UNIVERSITY OF NEWCASTLE
FACULTY OF HEALTH
SCHOOL OF BIOMEDICAL SCIENCE AND PHARMACY

DIFFERENTIAL EFFECTS OF SATURATED FATTY ACIDS OF VARYING CHAIN LENGTH ON LIPID PROFILES IN HEALTHY INDIVIDUALS.

Nisha Panth (MSc)
MSC candidate

Professor Manohar Garg
Supervisor

Dr. Katie Wynne
Co-supervisor

Dr. Cintia Dias
Co-supervisor

Thesis submitted to the Faculty of Health and Medicine at the University of Newcastle in fulfilment of the requirement to obtain the degree of Master of Philosophy in Nutritional Biochemistry.

August 2018

Statement of originality

I hereby certify that the work embodies in the thesis is my own work, conducted under normal supervision.

The thesis contains published scholarly work of which I am a co-author. For each such work a written statement, endorsed by the other authors, attesting to my contribution to the joint work has been included.

The thesis contains no material which has been accepted, or is being examined, for the 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.

Statement of authorship

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.

Nisha

_____

Nisha Panth

ACKNOWLEDGEMENTS

It is a great pleasure to acknowledge those people who have assisted me to search, to see and to reach the light at the end of the tunnel. Specifically, I want to thank following people who have assisted me in one way or another along my master's journey over the two years:

I have completed this work with the advice of my supervisors, Professor Manohar Garg, Dr. Katie Wynne and Dr. Cintia Dias, throughout my journey. First, I am indebted to my supervisor Prof. Manohar Garg, whose guidance, feedback and constant availability inspired me to finish the studies. I am thankful to my co-supervisot Dr. Katie Wynne for giving me insightful comments in my academic writing and occasionally challenging me beyond my limits, which I believe strengthened the end product. Katie, you are a remarkable role model that has inspired me with your knowledge. Thank you Dr. Cintia Dias for lending your expertise throughout my Journey.

Cintia, Kylie, Jess, Mel, Alex, Rohith; it was a good journey with fun and laughter (so many funny stories, word etc), coffee and cake. Kylie, I really appreciate your guidance on research methodology for systematic review. Thanks, Sikha for helping me making test biscuits for my trial and friendship that I can cherish my whole life.

My research is supported by a University of Newcastle International Postgraduate Research Scholarship; therefore, I am thankful for the financial support from the University of Newcastle, Australia.

To my husband, Keshav, thank you for understanding and giving me the strength in moments of stress and doubt and for your unconditional love. Keshav, I am forever appreciative of our happy family members for supporting us in many ways at different stages of our journey. Also, thanks to my precious child, PRISHA, for coming into our lives for good reasons.

And finally, I would like to thank all the volunteers who participated in my study and it was truly a pleasure to meet volunteers. I would also like to acknowledge several people who are not mentioned here, but somehow helped in my journey and consequently to my success.

…..we know how to wait…. ….

…..let the stars keep on turning….. ….

…..from little things….big things grow…..

Paul Kelly

LIST OF RESEARCH PUBLICATIONS INCLUDED IN THE THESIS

1. **Chapter 3**

Panth N, Abbott KAA, Dias C, Wynne Katie, Garg ML. Differential effects of medium and long-chain saturated fatty acids on blood lipid profile: a systematic review and meta-analysis; The American Journal of Clinical Nutrition. 2018; 108(4): 675-687. DOI: 10.1093/ajcn/nqy167

2. **Chapter 4**

Panth N, Dias C, Singh H, Wynne Katie, Garg ML. Medium-chain fatty acids lower postprandial lipemia: a randomized crossover trial. 2018; submitted to Clinical Nutrition.

- TABLE OF CONTENTS -

ABREVIATIONS xi

LIST OF FIGURES xv

LIST OF TABLES xvii

SINOPSIS xviii

THESIS LAYOUT xx

CHAPTER 1: GENERAL LITERATURE REVIEW

1.1. Introduction 2

1.2. Fatty acids 2

1.2.1. Saturated fatty acids 3

1.2.2. Monounsaturated fatty acids 4

1.2.3. Polyunsaturated fatty acids 4

1.2.4. Trans fatty acids 6

1.3. Endogenous and dietary fatty acids 7

1.4. Lipoprotein metabolism 9

1.4.1. Structure and functions 9

1.4.2. The exogenous pathway 10

1.4.3. The endogenous pathway 15

1.4.4. HDL metabolism and reverse cholesterol transport 18

1.5. Postprandial lipid metabolism 20

1.6. Dietary fats and blood lipids 21

1.6.1. Unsaturated fats 22

1.6.2. Saturated fats 23

1.7. Implication of MCFAS for health 25

1.7.1. Fasting plasma lipids 25

1.7.2. Postprandial plasma lipids 26

1.8. Research rationale, objectives and significance 27

1.8.1. Rationale 27

1.8.2. Objectives 28

1.8.3. Significance 28

CHAPTER 2: METHODS 30

2.1. Study 1: Systematic review and meta-analysis 31

2.1.1. Search strategy, selection criteria and selection procedures 31

2.1.2. Critical appraisal and data extraction and reporting 32

2.2. Intervention 33

2.2.1. Acute fat challenge study 33

2.2.1.1. Research study design 33

2.2.1.2. Participants 33

2.2.1.3. Inclusion/Exclusion criteria 33

2.2.1.4. Ethics and trial registrations 33

2.2.1.5. Anthropometric measurements 34

2.2.1.6. Medical Questionnaire 35

2.2.1.7. Physical activity assessment 35

2.2.1.8. Blood pressure 35

2.2.1.9. Dietary recall (24-hour) 36

2.2.1.10. Sample size 36

CHAPTER 3: Differential effect of medium and long-chain saturated fatty acids on blood lipid profile: a systematic review and meta-analysis 37

Statement of authorship/ Appendix 1 120

Abstract 38

3.1. Introduction 39

3.2. Methods 40

3.2.1. Search strategy 40

3.2.2. Eligibility criteria 41

3.2.3. Selection process and quality assessment 41

3.2.4. Data extraction 41

3.2.5. Statistical analysis 42

3.3. Results 43

3. 3.1. Study and participant characteristics 43

3.3.2. Overall effects of ther MCFAS diet compared with those of the LCSFAS diet on lipid profile and study heterogeneity 46

3.3.3. Subgroup analysis 53

3.3.4. Assessment of risk of bias 60

3.3.5. Publication bias 60

3.4. Discussion 68

CHAPTER 4: Medium chain fatty acids lower postprandial lipemia: a randomized crossover trial 72

Statement of authorship/ Appendix 2 122

Abstract 72

4.1. Introduction 73

4.2. Materials and methods 75

4.2.1. Study participants 75

4.2.2. Study design and experimental protocol 75

4.2.3. Measure 76

4.2.4. Statistical analysis 77

4.3. Results 77

4.4. Discussion 86

CHAPTER 5: GENERAL DISCUSSION 91

5.1. Key finding 92

5.2. Strengths and limitations of the research 93

5.3. Impact to nutrition research 94

5.4. Conclusion 94

REFERENCES 95

APPENDICES 120

Appendix 1 121

Appendix 2 123

Appendix 3 125

Appendix 4 126

Appendix 5 133

Appendix 6 134

Appendix 7 138

Appendix 8 140

Appendix 9 141

Appendix 10 145

Appendix 11 146

ABBREVIATIONS

CVD	Cardiovascular disease
SFA	Saturated fatty acid
SCFA	Short-chain saturated fatty acid
MCFAS	Medium-chain saturated fatty acid
LCFA	Long-chain saturated fatty acid
apo A-I	Apolipoprotein A-I
TG	Triglyceride
TC	Total cholesterol
LA	Linoleic acid
AA	Arachidonic acid
PUFA	Polyunsaturated fatty acids
n-3PUFA	Omega-3 polyunsaturated fatty acids
n-6PUFA	Omega-6 polyunsaturated fatty acids
sdLDL	Small dense low-density lipoprotein
CLA	Conjugated linoleic acid
EPA	Eicosapentaenoic acid
DPA	Docosapentaenoic acids
DHA	Docosahexaenoic acid
TFA	Trans-fatty acids
NEFA	Non-esterified fatty acids
ACC	Acetyl-CoA carboxylase
FASN	Fatty-acid synthase
CoA	Coenzyme A
VLDL	Very low-density lipoprotein
IDL	Intermediate-density lipoprotein

MUFA	Monounsaturated fatty acids
HMG-CoA	3-hydroxy-3-methyl-glutaryl-CoA
apoC-III	apolipoprotein C-III
apo B	Apolipoprotein B
apo A-IV	apolipoprotein A-IV
apoE	apolipoprotein E
apo B-100	Apolipoprotein B-100
apo B-48	Apolipoprotein B-48
FFA	Free fatty acid
LPL	Liporotein lipase
ABCA-1	ATP-binding cassette sub-family A member 1
CETP	Cholesteryl ester transfer protein
LCAT	Lecithin-cholesterol acyltransferase
SR-B1	Scavenger receptor class B type 1
TRL	Triglyceride rich lipoprotein
iAUC	Incremental area under the curve
AUC	Area under the curve
MCT	Medium chain triglyceride
PPARs	Peroxisome proliferator activated receptors
LXRs	Liver X receptors
HNF 4	Hepatic nuclear factor 4
SREBPs	Sterol regulatory element binding proteins
HDL	High density lipoprotein
HDL-C	High density lipoprotein cholesterol
HDL-P	High density lipoprotein particles
HDL-TG	High density lipoprotein triglycerides

iAUC	Incremental area under the curve
IDL-C	Intermediary density lipoprotein cholesterol
IDL-TG	Intermediary density lipoprotein triglycerides
LCAT	Lecithin-cholesterol acyltransferase
LDL	Low density lipoprotein
LDL-C	Low density lipoprotein cholesterol
LDL-P	Low density lipoprotein particle
LDLr	Low density lipoprotein receptor
LDL-TG	Low density lipoprotein triglycerides
MUFA	Monounsaturated fatty acids
NEFA	Non-esterified fatty acids
n-6 PUFA	Omega-6 polyunsaturated fatty acids
n-3 PUFA	Omega-3 polyunsaturated fatty acids
PPAR	Peroxisome proliferator-activated receptor
SREBP-1c	Sterol regulatory element-binding protein 1c
TAG	Triacylglycerol
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
CHD	Coronary heart disease
HDL: LDL-C	HDL-to-LDL cholesterol ratio
LDL: HDL	LDL-to-HDL cholesterol ratio;
Lp(a)	Lipoprotein (a)
mRNA	Messenger RNA
RCT	Reverse cholesterol transport

TC: HDL	Total cholesterol-to-HDL ratio
CB	Coconut biscuit
BB	Butter biscuit
LB	Lard biscuit

LIST OF FIGURES

Chapter 1

Figure 1.1: General structure of triglyceride. 3

Figure 1.2: Lauric acid ($C_{12}H_{24}O_2$) structural formula. 4

Figure 1.3: Oleic acid ($C_{18}H_{34}O_2$) strutural formula. 4

Figure 1.4: Linoleic acid ($C_{18}H_{32}O_2$) strutural formula. 5

Figure 1.5: α- Linoleic acid acid ($C_{18}H_{30}O_2$) strutural formula. 5

Figure 1.6: EPA ($C_{20}H_{30}O_2$) strutural formula. 5

Figure 1.7: Elaidic acid ($C_{18}H_{34}O_2$) strutural formula. 6

Figure 1.8: Fatty acid biosynthesis from proteins and carbohydrates 8

Figure 1.9: Digestion and absorption of short, medium and long-chain saturated fatty acid 12

Figure 1.10: Metabolism of fatty acids in the liver and mitochondria 13

Figure 1.11: Metabolism of chylomicron 14

Figure 1.12: Metabolism of VLDL 17

Figure 1.13: Reverse cholesterol transport 19

Chapter 3

Figure 3.1. Study flow diagram. 58

Figure 3.2: Meta-analysis of the effect of MCFAS intervention on HDL-C (A) and apo A-I (B) levels. 60

Figure 3.3: Meta-analysis of the effect of MCFAS intervention on LDL-C (A), VLDL-C (B), and apo B (C) levels. 63

Figure 3.4. Funnel plots of (A) TG; (B) LDL-C; (C) HDL-C; (D) TC 67

Figure 3.5. Fractional polynomial regression analysis of dose-response effect of MCFAS intake on change in HDL-C concentration 69

Chapter 4

Figure 4.1. Study flow diagram. 84

Figure 4.2: Postprandial TG levels (A) and net AUC (B) over 6 hours in response to the ingestion of the butter, coconut or lard biscuits. 85

Figure 4.3: Postprandial LDL-C levels (A) and net AUC (B) over 6 hours in response to the ingestion of the butter, coconut or lard biscuits. 87

LIST OF TABLES

Chapter 3

Table 3.1: Characteristice of included studies. 48

Table 3.2: Summary of outcome measures showing differential effects of MCFAS and LCSFAS on blood lipid. 52

Table 3.3: Subgroup analysis comparing the effects of MCFAS and LCSFAS on lipid profile 55

Table 3.4: Risk of bias of included studies 57

Chapter 4

Table 4.1. Participant characteristics at baseline 80

Table 4.2: Fasting blood lipids and nutrient intake 24 hours prior to each postprandial intervention 81

Table 4.3: Fasting lipid profile at baseline, changes over 6 hours postprandially and net area under the curve for change values (**Δ** netAUC) following consumption of butter, coconut or lard biscuit. 82

SYNOPSIS

Saturated fatty acid (SFA) intake has been linked with increased blood lipid concentrations and increased cardiovascular disease (CVD) risk. Current dietary guidelines consider all SFA as a single group and encourage to reduce saturated fat consumption to 10% of daily energy intake. However, not only the number, position and configuration of double bonds, but also the chain length of SFA have been shown to be a major determinant of their metabolic fate. Short-chain (SCFA, 2-4 carbons long, found in butter and products of fibre and resistant starch fermentation) and medium-chain (MCFAS, 6-12 carbons long, found in coconut and palm kernel oil) are absorbed directly through the villi of the intestinal mucosa and transported to the liver via the portal circulation. In contrast, long-chain (LCSFAS, >12 carbons, found in animal fats and dairy products) follow complex metabolic pathways including chylomicron synthesis in the intestinal villi, secretion into the thoracic lymph, hydrolysis of some triglycerides by lipoprotein lipase into free fatty acids which are transferred to the peripheral tissues (including muscle) followed by chylomicron remnants being taken up into the liver for further metabolism. This, together with the fact that humans spend most of their time in the postprandial state, suggests that saturated fat type may play an important role in overall lipid metabolism and modulation of CVD risk. Therefore, it was hypothesised that consumption of SCFA and MCFAS reduce blood lipid levels compared with LCSFAS.

Our first aim, addressed in chapter 3, was to to establish the basis for our hypothesis, conducting systematic review and meta analysis of the literature assessing the differential effects of chain lengths of SFA on blood lipids. The findings from this chapter demonstrate that the consumption of MCFAS enriched diets increased high-density lipoprotein cholesterol (HDL-C) and apolipoprotein A-I (apo A-I) levels compared to LCSFAS diet without any significant effect on triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) levels.

In chapter 4, we aimed to determine if SFA of different chain lengths would differentially influence postprandial lipid levels. In a randomised cross-over design, we investigated the effect of a meal (sweet biscuits) rich in either SCFA or MCFAS or LCSFAS on postprandial lipids (TG, TC, LDL-C, and HDL-C). The results presented in this chapter demonstrate that the postprandial triglyceride response following MCFAS

was lower compared to LCSFAS and that predominant fatty acid in the meal is a determinant of the lipemic response.

In conclusion, while this study has highlighted the differential effects of chain lengths of SFA on blood lipids. These results draw attention to the evidence that guidelines on SFA must consider the fatty acid chain length and importantly, the diverse SFA containing foods (processed and unprocessed meats, dairy products, eggs, coconut and palm oils, chocolate) that may possess harmful, neutral or even beneficial effects in relation to cardiovascular health.

THESIS LAYOUT

This thesis by publication is organised in 5 chapters, including one published study in the American Journal of Clinical Nutrition. This thesis contains a general introduction and literature review section (Chapter 1) followed by the methodology undertaken in the conduct of the research (Chapter 2). The introduction, methods, results, discussion of the research conducted for this thesis are then demonstrated as Chapter 3 and Chapter 4. This thesis presents work from a body of research comprised of one systematic review/ meta-analysis and one human research study; (i) differential effects of medium and long-chain saturated fatty acids on blood lipid profile: a systematic review and meta-analysis (Chapter 3) and (ii) medium chain fatty acids lower postprandial lipemia: a randomized, crossover trial (Chapter 4). An overall discussion of the findings from this body of research and its implications are demonstrated in the final chapter of the thesis (Chapter 5).