CURCUMIN AND LONG-CHAIN OMEGA-3 POLYUNSATURATED FATTY ACIDS: EFFECTS ON GLYCAEMIC CONTROL AND BLOOD LIPIDS

Rohith N Thota

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Nutraceuticals Research Program, Department of Pharmacy and Experimental Pharmacology, School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, The University of Newcastle

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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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_____

Mr Rohith N Thota

13/04/18

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2. Chapter 4

Thota RN, Dias CB, Abbott KA, Acharya SH, Garg ML. Curcumin alleviates postprandial glycaemic response in healthy subjects: A cross-over, randomized controlled study. Manuscript submitted MAR 2018.

3. Chapter 5

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4. Chapter 6

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Other co-authored publications

1. Jameel F**, Thota RN,** Wood LG, Plunkett B, Garg ML. Sex-dependent association between circulating irisin levels and insulin resistance in healthy adults. Journal of Nutrition & Intermediary Metabolism 2015; 2(3–4):86-92.
2. Abbott KA, Burrows TL, **Thota RN**, Acharya S, Garg ML. Do omega-3 PUFAs affect insulin resistance in a sex-specific manner? A systematic review and meta-analysis of randomized controlled trials. American Journal of Clinical Nutrition 2016; 104 (5):1470-84.
3. **Thota RN**, Ferguson JJA, Abbott KA, Dias CB, Garg ML. Science behind the health benefits of omega-3 polyunsaturated fatty acids: biochemical effects vs clinical outcomes. Food and Function 2018; doi:10.1039/c8fo00348c.

Conference presentations

1. Oral presentation on "Sex-dependent relationship between n-3 long-chain polyunsaturated fatty acids and insulin resistance: A Systematic review and Meta-analysis" at Joint Annual Scientific Meeting of the Nutrition Society of NZ and the Nutrition Society of Australia. 1-4 December 2015; Wellington, New Zealand.
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3. Publication of the month OCT 2017 – School of Biomedical Sciences and Pharmacy

Media activities

1. Turmeric + fish oil could prevent type 2 diabetes – ABC NEWS 15 JUL 2015
2. Trial tests the healing properties of turmeric – Newcastle Herald 15 JUL 2015
3. Australian scientists to test Indian spice in type 2 diabetes prevention – China.org.au 17 JUL 2015
4. Australian study investigating Indian spice as type 2 diabetes treatment – Diabetes UK 17 JUL 2015
5. Turmeric may help treat diabetes – Press Club of India – 18 JUL 2015
6. Turmeric and Omega-3 could prevent type 2 diabetes – Saturn Herald 29 JUL 2015
7. Invited to present research during Hunter Science Festival, Science Week – AUG 2015
8. Radio Interview – 'Reducing the risk factors for type 2 diabetes' 2NURFM – 24 NOV 2015 (https://www.youtube.com/watch?v=zVu-uB29Dc8)
9. Radio Interview – Diabetes survey – 2NURFM – 4 JUL 2016 (https://www.youtube.com/watch?v=xzlNLrB0WFo)
10. Radio Interview – Curcumin and Omega-3 fatty acids for management of heart disease risk in type 2 diabetes - 2NURFM – 3 MAR 2017
11. Radio Interview – Curcumin and Omega-3 fatty acids for management of heart disease risk in individuals with type 2 diabetes - ABC Newcastle Drive – 19 OCT 2016

Table of contents

Acknowledgements iii
List of research publications included in the thesis v
Other co-authored publications vi
Conference presentations vi
Scholarships vi
Awards and media appearances vii
Media activities vii
List of figures xiii
List of tables xiv
List of appendices xv
Abbreviations xvi
Synopsis xx
Thesis layout xxiii
CHAPTER 1 General Introduction 25
1.1 Overview 26
1.2 Background and context 26
1.2.1 Prevalence and diagnostic criteria of T2D and prediabetes 26
1.2.2 Economic burden and health consequences of T2D 28
1.3 Multi-stage model development of T2D 28
1.4 Necessity for the improved markers 34
1.5 Current available interventions for prevention and management of T2D 35
1.6 Role of bio-actives in prevention of T2D 36
1.6.1 Curcumin 36
1.6.2 Long chain omega-3 polyunsaturated fatty acids (LCn-3PUFA) 41
1.6.3 Chlorogenic acid 47
1.6.4 Resveratrol 48
1.7 Conclusion of the literature review and project rationale 49
1.8 Research Aims 52
CHAPTER 2 General Methods 54
2.1 Participant Recruitment 55
2.2 Anthropometry and Body Composition 55
2.3 Questionnaires 55

2.3.1 Medical History 55
2.3.2 Nutrition assessment 56
2.3.3 Physical Activity 56
2.4 Randomisation 56
2.5 Interventions 56
2.6 Clinical Trials 57
2.6.1 Trial 1- Acute postprandial cross over study 57
2.6.2 Trial 2 - **C**urcumin and/or **O**mega-3 polyunsaturated fatty acids for **P**revention of T2**D** (COP-D trial) 58
2.6.3 Trial 3 - **C**urcumin **A**nd/or **L**ong-chain omega-3 polyunsaturated fatty acids **FOR** management of **C**ardio**V**ascular health in individuals with T2**D** (CALFOR-CVD trial) 60
2.7 Blood collection 62
2.8 Biomarker analysis 62
2.8.1 Markers of glycaemic control 62
2.8.2 Lipid parameters 63
2.8.3 Inflammation and other markers 63
2.8.4 Formula based markers 63
2.8.5 Safety monitoring and compliance 63
CHAPTER 3 InsuTAG: A novel physiologically relevant predictor of insulin resistance and metabolic syndrome 64
3.1 Abstract 65
3.2 Introduction 66
3.3 Methods 67
3.4 Results 69
3.5 Discussion 77
CHAPTER 4 Curcumin alleviates postprandial glycaemic response in healthy subjects: A cross over, randomised controlled study 80
4.1 Abstract 81
4.2 Introduction 82
4.3 Methods 83
4.3.1 Subjects 83
4.3.2 Standard meal 83

4.3.3 Study design 83
4.3.4 Test day protocol 84
4.3.5 Statistics 84
4.4 Results 85
4.4.1 Participant characteristics 85
4.4.1 Physical activity and dietary intake 85
4.4.2 Postprandial glucose levels 85
4.4.3 Postprandial insulin 86
4.4.4 Postprandial triglycerides 86
4.4.5 Confounding variables 86
4.5 Discussion 92
CHAPTER 5 Curcumin and long-chain Omega-3polyunsaturated fatty acids for prevention of type 2 Diabetes (COP-D): Study protocol for a randomised controlled trial 95
5.1 Abstract 96
5.2 Background 97
5.3 Method/design 99
5.3.1 Study Aims 99
5.3.2 Inclusion criteria 99
5.3.3 Exclusion criteria 100
5.3.4 Sample size calculation 100
5.3.5 Participant recruitment 100
5.3.6 Baseline assessments 101
5.3.7 Randomisation 101
5.3.8 Intervention 101
5.3.9 Interim visit 102
5.3.10 Post intervention visit 102
5.3.11 Safety and compliance monitoring 102
5.3.12 Ethics 103
5.3.13 Data collection and outcome measures 103
5.3.14 Data analysis 104

5.4 Discussion 104
5.5 Strengths and limitation of study design 108
CHAPTER 6 Effects of curcumin and/or omega-3 polyunsaturated fatty acids on glycaemic control and blood lipids in individuals with high risk of type 2 diabetes: A randomised controlled trial 111
6.1 Abstract 112
6.2 Introduction 113
6.3 Methods 114
6.3.1 Subjects 114
6.3.2 Study design 115
6.3.3 Data collection and outcome measures 115
6.3.4 Statistical analysis 116
6.4 Results 117
6.4.1 Baseline characteristics 117
6.4.2 Body composition 117
6.4.3 Glycaemic indices 117
6.4.4 Blood lipids 118
6.4.5 Inflammation and blood cell count 118
6.4.6 Dietary intake, physical activity, compliance and adverse effects 118
6.5 Discussion 128
CHAPTER 7 Curumin and/or omega-3 polyunsaturated fatty acids for amelioration of diabetic dyslipidaemia: A pilot randomised controlled trial 132
7.1 Abstract 133
7.2 Introduction 134
7.3 Methods 135
7.3.1 Subjects 135
7.3.2 Study Design 136
7.3.3 Data collection and outcome measures 137
7.3.4 Statistical analysis 138
7.4 Results 138
7.4.1 Baseline participant characteristics 138
7.4.2 Blood lipids 139
7.4.3 Glucose control and other secondary outcome measures 139

7.4.4 Dietary intake, physical activity, compliance and fatty acid changes 140
7.5 Discussion 147
CHAPTER 8 General Discussion 151
8.1 Key findings 152
8.1.1 Novel marker, InsuTAG, shows superiority in identifying IR and MetS than the existing markers 152
8.1.2 Curcumin effectively lowered PBG levels, but did not show any effect on FPG and HbA1c 153
8.1.3 Curcumin effectively lowered both postprandial & fasting insulin levels and improved IS 154
8.1.4 Magnitude of reduction in blood lipids by curcumin was higher in individuals with T2D compared with the reductions in those with high risk of T2D 156
8.1.5 LCn-3PUFA lowers FPG in individuals with baseline FPG level above 5.5 mmol/L 157
8.1.6 LCn-3PUFA supplementation reduces erythrocyte arachidonic acid levels, but has no effect on CRP 157
8.1.7 Both LCn-3PUFA and curcumin exhibit similar magnitude of reduction in InsuTAG 158
8.1.8 Complementary or added benefits with curcumin and LCn-3PUFA were not observed in all the three intervention studies 160
8.2 Limitations 161
8.2.1 Feasibility of recruiting study participants with pre-diabetes and T2D 161
8.2.2 Heterogeneity in the study populations 161
8.2.3 Different available formulations of curcumin and fish oil 162
8.3 Conclusions 162
References 164

List of figures

Figure 1.1: Multi-stage model development of T2D 34
Figure 1.2: Structures of a. curcumin b. demethoxycurcumin c. bisdemethoxycurcumin..... 36
Figure 1.3: Structures of Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA)..... 41
Figure 1.4: Structure of chlorogenic acid 47
Figure 1.5: Structure of resveratrol..... 48
Figure 1.6: Rationale for combination of curcumin and LCn-3PUFA 51
Figure 1.7: Summary of literature review and research aims 53
Figure 3.1: Scatterplot of InsuTAG and HOMA-IR. 76
Figure 3.2: Receiver operating characteristic (ROC) curves for identifying Insulin Resistance (IR) using surrogate markers of IR. 76
Figure 3.3: Receiver operating characteristic (ROC) curves for identifying Metabolic Syndrome (MetS) using InsuTAG and HOMA-IR. 77
Figure 4.1: CONSORT flow chart..... 90
Figure 4.2: Changes in the mean blood glucose levels (Δglucose) over 120 minutes 91
Figure 4.3: Changes in the mean serum insulin levels (Δinsulin) over 120 minutes 91
Figure 4.4: Changes in the mean serum triglyceride levels (Δtriglycerides) over 120 minutes. 92
Figure 5.1: Trial protocol flow chart..... 109
Figure 6.1: Consolidated Standards of Reporting Trials (CONSORT) flow chart 119
Figure 6.2: Changes in the outcome measures from baseline to post intervention with-in and between double placebo (PL), curcumin (CC), fish oil (FO) and curcumin + fish oil (CC-FO) 120
Figure 6.3: Changes in the outcome measures from baseline to post intervention and between double placebo (PL), curcumin (CC), fish oil (FO) and curcumin + fish oil (CC-FO); 121
Figure 6.4: Changes in the outcome measures from baseline to post-intervention in people with FPG >5.5 and <5.5 in PL, CC, FO and CC-FO groups. 127
Figure 7.1: Consolidated Standards of Reporting Trials (CONSORT) flow chart 145
Figure 7.2: Changes in the blood lipids from baseline to post-intervention with-in and between the PL, CC, FO, and CC-FO. 146
Figure 8.1: Summary of the effects of curcumin on glycaemic control and blood lipids..... 155
Figure 8.2: Summary of the effects of LCn-3PUFA on glycaemic control and blood lipids .. 159

List of tables

Table 1.1: Diagnostic criteria for T2D and prediabetes 27
Table 3.1: Participant characteristics of all participants and for participants stratified into subgroups by IR status 72
Table 3.2: Correlations between contributors to insulin resistance, InsuTAG, and other surrogate markers of insulin resistance 73
Table 3.3: Regression models for predicting IR 74
Table 3.4: Predictive values of proposed InsuTAG cut-off of 11.2 for the identification of IR and MetS 75
Table 3.5: Participant characteristics and metabolic parameters of participants stratified into subgroups according to the proposed InsuTAG cut-off of 11.2 75
Table 4.1: Baseline characteristics of the participants 88
Table 4.2: Composition of the habitual diets as consumed 24 hours before the PL, FO, CC and CC-FO test days 89
Table 4.3: Baseline values, change area under the curve (AUC) for glucose, insulin, triglycerides in response to PL, FO, CC and CC-FO 89
Table 5.1: Study timeline and Assessments 110
Table 6.1: Baseline general characteristics of the study population: 122
Table 6.2: Baseline blood parameters of the trial participants 123
Table 6.3: Changes in the outcome measures from baseline to post-intervention 124
Table 6.4: Changes in the dietary intake, physical activity and fatty acid composition of the red blood cells of the participants 125
Table 6.5: Mean changes in outcome measures stratified by sex within PL, CC, FO and CC-FO groups 126
Table 7.1: Baseline general characteristics of the trial participants 141
Table 7.2: Baseline blood parameters of the study participants 142
Table 7.3: Changes in the outcome measures from baseline to post-intervention 143
Table 7.4: Changes in the dietary intake, physical activity and fatty acid composition of the study participants 144

List of appendices

Appendix 1: Recruitment flyer **–** Acute postprandial study 200
Appendix 2: Recruitment flyer – COP-D study 201
Appendix 3: Recruitment flyer – CALFOR-CVD Study 202
Appendix 4: Participant information sheet - Acute postprandial study 203
Appendix 5 : Participant information sheet – COP-D study 208
Appendix 6: Participant information sheet – CALFOR-CVD study 213
Appendix 7: Participant consent form – Acute post-prandial study 219
Appendix 8: Participant consent form – COP-D study 220
Appendix 9: Participant consent form – CALFOR_CVD trial 221
Appendix 10: Medical history and screening form – Acute postprandial study 222
Appendix 11: Medical history and screening form – COP-D study 227
Appendix 12: Medical questionnaire – CALFOR-CVD study 231
Appendix 13: 24 hour food recall – Acute postprandial study 234
Appendix 14: 3-day food record – COP-D and CALFOR-CVD trial 236
Appendix 15: International Physical Activity Questionnaire – Short form 240
Appendix 16: International Physical Activity Questionnaire – Long form 241
Appendix 17: The Australian Type 2 Diabetes Risk Assessment Tool 242
Appendix 18: SPIRIT checklist for trial protocol 243
Appendix 19: Statement of contribution and collaboration for chapter 3 249
Appendix 20: Statement of contribution and collaboration for chapter 4 250
Appendix 21: Statement of contribution and collaboration for chapter 5 251
Appendix 22: Statement of contribution and collaboration for chapter 6 ……251

Abbreviations

ABS	Australian Bureau of Statistics
AE	Adverse events
AIHW	Australian Institute of Health and Welfare
AIP	Atherogenic index of plasma
ALT	Alanine transaminase
AMPK	5' adenosine monophosphate-activated protein kinase
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ANZCTR	Australian New Zealand Clinical Trials Registry
AST	Aspartate transaminase
ATF2	Activating transcription factor 2
AUSDRISK	The Australian Type 2 Diabetes Risk Assessment Tool
BFM	Body fat mass
BMI	Body mass index
CALFOR-CVD	Curcumin And Long-chain omega-3 polyunsaturated fatty acids for management of CardioVascular health in type 2 Diabetes.
CC	Curcumin alone
CC-FO	Curcumin plus fish oil
CCL-2	CC-chemokine ligand 2
COP-D	Curcumin and/or Omega-3 polyunsaturated fatty acids for Prevention of type 2 Diabetes
CRP	C-reactive protein
CVD	Cardiovascular disease
DHA	Docosahexanoic acid
DPP-4	Dipeptidyl peptidase 4
EDTA	ethylenediaminetetraacetic acid

ELK-1	E26 transformation specific containing domain protein 1
EPA	Eicosapentaenoic acid
ER	Endoplasmic reticulum
FFA	Free fatty acids
FO	Fish oil alone
FPG	Fasting plasma glucose
GIP	Glucose-dependent insulinotropic polypeptide
GISSI-prevenzione	Gruppo Italiano per lo Studio della Sopravvivenza nell'infarto
GLP-1	Glucagon like peptide-1
GPR	G-protein coupled receptors
HbA1c	Glycosylated haemoglobin
HDL-C	High density lipoprotein cholesterol
HEC	Hyperinsulinemic euglycemic clamp
HMRI	Hunter medical research institute
HNEHREC	Hunter New England Human Research Ethics Committee
HOMA	Homeostatic model assessment
HOMA	Homeostatic model assessment
HOMA-IR	Homeostatic model assessment of IR
ICML	Intramyocellular lipids
IDF	International diabetes federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IKK	Iκb kinase β
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
IQR	Interquartile range
IR	Insulin resistance
IRS-1	Insulin receptor substrate-1
IS	Insulin sensitivity

JELIS	The Japan Eicosapentaenoic acid Lipid Intervention Study
JNK	JUN N-terminal kinase
LCn-3PUFA	Long-chain omega-3 polyunsaturated fatty acids
LDL-C	Low density lipoprotein cholesterol
LXRα	Liver X receptor alpha
MCP-1	Macrophage chemoattractant protein-1
MDA	Malondialdehyde
MetS	Metabolic syndrome
MM	Muscle mass
NF-κB	Nuclear factor kappa B
NLRP3	NOD, LRR and pyrin domain–containing 3 inflammasome
OGTT	Oral glucose tolerance test
PBF	Percent body fat
PBG	Postprandial blood glucose
PI	Postprandial insulin
PL	Double placebo
PPAR-α	Peroxisome proliferator-activated receptor alpha sub unit
RHLS	Retirement Health and Lifestyle Study
ROS	Reactive oxygen species
SAE	Serious adverse event
SBP	Systolic blood pressure
SD	Standard deviation
SEM	Standard error of mean
SIRT1	Sirtuin1
SOSC3	Suppressor of cytokine signalling 3
STAT3	Signal transducer and activator of transcription 3
SREBP-1c	Sterol regulatory element-binding protein 1
T2D	Type 2 diabetes

TC	Total cholesterol
TG	Triglycerides
TLR4	Toll like receptor-4
TNF-α	Tumor necrosis factor - alpha
UoNHREC	University of Newcastle Human Research Ethics Committee
UPR	Unfolded protein response
VLDL-C	Very low density lipoprotein cholesterol
WC	Waist circumference
WHR	Waist – hip ratio

Synopsis

Type 2 diabetes (T2D) is the most common chronic metabolic disorder resulting from either deficit of insulin secretion and/or action. The transition of normal glucose tolerance to T2D is usually accompanied by a cluster of metabolic risk factors such as low-grade inflammation, oxidative stress, insulin resistance (IR) and dyslipidaemia. IR is one of the marked independent predictors among these cluster of metabolic abnormalities that mediates the transition in high risk states such as obesity, impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) to overt T2D. IR also is often associated with decreased clearance of lipids and lipoprotein abnormalities, together representing a greater risk of cardiovascular disease (CVD) in both high risk and individuals with T2D. Several studies have employed lipid ratios, homeostatic models, and anthropometric measures as surrogate markers for predicting IR. However, none of these accounted for both insulin and lipid availability in a single model to predict IR or metabolic syndrome (MetS).

Therefore, the first aim of my PhD project, presented in the chapter 3, was to develop a novel marker for IR and MetS that accounts for both insulin and lipid availability in a single model. We proposed and evaluated a novel physiologically relevant marker, InsuTAG (product of fasting insulin and fasting triglycerides) as a predictor of IR and MetS. Cross-sectional analysis of data from the Retirement Health and Life-style Study (RHLS, n=618) showed that InsuTAG is a strong predictor of IR over existing lipid based surrogate markers and anthropometric measures. Receiver operating curve analysis indicated InsuTAG (93%) as the favourable marker for IR over other lipid based surrogate markers and anthropometry measures. Prevalence of MetS was significantly higher in individuals with InsuTAG values above the optimal cut-off value of 11.2. InsuTAG exhibited a greater area under than curve than HOMA-IR for identifying MetS. Together these observations indicate the potential of InsuTAG for predicting IR and MetS.

Despite effective lifestyle and pharmacological interventions, the prevalence of T2D is growing at an alarming rate in Australia, in line with global prevalence. Failure of long term compliance to these interventions is a major barrier for their effectiveness in halting the transition to T2D in high risk state individuals, indicating a necessity for alternative effective approach.

Given the fact that pathogenesis of T2D is chronic, complex and often involving multiple pathological pathways, use of well tolerated dietary bio-active compounds appears to be a potential strategy for delaying the onset of T2D. Several pre-clinical and *in-vitro* studies have reported the ability of dietary bio-actives to down regulate multiple pathological mechanisms (chronic low-grade inflammation, IR, oxidative stress and β-cell dysfunction) that are involved in the pathogenesis of T2D. We hypothesised that a combination of two lipid-lowering and anti-inflammatory dietary bio-active compounds, curcumin and long-chain omega-3 polyunsaturated fatty acids (LCn-3PUFA), could potentially act in multiple pathways to improve the glycaemic control in individuals at high risk of developing T2D.

My second aim, presented in chapter 4, was to evaluate the acute effects of curcumin and/or LCn-3PUFA on glycaemic responses. Therefore, in a randomised, cross over trial we investigated the postprandial glucose and insulin response to a single dose of curcumin and/or LCn-3PUFA in healthy individuals. The glucose levels were reduced by curcumin at as early as 30 min, and the maximum effect was observed at 60 min post meal consumption. Curcumin was found to be effective for lowering the insulin demand to control postprandial glucose levels. Similar results were observed following dietary supplementation with curcumin plus LCn-3PUFA. It was apparent that the postprandial effects on glycaemic control were primarily due to curcumin even in the combined treatment group. Thus, providing basis for long-term supplementation study with curcumin for glycaemic control.

In chapter 5, a detailed study protocol for 2x2 factorial placebo controlled, double blinded randomised trial with long term (12 weeks) curcumin and LCn-3PUFA supplementation (COP-D trial) was presented. In chapter 6, we examined the effects of curcumin with or without LCn-3PUFA on glycaemic control and blood lipid levels in people at high risk of T2D. 12 weeks of supplementation with curcumin has effectively reduced the fasting insulin levels and IR in individuals with high risk of T2D. Parallel to these results, both curcumin and LCn-3PUFA were able to reduce the fasting triglycerides and atherogenic index of plasma, however the magnitude of reduction was greater with LCn-3PUFA supplementation. InsuTAG levels were also reduced with curcumin and LCn-3PUFA supplementation. However, this study failed to show any complimentary effects with concurrent administration of curcumin and LCn-3PUFA. Though IR and fasting triglycerides, were effectively reduced by these two bio-actives, we did not find any

beneficial effects of curcumin and LCn-3PUFA supplementation on fasting glucose and glycosylated haemoglobin levels.

In chapter 7, we designed a study to target commonly prevalent dyslipidaemia with curcumin and/or LCn-3PUFA in individuals with T2D (CALFOR-CVD trial). Participants were randomised to either placebo or curcumin or LCn-3PUFA, or curcumin plus LCn-3PUFA for six weeks. This pilot study has demonstrated that supplementation of curcumin can effectively reduce the TG. Contrasting to the results from chapter 6, magnitude of reduction in triglycerides in this study was higher with curcumin than LCn-3PUFA. Preliminary observations also presented a non-significant, but a noteworthy reduction of 0.5 mmol/L in total cholesterol and LDL-Cholesterol with curcumin supplementation. In line with observations from the COP-D trial, curcumin and LCn-3PUFA did not have any complimentary and/or added benefits.

In conclusion, the results presented in this thesis demonstrate that InsuTAG has the potential to predict IR and MetS. This provides a basis for further research to validate InsuTAG with gold standard technique for IR and a longitudinal data analysis to determine the ability of InsuTAG to predict T2D in general population. With regards to the intervention trials, our hypothesis of targeting multiple pathways (IR and dyslipidaemia) in high risk and T2D patients with curcumin and LCn-3PUFA supplementation was successful. However, this thesis failed to provide any evidence on beneficial effects of combining curcumin and LCn-3PUFA for better glycaemic control to delay the onset of T2D. This could partly be due to presence of any unknown interactions between the two bio-actives or may be due to uncertainties in co-administration of curcumin and LCn-3PUFA. Thus, paving a way for further research to investigate beneficial effects with single formulation (curcumin and LCn-3PUFA) for achieving glycaemic control. This thesis constitutes a noted contribution to the research area of biomarkers and novel intervention strategies for T2D, and also presents a set of riddles that provides an extensive scope for future research.

Thesis layout

This thesis by publication is presented as 7 chapters: general introduction and literature review, general methodology chapter, 2 papers published in peer reviewed journals, 2 papers submitted for publication (under review), and one is presented as an unpublished chapter.

- **Chapter 1- General introduction and literature review**

  This chapter describes prevalence, economic and health burden of T2D, prediabetes and pathological mechanisms involved in the progression of prediabetes state to overt T2D. This chapter also focuses on the role of bio-active compounds for prevention or delaying the onset of T2D.

- **Chapter 2 – General methodology**

  This chapter describes detailed methodology, trial design and statistical analysis employed in the clinical trials (Chapter 4, 6 and 7).

- **Chapter 3 – InsuTAG: A novel and physiologically relevant marker of insulin resistance and metabolic syndrome.**

  This chapter is presented as published paper "Thota RN, Abbott KA, Ferguson JJA, Veysey M, Lucock M, Niblett S, King K, Garg ML. InsuTAG: A novel physiologically relevant predictor for insulin resistance and metabolic syndrome. Scientific Reports. 2017; 7(1):15204." The paper presents necessity for developing a novel marker, cross sectional study methodology, statistical analysis, results and discussion on whether InsuTAG has the potential to identify insulin resistance and metabolic syndrome.

- **Chapter 4 – Curcumin alleviates postprandial glycaemic response in healthy subjects: A cross over, randomised controlled study.**

  This chapter is presented as a paper (submitted MAR 18). It describes the study aims, design, methods, results and discussion on the effects of curcumin and omega-3 fatty acids for controlling the post-prandial glucose responses to a standardised high carbohydrate-fat meal in healthy adults.

- **Chapter 5 – Curcumin and long-chain Omega-3 fatty acids for Prevention of type 2 diabetes (COP-D): study protocol for a randomised controlled trial.**

  This chapter is presented as a published paper 'Thota RN, Acharya SH, Abbott KA, Garg ML. **C**urcumin and long-chain **O**mega-3 polyunsaturated fatty acids for **P**revention of type 2 **D**iabetes **(COP-D)**: study protocol for a randomised controlled

trial. Trials. 2016; 17:565'. This chapter presents the detailed study protocol of COP-D trial and provides a rationale for conducting a clinical trial with curcumin and LCn-3PUFA for controlling risk factors associated with progression of T2D.

- **Chapter 6– Effects of curcumin and/or omega-3 polyunsaturated fatty acids on glycaemic control and blood lipids in individuals with high risk of T2D: A randomised controlled trial.**

  This chapter is presented as a submitted version ' Thota RN, Acharya SH, Garg ML. Effects of curcumin and/or omega-3 polyunsaturated fatty acids on glycaemic control and blood lipids in individuals with high risk of type 2 diabetes: A randomised controlled trial. (Manuscript submitted MAR 18). This chapter describes the aims, methods, results and discussion from COP-D trial.

- **Chapter 7 – Curcumin and/or omega-3 polyunsaturated fatty acids for amelioration of diabetic dyslipidaemia: a pilot randomised controlled trial**

  This chapter presents the study aims, design, methods and results from pilot randomised controlled trial, '**C**urcumin **A**nd **L**ong-chain omega-3 polyunsaturated fatty acids **FOR** management of **C**ardio**V**ascular health in individuals with type 2 **D**iabetes (**CALFOR-CVD** trial).

- **Chapter 8 – General discussion**

  This chapter focuses on discussion on overall results from the clinical trials, strengths and limitations of the respective trials, significance of this research in nutrition and diabetes research areas and future directions.