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DO LONG CHAIN OMEGA-3 POLYUNSATURATED FATTY ACIDS MODULATE DIETARY FAT INDUCED CHANGES IN PLASMA LIPID AND LIPOPROTEIN PROFILES?

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Thesis submitted to the Faculty of Health and Medicine at the University of Newcastle in fulfilment of the requirement to obtain the degree of Doctor of Philosophy in Pharmacy.

February 2016

Statement of originality

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution 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. I give my consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provision of the Copyright Act 1968.

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I hereby certify that this thesis is in the form of a series of published papers of which I am a joint author. I have included as part of my thesis a written statement from each co-author, endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

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Cintia Botelho Dias

ACKNOWLEDGEMENTS

Many should be acknowledged for the completion of this thesis, as I would never have accomplished this without God's guidance and the support of my wonderful supervisors, family, and friends.

I would like to thank my husband for supporting me during the development of this project, for understanding when I was too tired to talk about anything and for giving me the strength I needed to continue in moments of stress and doubt. Thanks for reminding me that we also need to celebrate the accomplishment of each milestone along the way. I also would like to acknowledge my parents and the rest of my family for their unconditional love and support, despite the distance.

I would never have completed this work without the support and advice of my supervisors, Professor Manohar Garg and Associate professor Lisa Wood, throughout my journey. Thank you, Manohar, for been always available to discuss my progress, for providing me with valuable advice, for providing me with the means to achieve my professional goals and for encouraging me to publish. Thank you, Lisa, for your always valuable advice, guidance and support. I also would like to thank my fellow postgraduate students Irene, Jency, Melinda, Amani, Brendan, Rohit, Jessica, Kylie, Fatima and our research assistant Melissa Fry for their support. I cannot forget our collaborators at the Biosfer Teslab team, especially Núria Amigó and Miguel Angelo Pardo.

I am grateful for the financial support from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Priority Research Centre in Physical Activity and Nutrition, the Hunter Medical Research Institute and the Faculty of Health and Medicine. I am also grateful to EPAX Norway AS for providing the fish oil concentrate capsules used in the interventions.

And finally I would like to thank all the volunteers who took some time to be part of my clinical interventions and made this title possible. My sincere thank you also to all those who are not mentioned here, but somehow contributed to my work and consequently to my success.

LIST OF RESEARCH PUBLICATIONS INCLUDED IN THE THESIS

1. **Chapter 3**

Dias CB, Garg R, Wood LG, Garg ML. Saturated fat consumption may not be the main cause of increased blood lipid levels. Medical Hypotheses. 2014; 82:187-95. DOI: 10.1016/j.mehy.2013.11.036

2. **Chapter 4a**

Dias CB, Wood LG, Phang M, Garg ML. Postprandial lipid responses do not differ following consumption of butter or vegetable oil when Consumed with omega-3 polyunsaturated fatty acids. Lipids. 2015; 50(4):339-47. DOI: 10.1007/s11745-015-4003-2

3. **Chapter 4b**

Dias CB, Wood LG, Phang M, Garg ML. Kinetics of omega-3 polyunsaturated fatty acids when co-administered with saturated or omega-6 fats. Metabolism - Clinical and Experimental. 2015; 64(12):1658-66. DOI: 10.1016/j.metabol.2015.08.012

4. **Chapter 5**

Dias CB, Wood LG, Garg ML. Effects of dietary saturated and n-6 polyunsaturated fatty acids on the incorporation of long chain n-3 polyunsaturated fatty acids into blood lipids. European Journal of Clinical Nutrition. 2016; in press (00):1-7. DOI: 10.1038/ejcn.2015.213

5. **Chapter 6**

Dias CB, Amigo N, Wood LG, Mallol R, Correig X, Garg ML. LDL particle size is increased by omega-3 polyunsaturated fatty acids irrespective of the major dietary fat. 2016; submitted to Journal of Lipid Research

6. **Chapter 7**

Dias CB, Amigo N, Wood LG, Mallol R, Correig X, Garg ML. Prior supplementation with long chain omega-3 polyunsaturated fatty acids does not prevent dietary fat

induced changes in lipid and lipoprotein profiles. 2016; submitted to Journal of Nutritional Biochemistry

OTHER PUBLICATIONS RELEVANT TO THE THESIS

Conference abstracts: published in peer-reviewed journals

Dias CB, Wood LG, Garg ML Saturated fat enhances incorporation of n-3 polyunsaturated fatty acids into plasma and erythrocyte lipids in healthy humans. Journal of Nutrition & Intermediary Metabolism. 2015

Dias CB, Wood LG, Garg ML Changes in blood lipid levels induced by different dietary fat types are not influenced by pre-supplementation with fish oil. Journal of Nutrition & Intermediary Metabolism. 2015

Dias CB, Wood LG, Garg ML, M.L. Differential effects of saturated and n-6 PUFA on blood lipids when co-administered with n-3 PUFA. Journal of Nutrition & Intermediary Metabolism. 2014, 1:10. DOI: http://dx.doi.org/10.1016/j.jnim.2014.10.025

Dias CB, Wood LG, Phang M, Garg ML. Postprandial lipemia following consumption of saturated fat and omega-6PUFA rich diets: interactions with omega-3PUFA. Australasian Medical Journal. 2013, 6(11):601.

Dias CB; Wood LG; Phang M, Garg, ML. Omega-3 polyunsaturated fatty acids alleviate dietary saturated fat-induced postprandial rise in blood lipid levels. In: Murphy, K.; Howe, P. Proceedings of the 2013 Meeting of the Australasian Section of the American Oil Chemists Society (AAOCS). Nutrients. 2012, 5:5065-5096. DOI: 10.3390/nu5125065

Conference abstracts: published in conference proceedings

Dias CB, Wood LG, Garg ML. Dietary Saturated Fat Promotes Omega-3 Polyunsaturated Fatty Acid Incorporation into Human Plasma and Erythrocytes. Proceedings of the 107th AOCS Annual Meeting & Expo, 2016.

Dias CB, Wood LG, Garg ML. Fish oil supplementation modulates lipoprotein profile irrespective of the dietary fat type. Proceedings of the 3rd International Conference on Food Structures, Digestion and Health, 2015.

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ABREVIATIONS

AA	Arachidonic acid
ALA	α-linolenic acids
AMP	Adenosine monophosphate
ApoA	Apolipoprotein A
ApoA-II	Apolipoprotein A-I
ApoA-II	Apolipoprotein A-II
ApoB	Apolipoprotein B
ApoB-48	Apolipoprotein B-48
ApoB-100	Apolipoprotein B-100
ApoC-II	Apolipoprotein C-II
ApoC-III	Apolipoprotein C-III
ApoE	Apolipoprotein E
ATP	Adenosine triphosphate
AUC	Area under the curve
CETP	Cholesteryl ester transfer protein
CHD	Coronary heart disease
CLA	Conjugated linoleic acid
COX	Cyclooxygenase
CoA	Coenzyme A
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
DSTE	Diffusion-ordered 1H-NMR spectroscopy
EPA	Eicosapentaenoic acid
FAD	Flavin adenine dinucleotide
$FADH_2$	Hydrogen flavin adenine dinucleotide

FAME	Fatty acid methyl ester
HAPS	Hunter area pathology service
GM-CSF	Granular-macrophage colony-stimulating factor
HDL	High density lipoprotein
HDL_2	Large high density lipoprotein
HDL_3	Small high density lipoprotein
HDL-C	High density lipoprotein cholesterol
HDL-P	High density lipoprotein particles
HDL-TG	High density lipoprotein triglycerides
HDL-Z	High density lipoprotein size (diameter)
HF	Heart failure
HMRI	Hunter medical research institute
HNF-4α	Hepatocyte nuclear factor-4α
hs CRP	High sensitivity C-reactive protein
iAUC	Incremental area under the curve
IκB	Inhibitor κB
ICAM-1	Intracellular adhesion molecule-1
IDL-C	Intermediary density lipoprotein cholesterol
IDL-TG	Intermediary density lipoprotein triglycerides
IL-6	Interleukin-6
IL-β1	Interleukin- β1
IQR	Interquartile range
LA	Linoleic acid
LCAT	Lecithin-cholesterol acyltransferase
LC-n-3PUFA	Long chain omega-3 polyunsaturated fatty acids
LDL	Low density lipoprotein

LDL-C	Low density lipoprotein cholesterol
LDL-P	Low density lipoprotein particle
LDLr	Low density lipoprotein receptor
LDL-P/oxLDL	ratio LDL particle concentration to oxidised LDL
LDL-TG	Low density lipoprotein triglycerides
LDL-Z	Low density lipoprotein size (diameter)
LOX	Lipoxygenase
MUFA	Monounsaturated fatty acids
NEFA	Non-esterified fatty acids
NAD^+	Niacin adenine dinucleotide
NADH	Hydrogen niacin adenine dinucleotide
NFκB	Nuclear factor κB
NMR	Nuclear magnetic resonance
n-6 PUFA	Omega-6 polyunsaturated fatty acids
n-3 PUFA	Omega-3 polyunsaturated fatty acids
PAD	Peripheral artery disease
PCSK9	Proprotein convertase subtilisin/kexin 9
PGE_2	Prostaglandin E series 2
PPAR	Peroxisome proliferator-activated receptor
PPi	Pyrophosphate
PUFA	Polyunsaturated fatty acids
RAE	Research awareness exercise
RBC	Red blood cell
SFA	Saturated fatty acids
SREBP-1c	Sterol regulatory element-binding protein 1c
TAG	Triacylglycerol

TNFα	Tumour necrosis factor – alpha
TXB_2	Thromboxane B series 2
VCAM-1	Vascular cellular adhesion molecule-1
VLDL	Very low density lipoprotein
VLDL-C	Very low density lipoprotein cholesterol
VLDL-P	Very low density lipoprotein particle
VLDL-TG	Very low density lipoprotein triglycerides
VLDL-Z	Very low density lipoprotein size (diameter)

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SYNOPSIS

The consumption of foods rich in saturated fats has been associated with elevated blood lipid levels and consequently with risk for numerous chronic diseases, such as coronary heart disease. However, understanding the effects of replacing saturated fat in the diet is complex, and the health effects of reducing saturated fat consumption clearly depend on what substitutions are made. Furthermore, studies using animal models have demonstrated that dietary saturated fats raise triglyceride levels only when the diet is deficient in omega-3 polyunsaturated fatty acids (n-3PUFA). The n-3PUFA are known for their potential to help in managing hyperlipidaemia for the prevention of coronary heart disease, as well as for their anti-inflammatory, anti-arrhythmic and anti-aggregatory potential. In addition, research in human and animal models has shown competition for important enzymes in the metabolism of omega-6 polyunsaturated fatty acids (n-6PUFA) and n-3PUFA, with high consumption of n-6PUFA leading to an increase in their metabolism at the expense of n-3PUFA. This leads to an increased production of n-6PUFA derived eicosanoids, which are pro-inflammatory and pro-aggregatory, in contrast with those derived from n-3PUFA, which are less inflammatory and aggregatory. Therefore, we hypothesised that consumption of saturated fats, would not adversely influence coronary heart disease risk factors (blood lipid levels, lipoprotein profiles, platelet aggregation and inflammation) when the diet was balanced with an adequate intake of n-3PUFA. Moreover, we hypothesized that the health benefits obtained with the consumption of n-3PUFA would be maximised by including foods rich in saturated fat and reducing the consumption of vegetable oils (rich in n-6PUFA) in the diet.

Our first aim, addressed in chapter 3, was to establish the basis for our hypothesis, analysing the literature on saturated fatty acids to identify the contradictions in the literature to date and to highlight the gaps in knowledge gained from previous interventional and epidemiological studies. We have observed that although many studies have associated saturated fatty acids with hyperlipidaemia and cardiovascular disease risk factors, there is still much contradiction on the subject with not all studies finding the same association. The key studies relating saturated fat consumption and heart health made no mention about the presence or absence of n-3PUFA as a possible confounding factor. This may have been due to the lack of knowledge about the

existence of n-3PUFA or an inability to determine n-3PUFA concentration in most of the early studies. Therefore the missing link in the research on cardiovascular disease risk and dietary fats could be an ignorance of the interactions between different dietary fats and the effect of this interaction.

In Chapters 4a and 4b we aimed to determine if LCn-3PUFA and the other dietary fats interact during digestion, absorption, re-esterification into triglycerides and assembly into chylomicrons to modulate circulating lipid levels postprandially. In a randomised cross-over design, we investigated the effect of feeding meals rich in either saturated fatty acids or n-6PUFA in conjunction with LCn-3PUFA on plasma lipid (triglycerides and total, low density lipoprotein and high density lipoprotein cholesterol) and fatty acid levels. The postprandial lipemic response and fatty acid kinetics were similar after the consumption of both meals and suggest that the competition between n-3 and n-6PUFA may be a longer term phenomenon, not just a postprandial effect.

The aim of Chapter 5 was then to determine if there were interactions between LCn-3PUFA and other dietary fats in the longer term (6 weeks). Therefore, in a randomized parallel design intervention we investigated the longer-term effects of LCn-3PUFA supplementation in subjects consuming diets enriched in either saturated fatty acids or n-6PUFA, on blood lipid profiles and on the incorporation of fatty acids into plasma and erythrocyte lipids. Long chain omega-3 polyunsaturated fatty acids were incorporated to a greater extent into the plasma and erythrocyte lipids of subjects consuming the saturated fat rich diet compared to the n-6PUFA rich diet, although total and low density lipoprotein (LDL) cholesterol were also increased.

Plasma samples of the subjects who completed the intervention in chapter 5 were then further analysed in chapter 6 for lipoprotein profiles, with the aim of determining if the increase in plasma cholesterol levels was due to changes in the lipoprotein particle concentration or size. The increase in LDL cholesterol was due to an increase in the less atherogenic, large, buoyant LDL particles rather than the small, dense LDL particles.

In chapter 7, the aim was to determine if pre-supplementation rather than co-supplementation with LCn-3PUFA would improve the effect of the major dietary fat groups on plasma lipids and lipoprotein profiles. Therefore, in a randomized parallel design clinical intervention, we examined the effect of increasing the omega-3 index of subjects before randomizing them to a diet rich in either saturated fatty acids or n-

6PUFA. The diet rich in saturated fatty acids increased, while the diet rich in n-6PUFA decreased, total and LDL cholesterol, independently of LCn-3PUFA supplementation. However, the saturated fatty acid rich diet caused a further increase in plasma and erythrocyte LCn-3PUFA compared to the n-6PUFA rich diet.

In conclusion, the results presented in this thesis demonstrate that the background dietary fat is a determinant of the degree of incorporation of LCn-3PUFA into plasma and tissue lipids. The consumption of a saturated fat rich diet did indeed cause an increase in plasma cholesterol levels. However, the rise in circulating cholesterol levels following saturated fat consumption is accompanied by an increase in the less atherogenic LDL particle size, when the LCn-3PUFA status is adequate, which is likely to reduce the detrimental effects. In addition, there was a concurrent increase in incorporation of LCn-3PUFA into plasma and erythrocytes, which may have benefits, independent of cholesterol or blood lipids. Hence, this thesis paves the way for further research to examine the impact of increased plasma and tissue LCn-3PUFA levels as a result of saturated fat consumption with adequate LCn-3PUFA intakes, on cardiovascular health risk indicators, such as inflammation, hypertension, platelet aggregation and endothelial function.

THESIS LAYOUT

This thesis by publication is organised in 9 chapters: a general introduction and literature review section, a methods section, 4 papers published in scientific journals, 2 papers submitted for publication and a general discussion section. Chapters are organized as follows:

- **Chapter 1:** describes a literature review on fatty acids, including their biological functions, metabolism, dietary requirements and health effects. Excerpts of this chapter were published in the following paper:

  Dias CB, Garg R, Wood LG, Garg ML. Saturated fat consumption may not be the main cause of increased blood lipid levels. Medical Hypotheses. 2014; 82:187-95.

- **Chapter 2:** describes detailed study designs, methods and statistical analysis employed to undertake the three clinical interventions discussed in chapters 4a to 7.

- **Chapter 3:** describes a literature review on the effects of saturated fatty acids on plasma lipid profiles and health. In this chapter the contradictions among publications on saturated fatty acids and cardiovascular health are discussed and a hypothesis is formulated to explain such contradictions.

- **Chapter 4a:** describes the results and discussion on the effects of the first clinical intervention, the "Acute fat challenge study" on blood lipid levels.

- **Chapter 4b:** describes the results and discussion on the effects of the first clinical intervention, the "Acute fat challenge study" on postprandial fatty acid kinetics.

- **Chapter 5:** describes the results and discussion on the effects of the second clinical intervention, the "Chronic fat manipulation study" on plasma lipid levels and on plasma and erythrocytes fatty acids.

- **Chapter 6:** describes the results and discussion on the effects of the second clinical intervention, the "Chronic fat manipulation study" on plasma lipopoprotein profiles.

- **Chapter 7:** describes the results and discussion on the effects of the third clinical intervention, the "Pre-supplementation study" on plasma lipid levels, plasma lipoprotein profile and on plasma and erythrocytes fatty acids.

- **Chapter 8:** focuses on general discussion and conclusion, future directions and limitations.