Influence of dietary fructose on lipid profile and glycaemic control in healthy individuals

Faizan Jameel

Master of Pharmacy

Thesis submitted in fulfilment of the requirements for the
Degree of Doctor of Philosophy

School of Biomedical Sciences and Pharmacy
The University of Newcastle, Australia

October 2015

THE UNIVERSITY OF
NEWCASTLE
AUSTRALIA

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 consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository subject to the provisions of the Copyright Act 1968.

Acknowledgments

The end of an arduous journey of completing a PhD thesis calls for a pause and rewind; and for expressing my heartfelt gratitude to those without whose support, this project would not have come to fruition. I want to acknowledge all those moments, circumstances and people who have helped me accomplish this rather ambitious goal.

Firstly, I owe the biggest debt of gratitude to Professor Manohar Garg, who has contributed significantly to my development through his guidance, encouragement and support. His intellectual inputs, expertise and positive outlook have inspired me to keep going, and those much needed pep talks have given me the confidence to overcome even the most difficult of challenges with relative ease.

I would also like to thank my co-supervisor, Associate Professor Lisa Wood for her patience, invaluable feedback and passionate mentoring. She is a remarkable role model who has inspired me to think laterally and aim higher.

My sincere thanks to the Hunter Medical Research Institute (HMRI) for helping me recruit participants for my research. I would like to express my sincere gratitude to all the wonderful participants who selflessly committed their time towards my research.

I will be ever indebted to Jennie Thomas Trust Fund (via HMRI) and the University of Newcastle scholarships without whose support completing a PhD would have remained a dream.

I have been very fortunate to be a part of Nutraceuticals Research Group. A big ‘thank you’ to my colleagues: Jency, Cintia, Rohith, Fatimah, Jess and Kylie. They have been immensely helpful, supportive and encouraging. Special thanks also to Melissa Fry and Melinda Phang, who always listened patiently to all my woes and provided their useful suggestions.

Last but not the least; I would also like to thank my family and friends for their ongoing encouragement, support and prayers and for believing in me when I had moments of doubt. A special thanks to my wife Sameena and my little boy Ayaan. Thank you Sameena for always believing in me and encouraging me to be the best that I can be. I would have never been able to come so far without your constant support and love and putting my work as a priority ahead of our life.

Publication and presentations central to this thesis

Refereed journal publication:

Jameel F, Phang M, Wood LG and Garg ML; Acute Effects of Feeding Fructose, Glucose and Sucrose on Blood Lipid Levels and Systemic inflammation Lipids in Health and Disease *2014,* **13***:195*

Conference Abstracts:

F. Jameel, M. Phang, L. G. Wood, M. L. Garg; Fructose consumption modulates postprandial lipid levels and inflammatory mediators in healthy subjects, Nutrition Society of Australia and New Zealand ASM (Dec 2013), Brisbane.

F. Jameel, M. Phang, L. G. Wood, M. L. Garg; Acute Effects of Feeding Fructose, Glucose and Sucrose on Blood Lipid Levels and Systemic Inflammation, Australasian Section of the American Oil Chemists Society (AAOCS) (Nov 2013), Newcastle.

F Jameel, LG Wood, ML Garg; Dietary supplementation with fructose or glucose does not influence blood lipids and C-reactive protein in healthy subjects, Nutrition Society of Australia and New Zealand ASM (NOV 2014), Hobart.

Table of Contents

List of figures 7
List of tables 8
Abbreviations 10
Synopsis 12
Thesis structure and overview 14

1. CHAPTER 1 - BACKGROUND LITERATURE REVIEW 15

1.1. Sugars and Sweeteners 16
1.2. Fructose 20
1.3. Food sources of fructose 21
1.4. Dietary intake of fructose 22
1.5. Absorption of Fructose 23
1.6. Comparison between Glucose and Fructose absorption 24
1.7. Hepatic metabolism of fructose 24
1.8. Comparison between Glucose and Fructose metabolism 26
1.9. Fructose and cardiovascular disease risk factors 28
1.10. Blood lipids 29
1.11. Insulin resistance 35
1.12. Inflammation 38
1.12.1. Role of fructose in inflammation 38
1.12.2. Inflammation mediating chronic diseases 43
1.12.2.1. Cardiovascular disease and inflammation 43
1.12.2.2. NAFLD and inflammation 44
1.12.2.3. Diabetes and inflammation 45
1.12.2.4. Cancer and inflammation 46
1.12.2.5. Neurological disease and inflammation 47
1.13. Obesity 54
1.14. Summary 57
1.15. Hypothesis & Aims 58
1.15.1. Hypothesis 58
1.15.2. Aims 58
1.15.2.1. Aim 1 58
1.15.2.2. Aim 2 59
1.15.2.3. Aim 3 59

2. CHAPTER 2: GENERAL METHODS 60

2.1 Study Design 61
2.2 Participants 61
2.3 Exclusion criteria 61
2.4 Ethics and trial registrations 62
2.5 Anthropometric measurements 62
2.6 Dietary Intakes 62
2.7 Laboratory methods 63
2.8 Statistical analyses 63

3. CHAPTER 3- ACUTE EFFECTS OF FEEDING FRUCTOSE, GLUCOSE AND SUCROSE ON BLOOD LIPID LEVELS AND SYSTEMIC INFLAMMATION 64

3.1 Thesis aim 65
3.1.1 Aim 1 65

3.1.2 Abstract 65
3.1.3 Introduction 66
3.1.4 Methods 67
3.1.4.1 Study population 67
3.1.4.2 Study Design 68
3.1.4.3 Laboratory methods 68
3.1.4.4 Statistical analysis 69
3.1.5 Results 69
3.1.6 Discussion 71

4. CHAPTER 4: CHRONIC STUDY 81

4.1 Thesis aim 82
4.1.1 Aim 2 82
4.1.2 Introduction 82
4.1.3 Methods 84
4.1.3.1 Study population 84
4.1.3.2 Study Design 84
4.1.3.3 Laboratory methods 85
4.1.3.4 Statistical analysis 85
4.1.4 Results 86
4.1.5 Discussion 91
4.1.6 Conclusion 96

5. CHAPTER 5 - FRUCTOSE RESTRICTED STUDY 104

5.1 Thesis aim 105
5.1.1 Aim 3 105
5.1.2 Introduction 105
5.1.3 Methods 107
5.1.3.1 Study population 107
5.1.3.2 Study Design 108
5.1.3.3 Laboratory methods 112
5.1.3.4 Statistical analysis 112
5.2 Results 113
5.3 Discussion 116
5.4 Conclusion 120

6. CHAPTER 6 - DISCUSSION AND CONCLUSION 126

6.1 Summary 127
6.2 Discussion and Limitations 127
6.3 Conclusion 132

REFERENCES 133

APPENDICES 152

Appendix one: Study 1 Information Statement & Consent Form 153
Appendix Two: Study 2 Information Statement & Consent Form 158
Appendix Three: Study 3 Information Statement & Consent Form 162
Appendix Four: Pre-Trial Medical Questionnaire 166
Appendix Five: 24 Hour Food recall Form 169
Appendix Six: 3 days food recall form 171
Appendix Seven: International Physical Activity Questionnaire 173

List of figures

Figure 1-1: The global sugar glut, taken from Lustig et al [36] 17

Figure 1-2: Splanchnic metabolism of fructose, taken from Tappy et al [80] 26

Figure 1-3: Comparison between glucose and fructose metabolism, taken from Feinman et al [83] 27

Figure 1-4: Excess fructose intake leading to obesity and insulin resistance, adapted from [152] 37

Figure 1-5: Extracellular initiating events through which NF-κB, a master controller of inflammation can be activated 41

Figure 3-1: Kinetics of change and area under the curve (AUC) for blood glucose (Mmol/L) and insulin (mIU/L) after the fructose, glucose or sucrose supplementations. Values without a common superscript are significantly different; $P<0.05$ 75

Figure 3-2: Kinetics of change for total cholesterol (mmol/L), LDL-C (mmol/L), HDL-C (mmol/L) and triglyceride (mmol/L) after the fructose, glucose or sucrose supplementations. Values without a common superscript are significantly different; $P<0.05$. 76

Figure 3-3: Kinetics of change and area under the curve (AUC) for CRP (mg/L) after the fructose, glucose or sucrose supplementations. Values without a common superscript are significantly different; $P<0.05$ 77

Figure 5-1: Study design 110

List of tables

Table 1-1: Selected sugar contents of food, (Grams per 100 g edible portion) 18

Table 1-2: in vitro, in vivo and human studies of investigating role of fructose in inflammation 49

Table 3-1: Baseline values of anthropometric measurements, blood biomarkers & energy and nutrients intakes of study participants 78

Table 3-2: Daily energy and nutrient intake 78

Table 3-3: AUC for blood lipids derived from kinetics of change 79

Table 4-1: Baseline values of anthropometric measurements, body composition & blood biomarkers of study participants 97

Table 4-2: Daily energy and nutrient intake 98

Table 4-3: Values of blood markers at baseline and post intervention & changes in their levels after glucose and fructose supplementation (Independent sample t-test) 99

Table 4-4: Values of anthropometric measurements and body composition at baseline and post intervention & changes in their levels after glucose and fructose supplementation (Independent sample t-test) 100

Table 4-5: Daily energy and nutrient intake at baseline and post intervention & changes in their levels after glucose and fructose supplementation (Independent sample t-test) 101

Table 5-1: Baseline values of anthropometric measurements, body composition and blood biomarkers – Baseline Characteristics 121

Table 5-2: Daily energy and nutrient intake at baseline and post intervention 121

Table 5-3: Effect of fructose restriction on anthropometric measurements, body composition & blood biomarkers. Baseline and post-intervention values of anthropometric measurements and body composition 122

Table 5-4: Baseline and post-intervention values of blood biomarkers 122

Table 5-5: Correlation coefficients between fructose intake, anthropometric, body composition and clinical parameters - At baseline 123

Table 5-6: Correlation coefficients between fructose intake, anthropometric, body composition and clinical parameters - Post intervention.....124

Table 5-7: Correlation coefficients between changes in fructose intake, anthropometric, body composition and clinical parameters 125

Abbreviations

AGEs	Advanced glycation end products
AOPP	Advanced oxidation protein products
AUC	Area Under Curve
BFM	Body Fat Mass
BIA	Bioimpedance analysis
BMI	Body Mass Index
CETP	Cholesteryl ester transfer protein
CKD	Chronic kidney disease
CVD	Cardiovascular disease
DHAP	Dihydroxy acetone phosphate
DNL	*de novo* lipogenesis
FAS	Fatty acid synthase
Ga-3-P	Glyceraldehyde-3-Phosphate
GLUT 2	Glucose Transporter 2
GLUT 5	Glucose Transporter 5
HDL	High Density Lipoprotein
HEAC	Human aortic endothelial cells
HFCS	High Fructose Corn Syrup
HOMA	Homeostatic model assessment
hs-CRP	high-sensitivity CRP
ICAM-1	Intercellular adhesion molecule-1
IFN-γ	Interferon gamma
IHCL	Intra hepatocellular lipids
IKKβ	IκB kinase□
IL-1β	Inter Leukin-1β
IL-6	Inter Leukin-6
IL-8	Inter Leukin -8
IMCL	Intra-myocellular lipids
JNKs	c-jun N-terminal kinase
LCAT	Lecithin cholesterol acyltransferase
LDL	Low Density Lipoprotein

LPL	Lipoprotein Lipase
LTs	Leukotrienes
MCP-1	Monocyte chemotactic protein 1
MS	Multiple Sclerosis
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NEFA	Non-esterified fatty acids
PBF	Percent body fat
PD	Parkinson's Disease
PDH	Pyruvate dehydrogenase
PFK	Phosphofructokinase
PGE2	Prostaglandin E2
ROS	Reactive oxygen species
SGLT1	Sodium Glucose Transporter 1
sICAM	Soluble intercellular adhesion molecule
SREBP-1	Sterol regulatory element binding protein-1
SREBP-1c	Sterol regulatory element binding protein-1 c
T2DM	Type II Diabetes Mellitus
TBARS	Thiobarbituric acid reactive substances
TCA cycle	Tricarboxylic acid cycle
TG	Triglyceride
TLR	Toll-like receptors
TNFα	Tumour Necrosis Factor α
USD	United States Dollar
WHR	waist: hip ratio

Synopsis

Fructose is commonly known as 'fruit sugar', but is also a major component of table sugar and high fructose corn syrup. The way the human body absorbs and metabolises fructose is different from any other sugar molecule. Absorption of fructose is enhanced in the presence of glucose and unregulated, due to its passive diffusion into the blood stream. Similarly, excess fructose intake is metabolised in a way that may contribute to the development of chronic diseases. A review of the literature has shown positive associations between high fructose intake and cardiovascular disease risk factors, i.e. increased TG, HDL, total cholesterol and LDL & reduced HDL blood lipids [1-4] [5-9], development of insulin resistance [10-15], alteration in the production of satiety hormones: insulin [6] [16], leptin [6, 17] and ghrelin [6, 18, 19], increase in the level of inflammatory biomarkers (TNF-α, IL-6 etc.) [20-23] and increase in body weight or obesity [10, 24-28], in some but not all studies.

Extensive literature review has revealed that no work has been done on restricting fructose intake and its effect on disease risk in healthy individuals. We hypothesised that restricting dietary fructose intake would result in improved glycaemic indices, reduced circulating lipid levels and low grade inflammation in healthy individuals.

Prior to testing the effect of restriction of fructose consumption in the diet, we looked at the effect of acute and chronic consumption of fructose in healthy individuals. In the acute study (Chapter 3, published: Lipids in Health and Disease *2014*, 13: *195*), fructose was consumed as the sole source of energy without an accompanying meal. The reason for the increase in postprandial levels of total, LDL and HDL cholesterol in subjects who consumed fructose in the acute study is not known. Since no nutrients, other than sugars, were included in the test beverages, the lipoproteins measured were almost exclusively of hepatic origin. The lipemic

effects of fructose may depend on the dose and duration of fructose feeding and whether fructose is consumed in the presence or absence of other energy nutrients and also whether consumed as a substitute for another sugar or as a supplement in excess of energy requirements.

In the chronic study (chapter 4), fructose was used to supplement usual diets for a period of 4 weeks. When fructose was consumed for 4 weeks in addition to the usual diet, it was found to cause significant changes in glucose metabolism without causing any significant change in lipid and hs-CRP levels. It appears that at the dose and duration used in chronic study, the type of sugar (fructose or glucose) consumed increases fasting blood glucose levels but does not modulate other CVD risk factors such as lipid profile, insulin and low grade inflammation in healthy individuals. Hence consumption of a diet containing fructose at these moderate levels does not increase CVD risk in healthy individuals.

Conversely, the fructose restriction study (chapter 5) demonstrated that consumption of a low fructose diet (< 8g/day, less than 2% energy from fructose) resulted in a statistically significant decrease in BFM, BMI and a small decrease in weight (statistically non-significant). This suggests the potential for clinically important weight reduction to be observed if the duration of intervention was increased. There was no significant difference in other parameters of anthropometric measurements, body composition and blood biomarkers of lipids or systemic inflammation.

In conclusion, as fructose is metabolised differently to other sugars, this becomes important in ascertaining the effect of sugar consumption on cardiovascular health [29]. However, fructose when substituted for glucose in isocaloric diets and not consumed as excess energy, may not increase the risk of developing cardio-metabolic disease.

Thesis structure and overview

This thesis consists of six chapters including one published study in Lipids in Health and Disease. The thesis begins with an introduction and review of the literature (Chapter 1) followed by the methodology undertaken in the conduct of the research (Chapter 2). The introduction, methods, results, discussion and implications of the research conducted for this thesis are then presented as a series of three chapters (Chapter 3 to 5). This thesis presents work from a body of research comprised of three human research studies; (i) an acute fructose supplementation study (chapter 3), a chronic fructose supplementation study (chapter 4) and a fructose restricted study (chapter 5). An overall discussion of the findings from this body of research and its implications are provided as the final chapter of the thesis (chapter 6).