n-3 LCPUFA in rat tissues than low ALA diets, mRNA abundance of the Δ6 desaturase (FADS2) and elongase 2 (ELOVL2) genes were increased only in animals fed the low PUFA reference diet compared to those fed diets with adequate to high PUFA levels. There was no correlation between the gene expression of desaturases, elongases or transcription factors and the levels

of EPA, docosapentaenoic acid (DPA, 22:5 n-3) or DHA in rat blood, liver and other tissues as a result of feeding increasing levels of ALA.

In barramundi however, while vegetable oils induced significant increases in mRNA abundance of FADS2 and ELOVL genes compared with those fed the fish oil-based commercial diet, the tissue EPA, DPA and DHA levels were not increased. It is therefore hypothesised that the enzyme activity of barramundi Δ6 desaturase was low and therefore limited the effectiveness of the enzymes in the LCPUFA pathway to produce EPA and DHA. Furthermore, a large amount of variation between individual fish in DHA levels among those fed the vegetable oil-based diets was found, and this may provide a possibility for a future breeding program of barramundi for better DHA production.

Barramundi FADS2 and ELOVL genes were also cloned into yeast cells and performed functional expression of the two enzymes. Results revealed that the barramundi Δ6 desaturase also showed Δ8 desaturase activity and the elongase showed a broad range of fatty acid specificity with the greatest activity with EPA. In addition, a significant amount of the desaturation and elongation fatty acid products could be detected in the culture medium at various time points after the addition of fatty acid substrates, and that it was important to take the levels of fatty acids in the medium into account when it came to calculating enzyme activity.

Declaration

This is to certify that the data contained in this thesis is my own work and the thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Wei-Chun Tu and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Publications:

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***Wei-Chun Tu**, Rebecca Cook-Johnson, Michael James, Beverly Mühlhäusler and Robert Gibson*

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***Wei-Chun Tu**, Rebecca Cook-Johnson, Michael James and Robert Gibson*

Wei-Chun Tu

Acknowledgements

After all these years, I've got quite a list of people who contributed in some way to this thesis, for which I would like to express thanks.

I am deeply indebted to my supervisor Prof. Robert Gibson whose expertise, understanding, and patience, added considerably to my graduate experience. His stimulating suggestions and encouragement helped me in all the time of research for and writing of this thesis in which he became more of a mentor and friend, than a professor. He was always there when I needed it. Words cannot describe how much grateful I am with the effort, time and knowledge you have given me... thank you so much Bob.

I would also like to acknowledge my co-supervisors, Prof. Michael James and Dr. Rebecca Cook-Johnson from the Rheumatology Unit, Royal Adelaide Hospital. They provided me with a great environment for molecular works. Your supports throughout my candidature has been wonderful. I would like to thank co-supervisor Dr. David Stone from South Australian Research and Development Institute for the assistance and guidance he provided for the barramundi study. You are the best fish expert David.

I must also express my gratitude to Dr. Beverly Mühlhaüsler for taking incredible amount of time out from her busy schedule to offer terrific help with advice in data interpretation, manuscript preparation and thesis writing at times of critical need. Bev, you are indeed very special, amazing and really awesome. Thank you for everything.

I would like to also thank the people that helped me with the technical and philosophical aspects of my study. David Apps, for providing me suggestions for fatty acid data processing, teaching me Australian slang, sharing an office with me during the final stages of my PhD and being a friend. Ela Zielinski, as a stand-in mother figure, for helping out with lipid extraction and maintaining our lab in a tidy and clean status all the time. Zhi Yi Ong, for keeping me somewhat sane and providing me with company. Zhi Yi, my special one, admit that you are the best and I truly enjoyed the time with you. Thank you for your faith in me. It is about time to start on that list of things to do. Dr Melissa Gregory, for providing me with so much help and heart-soothing chats during my late-stage of study. Mel, thank you.

And then there are all the other group members who have made FOODplus a very special place over all those years: Dr. Jo Zhou, Dr. John Carragher, Anna Seamark, Kirsten Katnich, and Pamela Sim, Lilik kartikasari, Ge Liu and many other students for their support and assistance they provided at all levels during my student life in Waite Campus.

A huge thank-you must go to my family and friends. Thanks to my parents for creating an environment in which following this path seemed so natural and my other family members for the support they gave me through my entire life and in particular, my husband, a life-long partner and also a best friend, without whose love and encouragement, I would not have finished this thesis. Thanks to all of my friends, your loyal friendship I cherish so deeply. I cannot wait to see you guys again.