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

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Master of Pharmacy

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

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The University of Newcastle, Australia

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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 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.

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Conference Abstracts:

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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
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

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

Dietary carbohydrates are needed for energy production to fuel physiological functions [30, 31]. Of carbohydrates, a significant portion comes into the diet from sugars (monosaccharides and disaccharides), an important constitutive part of the food that we consume. Sugars are in general omnipresent substances [31] that are either a naturally occurring component of our food, added during processing or preparation of foods, or consumed as table sugar. A healthy diet contains sugar in addition to other nutrients because it is an integral component of fruit, vegetables, dairy products, and many grains [32]. Further, sugars add desired palatability and promote enjoyment of foods [32]. People tend to overconsume food with increased palatability, thus contributing to increased caloric intake, in excess of caloric expenditure [33]. Further, humans have an innate preference and attractiveness for sweetness since birth [34], one of the reasons for substantial consumption of sugars [35]. More than 2100kJ/day are consumed from added sugar alone in many parts of the world [36] (Figure 1-1). The added sugar in human diets is either sucrose (50% fructose + 50% glucose) or high fructose corn syrup (HFCS) (usually contains 42 to 55% fructose). HFCS is the favoured additive in the preparation of processed food because it is sweeter than sucrose, improves colour and texture, offers a cheaper alternative and can be used as a preservative in food [33].
The amount of total sugar present in different food sources can be estimated as grams per 100 gram edible portion. Common fruits like grapes contain 18.1g of total sugar followed by banana (15.6g), cherry (14.6g), apple (13.3g), pineapple (11.9g) and watermelon (9.0g).

Further, common vegetables like carrots contain 6.6g of total sugar followed by onions (6.2g), peas (5.8g) and tomato (2.8g). However, processed foods can contain very high levels of sugar, for example toffee contains 55.4 g of total sugar per 100g, milk chocolate contains 52.1 g, fruit cake contains 43.1g, fruit cocktail contains 15.3g and cola contains 10.6g (Table 1-1) [37].
## TABLE 1-1: SELECTED SUGAR CONTENTS OF FOOD, (GRAMS PER 100 G EDIBLE PORION)

<table>
<thead>
<tr>
<th>Food</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Sucrose</th>
<th>Total Sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fruits</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple, raw</td>
<td>2.3</td>
<td>7.6</td>
<td>3.3</td>
<td>13.3</td>
</tr>
<tr>
<td>Banana, raw</td>
<td>4.2</td>
<td>2.7</td>
<td>6.5</td>
<td>15.6</td>
</tr>
<tr>
<td>Cherry, raw</td>
<td>8.1</td>
<td>6.2</td>
<td>0.2</td>
<td>14.6</td>
</tr>
<tr>
<td>Pineapple, raw</td>
<td>2.9</td>
<td>2.1</td>
<td>3.1</td>
<td>11.9</td>
</tr>
<tr>
<td>Watermelon</td>
<td>1.6</td>
<td>3.3</td>
<td>3.6</td>
<td>9.0</td>
</tr>
<tr>
<td><strong>Vegetables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrot, raw</td>
<td>1.0</td>
<td>1.0</td>
<td>3.6</td>
<td>6.6</td>
</tr>
<tr>
<td>Corn, sweet</td>
<td>0.5</td>
<td>0.3</td>
<td>1.5</td>
<td>2.6</td>
</tr>
<tr>
<td>Onion, raw</td>
<td>2.4</td>
<td>0.9</td>
<td>1.3</td>
<td>6.2</td>
</tr>
<tr>
<td>Peas, cooked</td>
<td>0.2</td>
<td>0.1</td>
<td>4.8</td>
<td>5.8</td>
</tr>
<tr>
<td>Tomato, raw</td>
<td>1.1</td>
<td>1.4</td>
<td>0</td>
<td>2.8</td>
</tr>
<tr>
<td><strong>Legumes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beans, w/pork</td>
<td>1.6</td>
<td>1.4</td>
<td>4.3</td>
<td>8.3</td>
</tr>
<tr>
<td>Chick-peas</td>
<td>0.1</td>
<td>0.1</td>
<td>1.2</td>
<td>4.8</td>
</tr>
<tr>
<td>Lentils, cooked</td>
<td>0</td>
<td>0.1</td>
<td>0.5</td>
<td>1.8</td>
</tr>
<tr>
<td>Peanuts, dried</td>
<td>0.2</td>
<td>0</td>
<td>3.8</td>
<td>4.3</td>
</tr>
<tr>
<td>Soybeans</td>
<td>0.1</td>
<td>0.2</td>
<td>0.5</td>
<td>3.0</td>
</tr>
<tr>
<td><strong>Sweets</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn syrup</td>
<td>14.9</td>
<td>1.2</td>
<td>2.2</td>
<td>37.0</td>
</tr>
<tr>
<td>Honey</td>
<td>33.8</td>
<td>42.4</td>
<td>1.5</td>
<td>81.9</td>
</tr>
<tr>
<td>Molasses</td>
<td>7.4</td>
<td>7.9</td>
<td>26.9</td>
<td>42.8</td>
</tr>
<tr>
<td>Maple syrup</td>
<td>2.3</td>
<td>0.9</td>
<td>59.1</td>
<td>62.3</td>
</tr>
<tr>
<td>Brown sugar</td>
<td>5.2</td>
<td>0.4</td>
<td>84.3</td>
<td>89.9</td>
</tr>
<tr>
<td><strong>Processed Foods</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit cocktail</td>
<td>6.0</td>
<td>6.0</td>
<td>3.3</td>
<td>15.3</td>
</tr>
<tr>
<td>Orange Juice</td>
<td>5.3</td>
<td>4.6</td>
<td>0.7</td>
<td>10.6</td>
</tr>
<tr>
<td>Bread white</td>
<td>1.8</td>
<td>1.5</td>
<td>0.1</td>
<td>3.9</td>
</tr>
<tr>
<td>Fruit cake</td>
<td>11.3</td>
<td>11.3</td>
<td>20.5</td>
<td>43.1</td>
</tr>
<tr>
<td>Cola</td>
<td>4.0</td>
<td>4.4</td>
<td>2.1</td>
<td>10.6</td>
</tr>
<tr>
<td>Milk Chocolate</td>
<td>0.2</td>
<td>0.1</td>
<td>46.8</td>
<td>52.1</td>
</tr>
<tr>
<td>Toffee</td>
<td>6.7</td>
<td>5.2</td>
<td>40.9</td>
<td>55.4</td>
</tr>
<tr>
<td>Raisin bran</td>
<td>7.3</td>
<td>8.2</td>
<td>10.1</td>
<td>26.6</td>
</tr>
<tr>
<td>Shredded wheat</td>
<td>0.1</td>
<td>0</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Cherry brandy</td>
<td>16.5</td>
<td>16.1</td>
<td>0</td>
<td>32.6</td>
</tr>
</tbody>
</table>

Source: Selected sugar contents of food [37]

According to the American Heart Association, the maximum amount of added sugar recommended is 630kJ/day (39.3g or 9.4 teaspoons) for males and 420kJ/day (26.2g or 6.25 teaspoons) for females [38, 39]. However, the typical western diet is higher in sugar content, with an average intake of 49g fructose per day, of which only 8g is from natural sources [39,
The remaining 41g of added fructose is present as a component of sucrose (1314.6kj). This is well above the recommendation of American Heart Association for a healthy diet [38, 39]. Overall, there is an increase in caloric consumption in recent years; on average, an additional 630-1260kj per day is being consumed, almost 50% of which comes from liquid calories, in particular sugary drinks [41]. Concern has been expressed about the high intake of sugar [37, 42, 43] and its possible adverse effects on health [44]. Studies involving added sugar, particularly sugar sweetened beverages (SSBs) have shown a detrimental effect on body weight [45], obesity [46-49], diabetes [47], dyslipidaemia [50, 51], hypertension [52] and increased risk of cardiovascular disease [53, 54].

All forms of sugar break down into the smallest possible sugar unit with the help of digestive enzymes. Fructose and glucose, being monosaccharides, are already in the simplest form and do not require any chemical digestion prior to absorption in the small intestine. Sucrose after ingestion is hydrolysed by sucrase, an enzyme located in the brush border of the small intestine. This hydrolysis of sucrose yields one molecule of glucose and one molecule of fructose [55] to be metabolised further by the human body. As fructose is metabolised differently from glucose, this becomes important in ascertaining the effect of sugar consumption on cardiovascular health [29]. This thesis is focused on examining some of the cardio-metabolic effects of fructose in comparison to glucose.
1.2. Fructose

Fructose, also known as fruit sugar or levulose, has the same molecular formula as glucose i.e. C₆H₁₂O₆. It forms a part of the sucrose molecule with glucose linked through glycosidic linkage as shown below.

Fructose, the sweetest sugar, is up to 75% more (1.15-1.75 times) sweet than sucrose, but its sweetness depends on pH, temperature, and concentration [56, 57]. Fructose is a useful sweetener in soft drinks because its solution is sweetest when it is dilute, cool and slightly acidic [57]. Fructose is present in the human diet in three forms: as free fructose in the monosaccharide form (e.g. fruits & honey), as a part of sucrose (sugar cane, beet crop etc.) or in the polymerized form [58, 59]. Total fructose refers to the monosaccharide form plus the fructose that is bound to glucose in sucrose molecules [20]. The polymerised forms of fructose include fructans, oligo- and polysaccharides, which comprise short chains of fructose units with a single D-glucosyl unit at the non-reducing end [60-62]. Fructooligosaccharides
(FOS) or oligofructose are fructans with a short chain length (i.e., degree of polymerisation, DP) of 2 to 9 units. Fructans with a longer chain length of more than 10 units are called inulin [61, 63].

1.3. Food sources of fructose

Fructose in the diet of our ancestors used to be primarily consumed in whole fruits and vegetable. Since sugar plant farming was introduced, fructose primarily comes from the sugar plant or corn (HFCS) [64]. HFCS represents a significant percentage of the sweeteners added to foods and beverages and is the only caloric sweetener in soft drinks in the USA [65]. HFCS is produced from corn through conversion of corn starch into corn syrup (glucose syrup) and further isomerisation of glucose syrup to yield high fructose content, primarily HFCS-90, by isomerase. This is then mixed with glucose to yield HFCS-55 and HFCS-42 [66]. HFCS-55 contains 55%, while HFCS-42 contains 42% fructose [67].

Unlike in Australia, where there is sugar cane farming, in the USA there is higher dependency on corn and beet crops for sugar due to the lack of sugar cane plantations. In USA, beverages sweetened by sugar are the main source of fructose intake among children and adults, followed by grains, processed foods, fruits or fruit juices and vegetables [64, 68]. Notably, Australia does not have any other reported data for food sources of fructose except for one that exists for vegetables and fruits [69]. Among common Australian fruits, different varieties of apples contain 6.3 to 6.9g, bananas 2.2 to 5.4g and grapes contain 7.0 to 10.0 g of free fructose. Among common Australian vegetables, onion contains 0.8 to 6.0 g and different varieties of tomato contain 1.5 to 2.4 g of free fructose per 100 g fresh weight [69].
1.4. Dietary intake of fructose

Fructose may be economical for food manufacturers, as a relatively smaller amount of fructose is required to achieve the same sweetness in comparison to glucose or sucrose, particularly at low temperatures. Moreover, it was initially thought that fructose could be used to control energy intake, as a lesser amount provides the same sweetness. However, there has been an overall increase in the caloric consumption of sugar in recent years, most of which comes from sugary drinks made with fructose [41]. The dietary food consumption surveys give some insight into dietary fructose intake. However, the presence of free and bound fructose makes this task difficult. While accurate data are impossible to achieve, some estimation has been done by different researchers [59]. Depending on dietary habits and use of fructose as a sweetening agent, daily per capita consumption of fructose varies across the globe [59].

Fructose consumption in children, adolescents, adults and particularly in obese individuals is high in the USA [64] [68]. Consumption of fructose from sucrose, HFCS and honey constitutes approximately 8% of total calorie intake in the USA [37]. Furthermore, the daily average intake of HFCS is 132kcal for Americans ≥ 2 years of age [65]. While data on sugar consumption in Australia is available, there is no reported data about fructose consumption in the Australian population except the recently published data about consumption of fructose in Western Australian adolescents. This data shows that the contribution of fructose to total energy intake was 9.1% among adolescents [70]. Therefore, fructose intake in Australian adult populations merits investigations in the light of the fact that the major source of fructose in Australia is likely to be table sugar (sucrose) rather than HFCS.
The world market for HFCS has grown from USD 2858.56 million in 2001 to 3983.04 million in 2011 [71]. Fructose consumption has increased in the USA, especially from HFCS in previous decades. HFCS consumption in the USA has increased significantly between 1970-1990 [72]. In 1997, consumption of fructose from the total intake of sucrose and HFCS was 81g/day, compared to 64g/day in 1970 [73].

Although we don’t have any reported data for fructose consumption in Australia, reported data shows an increase in total sugar consumption [42, 43] and increase in the volume and value of imported sweetened products [74]. Moreover, the Australian market for HFCS has grown from USD 31.27 million in 2001 to 41.66 million in 2011. The Australian market constitutes 84.55% of the entire Oceania region for HFCS. Sydney was identified as the top market for HFCS in Australia holding 29.32% of the market share in 2005, followed by Melbourne and Brisbane [71].

1.5. Absorption of Fructose

When fructose reaches the gut either as free fructose, sucrose or HFCS, it is transported passively from the lumen by fructose specific hexose transporter, glucose transporter 5 (GLUT5) to the epithelium where it is absorbed into the systemic circulation [75, 76]. Fructose is released into the cytoplasm through facilitated diffusion by glucose transporter 2 (GLUT2) [75].

Free fructose absorption is quantitatively limited, ranging from 5-50g in healthy subjects [77]. However, when fructose is consumed bound to glucose, as in sucrose or HFCS, its absorption capacity is considerably enhanced in a dose dependent manner [77, 78].

![Fructose Absorption Diagram]

Fructose $\xrightarrow{\text{GLUT5}}$ Fructose $\xrightarrow{\text{GLUT2}}$ Fructose

(Lumen) (Epithelium) (Cytoplasm)
1.6. Comparison between Glucose and Fructose absorption

After digestion, glucose is taken up from the lumen of the small intestine into the enterocyte for absorption through a Na⁺coupled transport protein called Na⁺glucose transporter (secondary active transport symporter) 1 (SGLT1) [75, 79]. This contrasts with the absorption of fructose (as described above), which is transported passively by facilitated diffusion transporter GLUT5. The low intracellular concentration of fructose is maintained by its conversion into glucose and lactic acid. This low intracellular concentration helps in its continued absorption from the lumen. As glucose is absorbed by active transport by Na-K-ATPase against the concentration gradient, there is a regulation of glucose absorption. Fructose however, is passively absorbed, then delivered to the liver through the hepatic portal vein for metabolism, hence there is no regulation.

1.7. Hepatic metabolism of fructose

The majority of fructose in the portal vein is taken up by the liver for conversion into glucose, glycogen and lactate [80]. A comparatively small amount of fructose may be oxidized or converted into fatty acids [80]. The fatty acids formed are either secreted as VLDL-TG or stored within the liver as intra-hepatocellular lipids (IHCL) (Figure 1-2) [80]. Phosphofructokinase is an enzyme that phosphorylates fructose-6-phosphate, which is a key regulatory step in the glycolysis (Figure 1-3). Phosphofructokinase acts on fructose once it enters into liver and is not under negative feedback control of citrate and ATP unlike glucose metabolising enzymes[81]. This allows fructose to be an uncontrolled source for de novo lipogenesis [10].
Absorbed glucose, or glucose that is produced in the liver from fructose or from other precursors, is either processed in the liver or sent to the circulation and further to extra hepatic tissues [82]. Absorbed fructose is metabolised in the liver, with little fructose delivered to systemic circulation or peripheral tissues [82]. Most absorbed fructose is cleaved into glyceraldehyde and DHAP, and these trioses go to glycerol phosphate and pyruvate metabolic pathways, respectively [82]. The conundrum in understanding hepatic fructose metabolism is to determine the origin of carbon in triose-phosphates, whether they came from fructose or glucose [83].

Fluxes of carbon between different metabolic pathways and the metabolic fate of dietary fructose has been studied with the help of isotope tracer studies [82]. A review of these isotope tracer studies indicated that the mean oxidation rate of dietary fructose was 45% and the mean conversion rate of fructose to glucose was 41% of ingested fructose in normal subjects. In addition, approximately 1/4 of ingested fructose could be converted into lactate in normal subjects [82]. However, the quantity of fructose that is converted to glycogen needs to be further clarified [82].

After intake of sugar, the usage of energy sources by the body changes. When the carbohydrate from external sources is used as a fuel source, the oxidation rates of stored energy, typically, endogenous carbohydrates and fat, will decrease [82]. In the resting state, fructose will be preferentially oxidized. However, in exercise conditions, glucose is likely to be preferentially oxidized over fructose [82]. Although, limited information is available about the shifting of energy sources under different conditions, studies involving participants under exercise provides some information regarding energy shifting after sugar ingestion [82]. Under exercise conditions, the mean oxidation rate of dietary fructose was 45.8%. Further,
when fructose was taken with glucose, the mean oxidation rate of mixed sugar increased to 66% in exercise conditions. Glucose containing drinks were shown to decrease the oxidation rates of fat and endogenous carbohydrate by 22-42% and 9-32% respectively compared to control (zero gram of sugar intake). Fructose containing drinks also decreased the oxidation rates of fat and endogenous carbohydrate by a similar proportion (20-47 and 13-32% respectively compared to control) [82]. The decreases in oxidation rates were positively correlated with sugar intake levels and the ratio of fructose in the mixed sugar drinks [82]. Although, data derived from tracer studies does not directly represent real-life diets, it does provide a basic understanding of the utilization of fructose by the human body after its consumption.

![Splanchnic Metabolism of Fructose](image)

**FIGURE 1-2:** SPLANCHNIC METABOLISM OF FRUCTOSE, TAKEN FROM TAPPY ET AL [80]

### 1.8. Comparison between Glucose and Fructose metabolism

Fructose and glucose, being carbohydrates, are processed by carbohydrate metabolic pathways. In the liver, fructose and glucose metabolism converge at triose phosphate (DHAP & Glyceraldehyde-3-Phosphate (Ga-3-P)) levels. The crucial steps in fructose metabolism are derived from these intermediates, like glycerol-3-P for TG synthesis, generation of acetyl-
CoA for the TCA cycle and DNL [83]. It is suggested that any obvious adverse effects of fructose are likely to be mediated by an increase in the intermediates of carbohydrate metabolism [83] (Figure 1-3).

The key differences in glucose and fructose metabolism lie in the metabolic pathways that fructose and glucose follow (Figure 1-3). Metabolism of glucose is limited by glucokinase. After phosphorylation it is negatively regulated by the energy status of the cell by the phosphofructokinase regulatory step of glycolysis, while fructose by-passes this regulatory step and can continuously enter the glycolytic pathway. As a consequence of high influx of fructose to the liver, fructose increases hepatic pyruvate and lactic acid production [37, 57], activates pyruvate dehydrogenase (PDH), and shifts the balance from oxidation to esterification of unbound fatty acids, which can cause an increase in VLDL synthesis [84].

FIGURE 1-3: COMPARISON BETWEEN GLUCOSE AND FRUCTOSE METABOLISM, TAKEN FROM FEINMAN ET AL [83]

Glucose is first preferentially used by Krebs cycle for energy production, then it may be stored as glycogen, or can be utilised by other organs and if it is still available in excess, it is
converted to fatty acids. On the contrary, when fructose is consumed in excess of energy requirements, it is preferentially converted to fatty acids.

1.9. Fructose and cardiovascular disease risk factors

High intake of fructose raises questions about its metabolic effects. Of particular concern are the cardiovascular disease risk factors. These risk factors are elevated blood lipids, altered satiety hormones, insulin resistance, inflammation and obesity.

Epidemiological studies have provided evidence of adverse health effects of SSB consumption, including dyslipidaemia, obesity, visceral adiposity, hypertension, diabetes and insulin resistance [54, 85-96] and fructose has been implicated as it is the major sweetener in SSB [97]. Conversely, other epidemiological studies have reported that sugar consumption (fructose and non-fructose sugar) was not positively associated with indicators of metabolic syndrome [98] and an inverse association of high fructose intake with non-alcoholic fatty liver disease (NAFLD) was shown [99]. This controversy in the epidemiologic evidence suggests that evidence from clinical studies is needed to provide further insights into potential etiologies.

There is evidence emerging from systematic reviews and meta-analyses of controlled fructose feeding trials showing no adverse effects of fructose under isocaloric exchange of fructose for other carbohydrates, on body weight, fasting or postprandial lipids, non-alcoholic fatty liver disease (NAFLD), blood pressure and uric acid and showing benefit on glycaemic control [100-107]. At the same time, feeding fructose in hypercaloric diets appears to promote adverse effects of fructose on body weight, fasting and postprandial lipemia, uric acid and NAFLD [100-107]. Adverse health effects of fructose under hypercaloric feeding conditions
appear more attributable to excess energy than the fructose itself. In summary, direct experimental evidence of fructose consumption in the development of adverse health effects in humans is equivocal [13, 14, 78, 81, 108, 109] and more research is required. The available systematic reviews and analysis have suggested that longer, larger and high quality clinical trials are needed to ascertain the effects of fructose on human health.[100, 102, 104-106].

1.10. Blood lipids

The rate of fatty acid synthesis is affected by the nutritional status of the individual and the type of carbohydrate (sugar) consumed has an overall profound influence on lipogenesis [110]. Hepatic metabolism of fructose promotes lipogenesis, and as such, experimental studies have shown changes in blood lipid levels following fructose consumption [111]. Animal studies have shown overwhelming evidence of increased lipogenesis following feeding of fructose rich diets [110, 112, 113], resulting in significant elevation in blood cholesterol, VLDL, LDL cholesterol and a reduction in HDL [114-123]. In contrast, few animal studies have shown no effect of fructose consumption on triglyceride, LDL and total cholesterol levels [124-126]. This heterogeneity could be due to different doses used, different duration of studies, different types of animals etc.

A high fructose diet has been shown to cause hepatic insulin resistance, an increase in intra hepatocellular lipids (IHCL) and stimulation of hepatic de novo lipogenesis within a few days [4, 110]. When high fructose or sucrose diets are consumed over a long period of time it has been shown to induce hepatic steatosis [115, 127] and whole body insulin resistance with an associated build-up of intra-myocellular lipids (IMCL) [4, 128, 129]. Further, animal studies have also shown development of a complete metabolic syndrome following consumption of large amounts of fructose [97, 124-126, 130].
There are many mechanisms by which fructose are likely to promote hyperlipidaemia. Suggested mechanisms include increased hepatic *de novo* lipogenesis (DNL) [10, 131, 132] and decreased LPL activity, which reduces the rate at which TGs are cleared peripherally [38, 131, 133] thus contributing to increased TG concentrations. Fructose increases apoCIII (an inhibitor of LPL) production and reduces hydrolysis of TG [134, 135] which is a plausible mechanism for reduced hepatic clearance of TG. A decrease in LPL and LCAT (an enzyme that acts on HDL), key enzymes for HDL synthesis could all lead to reduction in HDL levels. LPL is sensitive to the effects of insulin and low levels of LPL can be related to insulin resistance caused by fructose [123].

Another possible mechanism leading to altered lipid metabolism has been shown in mice fed a high fructose diet, which led to an increase in hepatic sterol regulatory element binding protein (SREBP-1) expression, a transcription factor responsible for the regulation of cholesterol and fatty acid biosynthesis. Furthermore, SREBP-1 also regulates lipogenic gene expression, including fatty acid synthase (FAS) and acetyl Co-A carboxylase in mice [136].

An additional proposed mechanism relates to metabolic pathways of fructose. The starting point for fructose metabolism is fructokinase catalysed phosphorylation. Fructose enters into glycolysis at the triose phosphate level, i.e. dihydroxyacetone phosphate and glyceraldehyde-3-phosphate [81] bypassing rate limiting step of PFK (phosphofructokinase). The rate controlling step of conversion of glyceraldehyde-3-phosphate to pyruvate and lactate is catalysed by pyruvate kinase [137]. Fructose-1-P causes enhanced activation of pyruvate kinase compared to F-1,6-BP [138]. Consequently, hepatic pyruvate and lactate formation increases, activation of pyruvate dehydrogenase occurs and the balance shifts to
esterification of non-esterified fatty acids (NEFA), leading to higher secretion of VLDL [137]. Further, fructose conversion to triose phosphate is an insulin independent (contrary to glucose, which is insulin dependent) rapid process because of low Km (Michaelis constant) ~0.5 mM of fructokinase for fructose and no negative feedback by ATP or citrate [33]. This allows fructose to be an uncontrolled source of both glycerol-3-phosphate and acetyl-coenzyme A (CoA) for lipogenesis to occur in the liver and can lead to large amounts of triglyceride (TG) being packaged as VLDL by the liver [81].

In summary, fructose intake can promote hyperlipidaemia because, firstly, the liver is the main site where fructose is metabolised [139]; secondly, fructose enters into glycolysis at the triose phosphate level, thus allowing fructose to provide an uncontrolled source of both glycerol-3-phosphate and acetyl-coenzyme A (CoA) for lipogenesis to occur in the liver, leading to the production of large amounts of VLDL [81], and thirdly, fructose can stimulate hepatic sterol regulatory element binding protein-1 c (SREBP-1c), independent of insulin, which can activate genes involved in lipogenesis [97, 108, 136].

Studies carried out in human subjects [3, 5, 7, 9, 20] have also reported adverse effects of fructose on lipemia. In a short-term fructose consumption in healthy subjects, increased fractional DNL and plasma triglyceride levels were observed. DNL was speculated to be responsible for the observed increase in TG level. However, the dose of fructose given to participants was very high (3g/kg body weight/day), so clinical relevance of this study may be questionable [15]. In another study involving healthy subjects on a diet containing 20% energy from fructose, total cholesterol and LDL-cholesterol were increased compared to isocaloric starch after 4 weeks [9]. Further, there was a transient increase in triglyceride levels after only one day of the intervention in the fructose-rich diet group. Therefore, it can
be concluded that fructose-induced hypertriglyceridemia can be observed in as little as 24 hours [9]. In a longer intervention (6 weeks) study consisting of healthy subjects, the effects of a diet containing 17% energy from fructose or glucose were compared. The fructose group had a transient increase in levels of fasting plasma cholesterol and LDL-cholesterol on day 28 in comparison to glucose [140]. Further, it has been shown in healthy subjects that high fructose diets inhibit several pathways of lipid catabolism (lipolysis and lipid oxidation) besides the increase in TAG concentration [141]. In a study examining post prandial effects of fructose consumption in healthy subjects after a breakfast and subsequent lunch (more representative of normal dietary pattern where a person eats every 4-6 hours), lipogenesis were measured. The data showed that acute intake of fructose (in a mixture of sugars) in the fasted state stimulates early lipogenesis and augments triglyceride synthesis post-prandially. Further, the authors suggested that the additional post lunch increase in TGs was due to a carry-over effect from the morning, indicating the creation of a metabolic milieu by fructose consumption that interferes with handling of nutrients going to liver [132].

In other studies, consumption of fructose has been shown to increase circulating triglyceride levels in human subjects [2] [1]. Further, fructose intake has been reported to increase fasting, postprandial and day long levels of plasma triacylglycerol in comparison to glucose [3, 4]. Fructose sweetened beverages have been reported to cause a significant elevation in triglyceride levels in comparison to glucose sweetened beverages taken with meals in a long term [6] and a short term study [7]. Additionally, total plasma cholesterol and LDL cholesterol were reported to be elevated in subjects with a high fructose intake in comparison to controls [8] [9]. Moreover, few studies have shown reduction in HDL-cholesterol level following fructose consumption compared to baseline [142, 143]. Consumption of fructose sweetened beverages was found to be associated with a reduction in LDL particle size.
(smaller, dense LDL particles are more atherogenic) in comparison with glucose and sucrose sweetened beverages in healthy subjects [20]; however, this study was a cross sectional, not interventional. Interestingly, one study has reported a reduction in total cholesterol level when fructose was compared with sucrose consumption[142]. The author has reported that reduction in total cholesterol level could be because of the low level of cholesterol and fat in the test diet[142].

In contrast to the studies mentioned above, some studies have demonstrated no effects of consumption of fructose on blood lipids. In a study examining the effects of fructose substitution for sucrose in the diet reported no increase in fasting plasma triglyceride level. Sucrose was given at baseline (3-4 days) and then fructose (63-99g/day) was substituted for the sucrose during the study period of 14 days[142]. Sugars in this study were added into standard food products and were also provided as an added sweetener. In a study where 30-60g (≈4—12% of energy) fructose was added to the diet, no significant effects on lipid levels were reported [17, 144]. Similarly, consumption of isoenergetic (fructose=21%, sucrose= 23% and almost all carbohydrate energy as starch) fructose and sucrose diets for 8 days did not significantly increase triglyceride levels when compared with the starch diet in diabetic subjects [145]. Further, in a mixed population of hypertriglyceridemic and diabetic subjects, no effects of consumption of fructose for 2 weeks on the levels of triglyceride was shown[146].

In another consumption of fructose or sucrose incorporated into isocaloric diets showed no adverse effects. No change in triglyceride, LDL, HDL and total cholesterol levels were reported after 14 days of sucrose or fructose consumption [147]. The amounts of sugars consumed were 50-107g/day (1/3rd total carbohydrate intake).
Similarly, another study showed no change in triglyceride levels after 4 weeks of fructose versus glucose consumption. However, subjects were impaired glucose tolerant. Moreover, no change in total cholesterol level was shown in both normal and impaired glucose tolerant subjects in the same study. The glucose or fructose was given in the range of 45-83g/day in impaired and 45-112g in normal glucose tolerant subjects. Other sources of sugars or sugar added foods were excluded from the diets [148]. Yet another study showed no change in LDL, HDL and total cholesterol in a study involving 4 weeks of hyper energetic diets (150g of glucose or fructose/day) in addition to a weight maintaining diet [149]. In a study comparing fructose versus glucose consumption for 6 weeks no effect on LDL, HDL and total cholesterol levels were reported[140]. The diets were isoenergetic and identical, except for the addition of crystalline fructose (14% of energy) in one and glucose (14% of energy) in the other diet[140].

It is important to note, however, that some of these studies compared fructose with sucrose which is not optimal, as fructose is a component of sucrose.

As evident from the above mentioned studies, results on the relationship between a high fructose diet and elevation in blood lipid levels are varied. This therefore, requires further investigation to establish an association between fructose intake and blood lipids. To date, there has been no research carried out with a fructose restricted diet and its effect on blood lipids in healthy subjects. We hypothesise that restricting fructose intake will improve blood lipid values and result in reduction of one of the major cardiovascular disease risk factors.
1.11. **Insulin resistance**

In healthy individuals, insulin concentrations increase after a meal, which lowers blood glucose levels. This occurs due to the role of insulin in stimulating glucose uptake and metabolism. Insulin also affects circulating lipid levels by reducing hepatic VLDL-TG formation from free fatty acids [150]. This occurs due to insulin suppressing lipogenesis, which reduces the supply of free fatty acids to liver [151]. In addition, insulin also reduces lipolysis by inhibiting lipase [150]. Insulin resistance occurs when normal physiological concentrations of insulin are not able to produce effective uptake of nutrients by the cells. This can lead to control of glucose and circulating blood lipids being compromised [150].

Unlike glucose, fructose does not induce insulin secretion [6]. Reduced insulin secretion along with reduced leptin (transfers information on energy intake to central nervous system) and failure of fructose to suppress ghrelin (plays a role in the regulation of food intake), could lead to decrease in satiety and increased food intake, resulting in insulin resistance, possibly secondary to increase in BFM, following long term fructose consumption [6, 152].

Excess caloric consumption is one of the significant contributors to insulin resistance [153] and studies have reported that carbohydrate excess may contribute to the development of insulin resistance [153]. Sugar, in particular fructose may be of interest due to its potential to induce insulin resistance [153], as shown in animal studies carried out on rodents [114, 121, 130, 154-161], monkeys [162] and dogs [163]. Fructose-induced lipogenesis results in dyslipidaemia that leads to deposition of ectopic fat in muscles. Lipid metabolites generated from ectopic fat impair insulin signalling leading to insulin resistance [33]. Another proposed mechanism is that excessive intake of fructose causes formation of reactive oxygen species.
(ROS) that leads to build up of endoplasmic reticulum (ER) stress that causes impaired insulin signalling, leading to insulin resistance. In addition, excessive intake of fructose causes excess formation of uric acid, leading to a condition called hyperuricemia, which causes impairment in vasodilation that may lead to insulin resistance [33].

Although evidence of high fructose intake and development of insulin resistance is more limited in human subjects, few studies have shown a reduction in insulin sensitivity after high fructose intake in healthy, overweight/obese subjects [10] [11] and hyperinsulinemic subjects [12]. Short duration trials have also demonstrated that high intake of fructose may lead to development of insulin resistance [13-15].

The absence of any studies that demonstrate the impact of fructose-restricted diets on insulin resistance highlights the need for further research on human subjects. We hypothesise that by restricting intake of fructose, insulin sensitivity can be improved and that will ultimately lead to a reduction in one or more of the cardiovascular disease risk factors.
Excess Dietary Fructose

Hepatic de novo Lipogenesis

Hepatic VLDL-triglyceride Secretion & Extrahepatic VLDL-triglyceride Clearance

Hypertriglyceridemia

Ectopic Triglyceride Accumulation (Muscle & Liver)

Promotes

Obesity and Insulin Resistance

FIGURE 1-4: EXCESS FRUCTOSE INTAKE LEADING TO OBESITY AND INSULIN RESISTANCE, ADAPTED FROM [152]
1.12. Inflammation

Inflammation, although necessary to protect and defend the body against injury or infection, can cause or contribute to the damage of tissue and development of different disease conditions when occurring in excess [164]. Inflammatory mediators (such as IL-1β, IL-6, TNFα and CRP) produced following activation of transcription factors such as NFκ-B, play an important role in the development of chronic diseases.

There are several extracellular initiating events through which NF-κB, a master controller of inflammation, can be activated following excess fructose consumption (Figure 1-5). These encompass production of reactive oxygen species (ROS) and eicosanoids (PGE₂ and LTs), interaction with inflammatory cytokines (TNF, IL-1β, IL-6 and IFN-γ) and activation of pattern recognition receptors such as toll-like receptors (TLR) [165]. Many of these initiating signals are modulated by dietary factors [166] and fructose is hypothesised to be one of the dietary components responsible for the potentiation of inflammation.

1.12.1. Role of fructose in inflammation

Several studies conducted in vitro have documented that fructose can induce inflammation and oxidative stress. Fructose was shown to cause increased production of reactive oxygen species (ROS), an important mechanism that contributes to the development of inflammation, in a kidney (HK-2) cell line [23, 167, 168]. Increased activity of NF-κB, that fuels inflammation through various mechanisms, has been shown in the vascular smooth muscle cells of fructose fed rats[169]. Fructose can also induce the expression of monocyte chemotactic protein 1 (MCP-1), a chemo attractant that plays an important role in inflammation-mediated damage[170] in several cell types, including human kidney (HK-2) cells[23, 171] and human aortic endothelial cells (HEAC) [22]. Furthermore, an upregulation
of intercellular adhesion molecule-1 (ICAM-1), which is responsible for the entry of inflammatory cells to the site of inflammation, was reported in fructose supplemented human aortic endothelial cells (HEAC) [22]. This was demonstrated at a concentration of fructose that is readily achievable in humans following consumption of a large fructose-based meal[73]. Fructose has also been reported to promote significant leukocyte adherence to the endothelium at a concentration similar to what is achieved in human post prandial after a fructose meal [172]. The adhesion of leukocytes to vascular endothelium is a distinctive feature of the inflammatory process.

The effects of fructose have also been studied in a variety of in vivo studies. Fructose was found to cause an elevation in the levels of inflammatory biomarkers in animals consuming up to 60% of energy derived from fructose [171, 173-175]. A high fructose diet has been shown to increase plasma levels of cytokines including IL-1 and IL-6 in rats [174]. Furthermore, animal studies have shown induction of the c-jun N-terminal kinase (JNKs) signalling pathway by fructose feeding [176, 177], which activates the transcription factor c-Jun [178], contributing to inflammation [179]. The inflammatory cytokine, TNF-α, has been found to be elevated after fructose feeding in two animal studies [173, 175].

There is also evidence in the literature that overproduction/accumulation of ROS and consequent oxidative stress in response to fructose feeding in rats [180, 181] leads to inflammation by increasing plasma concentration of thiobarbituric acid reactive substances (TBARS, an index of lipid peroxidation), advanced oxidation protein products (AOPP) and advanced glycation end products (AGEs)[180, 181].
Recent epidemiological [11, 65, 81, 182] and clinical [182] studies in humans have also implicated increased fructose consumption in the development of inflammation. Plasma levels of CRP are an important biomarker to define systemic inflammation [183] and fructose consumption has been shown to increase CRP levels [20]. Interestingly, one study has shown that consumption of a low fructose diet can reduce inflammation by decreasing hs-CRP and soluble intercellular adhesion molecule (sICAM) levels in CKD patients [184]. On the other hand, ICAM levels were found to be elevated after fructose feeding in overweight or obese subjects [185]. Plasma concentrations of MCP-1 (chemo-attractant) have also been shown to be significantly elevated after fructose consumption in one study [186]. In the same study, a significant increase in soluble leukocyte adhesion molecule-1 (E-selectin) levels were shown [186], which is a pro-inflammatory mediator that is rapidly induced by cytokines [187]. However, no change in CRP, IL-6 or ICAM-1 was observed [186].

There is a paucity of published data looking at the effect of fructose on inflammation in human disease conditions. However, it is speculated that fructose may potentially affect inflammation in diabetes, cancer and neurological diseases by elevating inflammatory biomarkers involved in the initiation and progression of these diseases.
Interestingly, although it has been shown in various studies that fructose feeding elicits inflammation, there is a limited amount of evidence that fructose may also possess properties that protect the liver from inflammatory damage mediated by TNF-receptor-1 [188], possibly due to the extensive ATP depletion by fructose which prevents TNF-induced apoptosis [188, 189]. Indeed, in one study, fructose was reported to decrease levels of inflammatory mediators [189]. Thus while the majority of studies have shown potentiation of inflammation after fructose feeding, a few studies have shown a protective effect.

In conclusion, while there is some inconsistency in the data, the majority of the in vitro and in vivo animal and human studies support the argument that fructose is a pro-inflammatory nutrient (Table 1-2). Furthermore, there are a number of studies that implicate fructose as a nutrient that can potentiate a variety of chronic inflammatory diseases. Further studies are needed to explore the role of fructose as a pro-inflammatory nutrient, to determine whether
there is a need to develop strategies for restricting fructose intake in diet. Based on the observations described above, it can be suggested that the intake of a diet rich in fructose causes inflammation. However, human studies substantiating this hypothesis are lacking. Further clinical research on the impact of fructose restrictive diets on inflammatory biomarkers is required. We hypothesize that restricting intake of fructose will lead to improvement in the levels of inflammatory biomarkers.
1.12.2. Inflammation mediating chronic diseases

1.12.2.1. Cardiovascular disease and inflammation

There is growing evidence in the literature that inflammation plays a central role in the development of cardiovascular disease, from the initial lesion to thrombotic complications [190, 191]. Epidemiological studies have shown a positive association between elevated levels of circulating inflammatory bio-markers, such as CRP, TNF-alpha, IL-6 and increased risk of developing cardiovascular disease [192-198].

Adhesion molecules (like VCAM-1 or ICAM-1), pro-inflammatory cytokines (like TNF-α or IL-6), acute phase C-reactive protein (CRP), markers of oxidative stress and markers of endothelial dysfunction are associated with cardiovascular disease [33, 199-203]. In addition, epidemiological studies have found that elevated levels of cytokines, cellular adhesion molecules and acute-phase reactants such as CRP predict future cardiovascular events [197, 198, 200, 203-205]. Upon inflammatory insult by cytokines and acute phase proteins, expression of selectins and adhesion molecules (VCAM-1, ICAM-1) is induced, which heightens the risk of cardiovascular disease [206-210] by facilitating entry of monocytes into the arterial intima. Moreover, under conditions of oxidative stress, accumulation of oxidized lipids in the artery, such as oxidised LDL, can induce expression of adhesion molecules such as VCAM-1 in endothelial cells [211]. This pro-inflammatory state is associated with endothelial dysfunction [191, 212]. It has been shown that fructose fed rats develop endothelial dysfunction [213]. Furthermore, fructose can induce expression of monocyte chemotactic protein 1 (MCP-1) [23, 171], upregulate intercellular adhesion molecule-1 (ICAM-1) [22] and it has also been reported to promote significant leukocyte adherence to the endothelium [172].
1.12.2.2. **NAFLD and inflammation**

NAFLD is one of the major health problems in developed countries and estimated to affect 30% of the general population [214-216]. It is characterized by triglyceride accumulation in the liver leading to steatosis and hepatic inflammation [217]. NAFLD includes a spectrum of disorders, from fatty liver to non-alcoholic steatohepatitis (NASH) and cirrhosis [214, 218]. Epidemiological studies have shown an association of NAFLD with systemic inflammation [219] and association between hepatic steatosis (accumulation of fat in the liver; a common feature in NAFLD) and increased hs-CRP levels [220]. Feeding fructose is considered as a classical model for inducing NAFLD in rats with similarities in alterations caused by fructose in rodents and features of NAFLD observed in humans [221, 222]. Further, a fructose-fed rat model has been shown to be a better model for NASH than a fat-enriched diet [223]. Elevated levels of chemo-attractant protein-1 (MCP-1), IL-6, TNFα and CRP have been shown in NASH patients [224, 225]. In addition, elevated levels of IL-1α and TNFα have been shown in whole blood cell culture of non-alcoholic fatty liver disease (NAFLD) obese patients [226]. Experimental and epidemiological studies have shown that consumption of fructose is linked with NAFLD onset and progression [80, 222, 227]. Fructose consumption has been shown to cause hepatic inflammation in NAFLD subjects [228]. This is likely due to the conversion of fructose into fatty acids, after hepatic metabolism, which can be stored as TG in the liver [229]. Furthermore, there is no scientific evidence to articulate the dose of fructose responsible for development of NAFLD. However, literatures do blame excessive consumption of fructose for the development of NAFLD.
1.12.2.3.  **Diabetes and inflammation**

Epidemiological studies have shown that subclinical systemic inflammation (elevated levels of CRP and IL-6) is associated with increased risk of diabetes [192, 230-236]. Alteration in the levels of cytokines, chemokines and leukocytes, increased apoptosis and tissue fibrosis are indicators of the participation of inflammation in the pathogenesis of T2DM [237].

Studies have shown elevated levels of cytokines such as IL-1β, IL-6 and acute phase proteins such as CRP, in Type 2 diabetic patients [230, 238]. Various pathogenic factors cause insulin resistance in peripheral tissues via multiple metabolic pathways [239]. Stress-induced kinases, including IκB kinase-β (IKKβ) and JUN N-terminal kinase (JNK) [239], play a key role in the development of diabetes [240], as they activate NF-κB, that in turn induces TNF-α and IL-1β production [239]. In type 1 diabetes inhibition of islet of β cells in the pancreas by cytokines (IL-1, IL-6 and TNF-α) has been proposed as aetiological mechanism for the destruction of β cells [241, 242]. Fructose causes a modest increase in glucose and insulin levels after consumption [133, 243-245]. When fructose was exchanged with other sources of carbohydrate in diabetics, it was shown to improve long-term glycaemic control [107]. However, the potential impact of consuming large amount of fructose on lipid levels in diabetics is the matter of concern because they are already at higher risk of developing cardiovascular disease [78].
1.12.2.4. **Cancer and inflammation**

There is evidence in the literature that inflammatory cytokines and chemokines produced by the tumour cells may play a role in the advancement of malignancy [246, 247]. TNF, often with IL-1 and IL-6, can be detected in different types of cancers [246, 248, 249]. In addition, TNF has shown pro-cancer actions in animal models [246, 250-252] and in mice lacking genes for TNF, a resistance for skin carcinogenesis was observed [253]. Furthermore, mice deficient in the interleukins, IL-1β and IL-6, showed a resistance for hepatic metastases [254] and reduced occurrence of myeloma [246] respectively. Another interleukin, IL-8, is associated with tumorigenic and metastatic potential of pancreatic malignant cells [246].

In the epidemiological data of prospective Nurses Health Study (n=38,451 women), fructose intake was associated with a 2.1 fold increased risk of colorectal cancer [255]. On the contrary, in the Health Professionals Follow-up Study (n=47,781 men) an inverse association between fructose consumption with advanced and metastatic prostate cancer was reported [256]. However, this study was designed to test proposed interrelationship between dietary calcium, and fructose with vitamin D and prostate cancer; author did not look at proposed intervening steps to influence prostate cell differentiation and proliferation [256].

Data relating fructose consumption to cancer from in vitro and animal studies is also heterogeneous. Fructose treatment of cultured human breast cancer cells was shown to accelerate cellular migration and invasion [257]. On the contrary, another study demonstrated that fructose decreases colonic mucosal proliferation in comparison to sucrose, glucose or corn starch in rats [258]. In yet another study, fructose administration to rats has been shown to cause an increase in hepatocellular carcinoma [259].
1.12.2.5. **Neurological disease and inflammation**

Neuroinflammation has been linked to over nutrition in the form of excessive lipids and carbohydrates [260]. Neuroinflammation induced by over nutrition has been shown to be associated with a range of CNS dysfunctions in metabolic diseases [261-266] and contributes to neurodegeneration [267-270]. Pro-inflammatory molecules in the nuclear factor kB (NF-κβ)/IkB kinase β (IKKβ) pathway have been shown to be mechanistically responsible for this neurodegenerative disorder [268]. For example, IL-1 may affect neurophysiological processes of memory consolidation and IL-6 may affect memory and cognition by mediating hippocampal dysfunction [271, 272]. Furthermore, it has been shown in a study involving postoperative patients that elevated IL-6 and CRP levels were correlated with a short and medium term impairment of cognitive function [273]. In addition, TNFα, IL-6, IL-1β and other cytokines adversely affect the permeability of the blood brain barrier (BBB), making it more permeable and enabling migration of leukocytes into the brain [164, 274, 275].

There is growing evidence in the literature suggesting that neuroinflammation is associated with the onset of delirium [164, 276, 277]. Other neurodegenerative diseases like AD have been shown to be associated with elevated levels of IL-6, IL-1β, IL-8, TNF-α, MCP-1, macrophage inflammatory protein-1α (MIP-1α), macrophage colony-stimulating factor (M-CSF) and/or decreased levels of the anti-inflammatory cytokines like IL-1ra, and IL-10 [278-284]. Furthermore, AD brain has shown high levels of TNF-α in animal models [164, 285] and increased levels of TNF-α and IL-1 have been shown to be associated with development of this disease in human subjects (n=691) [286]. Another neurodegenerative disease, multiple sclerosis (MS), is associated with inflammation of nervous tissue i.e. neuroinflammation [164]. It has been shown that pro-inflammatory molecules contribute to the progression of MS [164, 287]. Furthermore, neuroinflammatory processes have been suggested to be a
contributory factor towards the development of PD [288]. TNF-α has been found increased in PD in one study [289]. In addition, IL-1β and IL-6 have been shown to be elevated in the cerebrospinal fluid of de novo PD patients [290].

It has been shown in animal models of dementia that excessive consumption of fructose present in sucrose or HFCS can advance pathogenesis of dementia through increased central neuronal insulin resistance and deposition of β amyloid (associated with Alzheimer’s disease) [291-293]. Supplementation of HFCS to rats fed a high fat and high glucose diet led to cognitive dysfunction [294]. Another animal study has also shown impairment in cognitive function by a high fructose diet [295]. Yet another animal study has shown that a high fructose diet impairs hippocampal dependent memory in rats [296]. In humans, a population based study (n=737) found an association between added fructose intake and cognitive decline [297].
### TABLE 1-2: IN VITRO, IN VIVO AND HUMAN STUDIES OF INVESTIGATING ROLE OF FRUCTOSE IN INFLAMMATION

<table>
<thead>
<tr>
<th>HUMAN STUDIES</th>
<th>Length of Study</th>
<th>Population</th>
<th>Health status/Cell type/Model of disease</th>
<th>Gender</th>
<th>Age group</th>
<th>Amount of fructose</th>
<th>Method of Intervention</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeberli, I., et al., American Journal of Clinical Nutrition, 2011. 94(2): p. 479-85.</td>
<td>3 weeks</td>
<td>Adult (n=29)</td>
<td>Healthy</td>
<td>M</td>
<td>26.3±6.6 y</td>
<td>MF: 40 g fructose/day, HF: 80 g fructose/day</td>
<td>High fructose beverage with meal</td>
<td>↑ CRP after the HF intervention</td>
</tr>
<tr>
<td>Brymora, A., et al., Nephrology, dialysis, transplantation, 2012. 27(2): p. 608-12.</td>
<td>12 weeks</td>
<td>Adult (n=28)</td>
<td>CKD</td>
<td>M: 17 F: 11</td>
<td>59±15 y</td>
<td>LFD: 12.0 g/24h, Regular: 60.0 g/24h</td>
<td>Switched from a regular (basal) to a LFD for 6 weeks, followed by a resumption of their regular diet for another 6 weeks.</td>
<td>↓ CRP and sICAM after LFD</td>
</tr>
</tbody>
</table>

**in vitro STUDIES**


49
<table>
<thead>
<tr>
<th>Authors</th>
<th>Model Type</th>
<th>Concentration</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanuri, G., et al.</td>
<td>Mouse model of the onset of fructose – induced NAFLD</td>
<td>30% solution of fructose</td>
<td>↑ ICAM-1, CCL2 and CCL19 expression</td>
</tr>
<tr>
<td>Kanuri, G., et al.</td>
<td>Mouse model of fructose-induced NAFLD</td>
<td>30% solution of fructose</td>
<td>↑ PAI-1 expression</td>
</tr>
<tr>
<td>Cirillo, P., et al.</td>
<td>Human kidney proximal tubular cells (HK-2)</td>
<td>Physiologic concentration (1 mM fructose)</td>
<td>↑ MCP-production and oxidative stress</td>
</tr>
<tr>
<td>Latta, M., et al.</td>
<td>Hepatocytes</td>
<td>50 mM</td>
<td>Inhibits TNF-induced cell death</td>
</tr>
<tr>
<td>Dhar, I., et al.</td>
<td>Aortic and renal cells</td>
<td>60% of total calories</td>
<td>↑NF-kB expression</td>
</tr>
<tr>
<td>Wei, Y., et al.</td>
<td>Hepatocytes</td>
<td>1 mmol/L fructose</td>
<td>↑ JNK activity</td>
</tr>
<tr>
<td>Spruss, A., et al.</td>
<td>Hepatocytes</td>
<td>30% fructose</td>
<td>↑ TNFα mRNA expression</td>
</tr>
<tr>
<td>Study</td>
<td>Condition</td>
<td>Fructose Percentage</td>
<td>Effect</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>----------------------------------</td>
<td>---------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Tsai, J., et al., 2009</td>
<td>Hepatocytes</td>
<td>60%</td>
<td>↓ hepatic IkB (inhibitor of NF-kB)</td>
</tr>
<tr>
<td>Wei, Y. and M.J. Pagliassotti, 2004</td>
<td>Hepatocytes</td>
<td>~1mM</td>
<td>↑ JNK activity</td>
</tr>
<tr>
<td>Aoyama, M., et al., 2012</td>
<td>Tubulointerstitial fibrosis</td>
<td>67% fructose (99% of which was fructose)</td>
<td>↑ MCP-1 expression</td>
</tr>
<tr>
<td>Haub, S., et al., 2010</td>
<td>Hepatocytes</td>
<td>30%</td>
<td>↑ TNFα expression</td>
</tr>
<tr>
<td>Cannizzo, B., et al., 2012</td>
<td>High-fructose induced insulin resistance</td>
<td>10%</td>
<td>↑ VCAM-1 expression</td>
</tr>
<tr>
<td>Study</td>
<td>Variable</td>
<td>Concentration</td>
<td>Outcome</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
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</tbody>
</table>

**ANIMAL STUDIES**

<table>
<thead>
<tr>
<th>Study</th>
<th>Variable</th>
<th>Concentration</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Armutcu, F., et al., Clinical biochemistry, 2005. 38(6): p. 540-7.</td>
<td>Fructose-induced steatohepatitis</td>
<td>Male Wistar albino rats</td>
<td>10% (w/v) fructose</td>
</tr>
<tr>
<td>Study</td>
<td>Weeks</td>
<td>Animals</td>
<td>Diet</td>
</tr>
<tr>
<td>-------</td>
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<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>Spruss, A., et al., Hepatology, 2009. 50(4): p. 1094-104</td>
<td>8 weeks</td>
<td>n=5-7 per group</td>
<td>Fructose-Induced Hepatic Steatosis</td>
</tr>
<tr>
<td>Nyby, M.D., et al., Hypertension research : official journal of the Japanese Society of Hypertension, 2007. 30(5): p. 451-7.</td>
<td>8 weeks</td>
<td>4 groups</td>
<td>Fructose-induced hyperinsulinemic rats</td>
</tr>
</tbody>
</table>

M: males; F: females; MF: medium fructose; HF: high fructose; ↑: increase; CKD: chronic kidney disease; LFD: low fructose diet; ICAM-1, intercellular adhesion molecule 1; CCL2, chemokine (C-C motif) ligand 2; CCL19, chemokine (C-C motif) ligand 19; TNFα, tumor necrosis factor α; PAI-1: Plasminogen activator inhibitor-1; MCP-1, monocyte chemotactic protein 1; NF-kB: Nuclear factor κB; JNK: c-Jun N-Terminal Kinase; ROS: reactive oxygen species; IL-6: Interleukin-6; IL-1β: Interleukin-1β; IkB: MCP-1: monocyte chemotactic protein-1; VCAM-1: vascular cell-adhesion molecule-1.
1.13. Obesity

Obesity is defined as a “condition in which there is an abnormal or excessive fat accumulation that may impair health” [298]. The simple and commonly used measure for obesity is BMI defined as weight/height$^2$ (kg/m$^2$) [299], a value of $\geq 25$-$<30$ being overweight and $\geq 30$ being obese. According to WHO, 1.9 billion people aged 18 years or over are overweight, of these over 600 million are obese [298]. Worldwide prevalence of obesity has more than doubled since 1980 [298]. Furthermore, 42 million children under the age of five were found to be overweight or obese in 2013. Overweight and obesity are associated with more deaths worldwide than underweight[298]. Overweight and obesity is one of the risk factor for developing CVD and CVD was the leading cause of death in 2012 [298].

Approximately 63% of Australian adults showed a combined incidence of overweight and obesity (comprised of 35.3% overweight and 27.5% obese) in the National Health Survey of 2011-2012. Prevalence of overweight and obesity has increased over time from 56.3% in 1995 and 61.2% in 2007–08[300]. The 2011-12 NHS survey identified that 55.7% women were overweight or obese which was less in comparison to 69.7% of men who were found overweight or obese. Furthermore, obesity has increased in all age groups over time, up from 18.7% in 1995 to 27.5% in 2011-12 [300].

Animal studies have suggested that high fructose intake causes obesity in rodents [114-116, 301] and monkeys [302]. Evidence from epidemiological studies has further strengthened this observation and has confirmed a positive association between the intake of fructose and prevalence of obesity [303-307]. High consumption of fructose in food (from both sucrose and HFCS) has been found to be a predisposing factor for the incidence of obesity [72, 182]. In
addition to this, the Department of Agriculture in the USA, reported that obesity rates increased in parallel with the increase in consumption of HFCS [65]. Aerated drinks containing sucrose caused a greater increase in weight in comparison to diet soft drinks in a randomized double-blind study in overweight subjects [25]. There was a significant increase in weight after administration of these drinks containing HFCS in comparison to those containing artificial sweetener in a trial involving normal weight human subjects [26].

Administration of fructose to diabetic subjects also showed an increase in weight [24]. Intake of fructose caused fat accumulation around the abdomen in comparison to glucose in obese subjects [10]. Consumption of soft drinks in overweight subjects caused an increase in body weight [27]. The effect on weight gain was greater in obese subjects in comparison to their leaner counterparts [28]. Furthermore, evidence suggests that reduction in consumption of aerated drinks can lead to weight loss. Human studies indicate that supplemental intake of food and beverages containing fructose do not cause a cutback in other sources of food. This is in agreement with the likelihood that fructose may not impact satiety as suggested by Teff et al [6]. Fructose administered subjects reported they were hungrier and consumed more calories in comparison to a starch administered group [6]. One mechanism has been proposed to explain how fructose is affecting satiety and resulting in development of obesity. Fructose by virtue of not stimulating acute insulin release, does not cause a substantial rise in postprandial leptin levels [6], which results in a failure for leptin to signal satiety centres in the brain with the sensation of fullness. This may lead to uncontrolled feeding that ultimately results in weight gain. Another mechanism put forward implicates ectopic TG accumulation in the muscle and liver, leading to obesity and insulin resistance following high dietary fructose consumption [150] (Figure 1-4).
On the contrary, according to one meta-analysis, the isocaloric trials did not show any change in weight after fructose intake, while hyper-caloric trials demonstrated a positive association between fructose intake and prevalence of obesity [104]. This meta-analysis has several limitations. The major drawback is that it encompasses trials involving administration of free fructose only. Free fructose is not the main source of fructose intake and it is important to include sucrose and HFCS as well. As this meta-analysis did not incorporate sucrose or HFCS, it is difficult to ascertain whether this analysis will persist with the same finding when trials involving sucrose or HFCS are also included. Another flaw in this analysis is dealing with changes in weight. This meta-analysis does not focus on changes in weight among trial groups, rather it concentrates on end differences in weight. Therefore, it is difficult to come to any concrete conclusion based on this meta-analysis.

The majority of the human studies mentioned above focus on intake of soft drinks and their effect on obesity. Studies that focus on consumption of fructose from all sources and its effect on weight gain are extremely limited. Studies have been carried out connecting high fructose intake with weight gain but no study has been carried out to investigate the impact of fructose restrictive diets on human subjects. We hypothesise that a fructose restrictive diet will lead to loss in weight and ultimately result in decline in obesity.
1.14. Summary

Fructose is commonly known as a 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 unlike any other sugar molecule, due to its passive diffusion into the bloodstream. Similarly, excess fructose intake is metabolised in a way that may contribute to the development of chronic inflammatory 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 hypothesise that restricting dietary fructose intake will result in improved glycaemic indices, reduced circulating lipid levels and low grade inflammation in healthy individuals.
1.15. Hypothesis & Aims

1.15.1. Hypothesis

A vast body of evidence from epidemiological, experimental and clinical studies suggest an important role of fructose consumption in elevating risk factors for the development of cardiovascular disease. However, no work has been done on restricting fructose intake and looking at its effect on development of cardiovascular disease in healthy individuals. Thus the research hypothesis of this thesis is that restricting dietary fructose intake will result in reduction in risk factors for the development of cardiovascular disease by improving glycaemic indices, reducing circulating lipid levels and inflammatory mediators in healthy individuals. Prior to testing the effect of restriction of fructose consumption in the diet, we will look at the effect of acute and chronic consumption of fructose in healthy individuals.

1.15.2. Aims

The primary aims of this thesis are to examine the effects of the consumption of fructose on glycaemic indices, postprandial lipemia and inflammatory biomarkers in healthy individuals and to examine whether a fructose restricted diet improves the above stated cardiovascular disease risk factors.

Specific aims of the presented research thesis

1.15.2.1. Aim 1

To examine the acute effects of fructose consumption on glycaemic indices, blood lipids and inflammatory biomarkers in healthy individuals. Information garnered from this study has
allowed us to determine if fructose consumed as a sole source of energy has adverse effects on human health.

1.15.2.2. **Aim 2**

To examine the chronic effects of fructose consumption on glycaemic indices, blood lipids and inflammatory biomarkers in healthy individuals. The study also aimed to examine:

- correlations between baseline fructose intake and body composition, lipid levels and inflammatory biomarkers
- correlations between change in fructose intake and change in body composition, lipid levels and inflammatory biomarkers

Information garnered from this study enabled us to determine if fructose consumed over and above the daily energy requirement has adverse effects on human health.

1.15.2.3. **Aim 3**

To examine the effects of restricting fructose intake on glycaemic indices, blood lipids and inflammatory biomarkers in healthy individuals. The study also aimed to see:

- correlations between baseline fructose intake and body composition, lipid levels and inflammatory biomarkers
- correlations between change in fructose intake and change in body composition, lipid levels and inflammatory biomarkers

Information garnered from this study has allowed us to assess the relevance of fructose restricted diets, as a dietary strategy for reducing the risk for development of cardiovascular diseases.
2. CHAPTER 2: General methods
2.1 Study Design

Three dietary intervention trials have been conducted in healthy subjects. These include:

- acute trial (single meal) investigating effects of fructose, glucose or sucrose consumption on lipid levels and systemic inflammation (Chapter 3)
- chronic trial (4 weeks) investigating effects of dietary supplementation of fructose or glucose on lipid levels and systemic inflammation (Chapter 4)
- fructose restricted trial (4 weeks) investigating effects of restricting fructose intake on lipid levels and systemic inflammation (Chapter 5)

Study designs for these trials have been described in the respective chapters as listed.

2.2 Participants

Healthy male and female adults between the ages of 18-60 years were recruited from the general community of Newcastle, NSW, Australia via advertisements on noticeboards of the University of Newcastle campus, through the Hunter Medical Research (HMRI) volunteer register and through advertisements in the media. Participants underwent study procedures at the Nutraceuticals Research Group Clinic rooms, University of Newcastle, NSW, Australia.

2.3 Exclusion criteria

Exclusion criteria were: diagnosed hyperlipidaemia, diabetes, gastrointestinal disorders, currently on fructose/sugar restricted diet, vegan diet or weight loss program, undergone any surgical procedure for obesity, pregnant or lactating mother, taking lipid-lowering or anti-inflammatory drugs and BMI ≥30 kg/m².
2.4 Ethics and trial registrations

All participants provided written informed consent prior to participation in each of the studies. The three studies were each approved by Human Ethics Registration Committee, The University of Newcastle with a reference number H-2011-0336. All the trials were registered with the Australian & New Zealand Clinical Trials Registry (Acute trial: ACTRN12614000431628; Chronic trial and fructose restricted trial: ACTRN12612000172808). The studies were conducted in accordance with The Declaration of Helsinki.

2.5 Anthropometric measurements

Height of each participant was measured at the commencement of the study using a wall-mounted stadiometer (Seca Wall Mounted Roll-Up Height Measurer / Stadiometer 0-220cm [SE206]). Body composition was assessed and body weight was measured by BIA using single frequency bioelectrical impedance apparatus (InBody 230, Maltron International, Essex, UK) at the commencement and at the end of the study. Measurements were conducted in the supine position, with participants wearing light clothing and without shoes, in the morning after a minimum 10 hours fast. The same research personnel measured anthropometry using the same equipment at all the visits to minimize variation in the results.

2.6 Dietary Intakes

Twenty-four-hour food recalls (Study 1) were collected by face to face interviews. Three-day food diaries (Study 2 & 3) were recorded by participants in the food diary provided. Food records collected from participants were entered into FoodWorks Version 7.0.291 database (Xyris
Software Pty Ltd, Queensland, Australia) to analyse daily energy and nutrient intake of participants.

2.7 Laboratory methods

Blood samples were collected into tubes pre-coated with EDTA, lithium heparin and sodium fluoride by venepuncture by a trained phlebotomist at the Nutraceuticals Research Group Clinic rooms or by a trained nurse at HAPS collection centre. EDTA blood tubes were centrifuged for 10 minutes at 3000 g at 4°C for separation of plasma and stored at -80°C for further use. The lithium heparin tubes were used for blood lipid profiles (total cholesterol, Triglyceride, LDL-cholesterol, HDL-cholesterol and Total/HDL-cholesterol ratio) and systemic inflammation (hs-CRP) and the sodium fluoride tubes were used for blood glucose and insulin measurement. All samples were analysed by the accredited Hunter New England Area Pathology Services (Newcastle).

2.8 Statistical analyses

Data calculations, sample size and statistical analyses were performed using SPSS software (version 21.0; SPSS Inc., Chicago, IL, USA) in all studies. Preliminary assumption testing was done to check for normality, linearity, outliers and homogeneity of variance with no serious violations observed for all test variables. Variables that were not normally distributed were log-transformed prior to analysis.
3. Chapter 3- Acute effects of feeding fructose, glucose and sucrose on blood lipid levels and systemic inflammation

The contents of this chapter are covered by the following publication entitled:

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
3.1 Thesis aim addressed in this chapter:

3.1.1 Aim 1 To examine the acute effects of fructose consumption on glycaemic indices, blood lipids and inflammatory biomarkers in healthy individuals. Information garnered from this study has allowed us to determine if fructose consumed as a sole source of energy has adverse effects on human health.

3.1.2 Abstract

Background: Recent studies have demonstrated a relationship between fructose consumption and risk of developing metabolic syndrome. Mechanisms by which dietary fructose mediates metabolic changes are poorly understood. This study compared the effects of fructose, glucose and sucrose consumption on post-prandial lipemia and low grade inflammation measured as hs-CRP.

Methods: This was a randomized, single blinded, cross-over trial involving healthy subjects (n = 14). After an overnight fast, participants were given one of 3 different isocaloric drinks, containing 50 g of either fructose or glucose or sucrose dissolved in water. Blood samples were collected at baseline, 30, 60 and 120 minutes post intervention for the analysis of blood lipids, glucose, insulin and high sensitivity C-reactive protein (hs-CRP).

Results: Glucose and sucrose supplementation initially resulted in a significant increase in glucose and insulin levels compared to fructose supplementation and returned to near baseline values within 2 hours. Change in plasma cholesterol, LDL and HDL-cholesterol (measured as AUC) was significantly higher when participants consumed fructose compared with glucose or sucrose (P < 0.05). AUC for plasma triglyceride levels however remained unchanged regardless of the dietary intervention. Change in AUC for hs-CRP was also significantly higher in subjects
consuming fructose compared with those consuming glucose (P < 0.05), but not sucrose (P = 0.07).

Conclusion: This study demonstrates that fructose as a sole source of energy modulates plasma lipids and hs-CRP levels in healthy individuals. The significance of increase in HDL-cholesterol with a concurrent increase in LDL-cholesterol and elevated hs-CRP levels remains to be delineated when considering health effects of feeding fructose-rich diets.

Registration number for clinical trials: ACTRN12614000431628

3.1.3 Introduction

Fructose, commonly known as fruit sugar, is also a major component of sweeteners such as table sugar, honey and high fructose corn syrup (HFCS). Fructose intake has quadrupled since the beginning of 20th century, partly because of the introduction of HFCS [308]. Increased fructose consumption can lead to a parallel rise in cardiovascular disease risk factors, i.e. increase in blood lipids [6, 9], development of insulin resistance [10, 14], alteration in the production of satiety hormones (insulin, leptin and ghrelin) [6], increase in inflammatory biomarkers [20, 186] and increase in obesity [10, 26]. Since similar effects do not occur following the intake of starch or glucose, it has been proposed that fructose-induced metabolic changes are not mediated by excessive sugar intake in general, but are specific to fructose. Precise underlying mechanisms by which fructose consumption may induce negative metabolic effects are not clear. One recent study demonstrated that in young healthy individuals, consumption of glucose and fructose drinks resulted in markedly different hemodynamic responses, with fructose stimulating a sustained increase in blood pressure [309]. These observations support the concept that diets with repeated fructose loads may, over time, contribute to increased cardiovascular disease risk.
The aim of this study was to investigate the effects of fructose compared to glucose and sucrose consumption, on postprandial lipemia and low grade inflammation in healthy subjects. Previous studies have examined the effects of feeding sugars as part of a meal on cardiovascular disease indices. In the current study, we looked at postprandial lipid and low grade inflammation following a single dose of sugary drink given as a sole source of energy after an overnight fast.

3.1.4 Methods

3.1.4.1 Study population

Healthy male and female adults (n=14) between the ages of 18-60 years were recruited by advertisement and underwent study procedures at the Nutraceuticals Research Group Clinic rooms, University of Newcastle, NSW, Australia. Exclusion criteria were: diagnosed hyperlipidaemia, diabetes, gastrointestinal disorders, currently on fructose/sugar restricted diet, vegan diet or weight loss program, undergone any surgical procedure for obesity, pregnant or lactating mother, taking lipid-lowering or anti-inflammatory drugs and BMI >30 kg/m². Participants were asked to complete a medical questionnaire, International Physical Activity Questionnaire (IPAQ) [310] and a 24 hr food record. Approval for the study was granted by the Human Research Ethics Committee of the University of Newcastle, Australia. All participants provided written informed consent and the study was conducted in accordance with The Declaration of Helsinki. The trial was registered with the Australian & New Zealand Clinical Trials Registry (ACTRN12614000431628). Body composition was assessed by BIA using single frequency bioelectrical impedance apparatus (Maltron International, Essex, UK). Measurements were conducted in the supine position, with participants wearing light clothing and without shoes, in the morning after a minimum 10 hours fast. Participants were asked to refrain from physical exertion and alcohol consumption for 24 hours prior to testing.
3.1.4.2 Study Design

The trial was a randomised, controlled cross-over intervention trial. Participants visited the research facility on three separate occasions, where they consumed a different sugary drink on each occasion, with a minimum of one week wash out period in between. The participants were randomised to consume: 1) 50g fructose dissolved in 300ml of water 2) 50g glucose dissolved in 300ml of water and 3) 50g sucrose dissolved in 300ml of water. Each sugar drink contained 10 ml of lemon juice to provide a more uniform and palatable taste. Participants were asked to consume the sweetened drinks within 2-3 minutes and compliance was observed. Block randomization technique was used for allocation of participants to treatment arms. During each visit, a fasting blood sample was collected prior to supplementation, then 30, 60 and 120 minutes following intake of the sugary drink. The participants remained in the research facility until the final sample was collected and were asked to limit physical activity during their time in the research facility.

3.1.4.3 Laboratory methods

Blood samples at base line, 30, 60 and 120 minutes were collected into tubes pre-coated with EDTA, lithium heparin and sodium fluoride by venepuncture. EDTA blood tubes were centrifuged for 10 minutes at 3000 g at 4°C for separation of plasma and stored at -80°C for further use. The lithium heparin tubes for blood lipids and sodium fluoride tubes for blood glucose and insulin measurement were analysed by Hunter New England Area Pathology Services (Newcastle). Twenty-four-hour food recalls were collected by face to face interview by an in-house dietician. Food records collected from participants were entered into FoodWorks Version 7.0.291 database (Xyris Software Pty Ltd, Queensland, Australia) to analyse daily energy and nutrient intake of participants.
3.1.4.4 Statistical analysis

All data are presented as mean ± SEM. Area under the curve (AUC) was calculated by the trapezoid method. Preliminary assumption testing was done to check for normality, linearity, outliers and homogeneity of variance with no serious violations noted for all test variables within the three different groups of fructose, glucose and sucrose. Comparisons between different groups were made with one-way repeated measures ANOVA and post hoc Tukey testing. A probability level of p<0.05 was adopted throughout to determine statistical significance unless otherwise mentioned. All statistical analyses were carried out with SPSS software (version 21.0; SPSS Inc., Chicago, IL, USA).

3.1.5 Results

All participants were healthy and their baseline values of anthropometric measurements and blood biomarkers were within normal range (Table 3-1).

As shown in Figure 3-1A, at 30 minutes, fructose consumption was followed by an increase in blood glucose levels that was significantly lower than following glucose and sucrose consumption. At 60 and 120 minutes there were no differences between the 3 groups. The overall change in blood glucose levels followed by fructose consumption measured as area under the curve (AUC), was significantly lower than following glucose supplementation (Figure 3-1B). Figure 3-1C demonstrates that after 30 minutes, fructose consumption led to a smaller increase in insulin levels than glucose or sucrose. At 60 minutes, insulin levels in the fructose fed group remained lower than the glucose fed group. However, at 120 minutes, there were no differences in insulin levels across intervention groups. The overall increase in the insulin levels, measured as AUC, was significantly lower when participants consumed fructose compared to glucose or sucrose (Figure 3-1D).
Fructose consumption led to an increase in total cholesterol compared to glucose and sucrose consumption (Figure 3-2A). The effects were still apparent at 60 minutes, however at 120 minutes there were no differences between groups. The overall increase in plasma total cholesterol measured as AUC was significantly higher when participants consumed fructose compared to glucose or sucrose (Table 3-3). At 30 minutes after fructose consumption an increase in LDL-cholesterol was observed compared to glucose and sucrose (Figure 3-2B). The effects were still apparent at 60 minutes. However, at 120 minutes there were no differences between groups. Overall, the increase in LDL cholesterol measured as AUC was significantly higher when participants consumed fructose compared to glucose or sucrose (Table 3-3). Similarly, fructose consumption was followed by an increase in HDL-cholesterol at 30 minutes in comparison to glucose and sucrose (Figure 3-2C). The effects were still apparent at 60 minutes; however, at 120 minutes there were no differences between groups. The overall increase in HDL cholesterol measured as AUC was significantly higher when participants consumed fructose compared to glucose or sucrose (Table 3-3).

Comparison of all treatment groups revealed no significant difference in TG levels at 30 minutes. At 60 minutes, subjects fed fructose had lower TG levels compared to glucose. At 120 minutes there were no differences between groups (Figure 3-2D). There were overall no significant differences in plasma triglyceride levels (measured as AUC) regardless of the dietary intervention (Table 3-3). Comparison between all treatment groups revealed no significant change in the ratio of total/HDL-cholesterol at all the time points (p>0.005). Furthermore, the overall change in the ratio of total/HDL-cholesterol measured as AUC was not different between groups (Table 3-3). Fig 3-3A demonstrates that fructose consumption was followed by an increase in hs-CRP level at 30 minutes when compared to glucose and sucrose. At 60 minutes, hs-CRP was not different compared to glucose or sucrose and at 120 minutes there were no differences between groups.
There was, however, an overall increase in hs-CRP levels measured as AUC in subjects who consumed fructose compared with those who consumed glucose (p<0.05), but not sucrose (Figure 3-3B).

3.1.6 Discussion

This study was designed to examine the metabolic consequences of sugar consumption when it is used as a sole source of energy. Participants consumed fructose in the fasting state in the morning; therefore, majority of the sugar consumed would be used to produce energy and/or partly used to replenish glycogen stores at this time of day [132]. Fructose was a sole source of energy without accompanying meal and there was no other nutrient to augment postprandial lipemia. Consumption of sugar sweetened beverages in the morning in the fasting state separated the effect of fructose from excess energy intake which may be a confounder in the overfeeding studies [132]. This is the first study to report the consequences of consuming a beverage containing fructose, glucose or sucrose as a sole source of energy, on postprandial lipid levels and inflammation markers. Acute fructose consumption in a single dose of 50g/day which provided approximately 8% of daily energy in the form of a beverage, resulted in a significant increase in the plasma levels of total, LDL and HDL cholesterol and the acute phase pro-inflammatory marker hs-CRP, compared to the same dose of glucose or sucrose. Interestingly, no significant change in TG levels was observed.

The change in fasting glucose and insulin responses was modest in fructose compared with glucose and sucrose groups. This modest increase in the glucose and insulin levels after fructose consumption is consistent with previous studies [133, 243, 244]. The blunted rise in insulin in response to fructose consumption is consistent with the blunted rise in blood glucose level, but may also be partly attributed to less release of intestinal incretin hormone that binds to β-cells of the pancreas leading to reduce secretion of insulin [311].
Changes in total, LDL and HDL-cholesterol levels were significantly higher when participants consumed fructose compared with glucose or sucrose sweetened beverage. Previous studies examining postprandial lipemia following fructose consumption were either focused on triglyceride levels only [132, 133, 312-315] or demonstrate heterogeneous results. Two studies showed no effects of fructose on plasma total, LDL or HDL-cholesterol levels [140, 147], while another 2 studies [8, 9] showed a significant increase in fasting serum total and LDL cholesterol following 4-5 weeks of consuming fructose-rich diets compared to the starch diet. The reason for the increase in postprandial levels of total, LDL and HDL cholesterol in subjects who consumed fructose in our 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. Whether fructose can influence total and LDL-cholesterol levels by blocking LDL-receptors or affect HDL cholesterol via CETP or reverse cholesterol transport is not known, therefore, merits further investigation.

Dietary fructose has been previously shown to cause either no change [9, 142, 147, 316] or an increase in fasting plasma triglycerides in healthy subjects [140, 317]. Our results showed no significant change in postprandial triglyceride levels irrespective of the type of sugar. These findings are in agreement with a meta-analysis reporting no significant change in post-prandial TG level unless the amount of fructose exceeds 50g/day [318]. On the other hand, the present results are in contrast with literature reporting an increase in TG after acute fructose consumption [5, 6, 132, 133, 312-314, 317]. This disagreement may be due to the accompanying meal with consumption of the fructose beverage in previously published studies. In our study, due to the absence of other energy yielding nutrients, the clearance rate of triglycerides can be expected to be higher, resulting in overall no change. Moreover the shorter duration (2 hours) of our study compared to the study by Bohannan et al [315] (5 hours) may account for the discrepancy in the two studies. Therefore, 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.

The pro-inflammatory biomarker that we examined in this study was hs-CRP. The area under the curve for hs-CRP level was significantly increased in the fructose group compared with glucose, but not with sucrose. This is the first time, to our knowledge, in healthy and normal weight adult subjects, that acute fructose consumption has been shown to increase hs-CRP levels. Previous studies reporting an increase in hs-CRP level were either conducted in a mixed population of lean and overweight individuals [243] or a long term intervention study [20]. The proposed mechanism of fructose-induced oxidative stress and inflammation markers (TNFα, IL-6, IL-1β)[319] potentially resulting in an increase synthesis of hs-CRP merits further investigation. Fructose has been shown to induce oxidative stress in cellular [23] and animal models [180], thereby, may result in elevated levels of pro-inflammatory mediators. Whether fructose can directly (without conversion to fat) influence inflammation pathways (leukotriene synthesis, expression of adhesion molecules etc) remains to be delineated. In conclusion, this study demonstrates that fructose as a sole source of energy modulates plasma lipids and hsCRP levels in healthy individuals. However, the significance of increase in HDL-cholesterol with a concurrent increase in LDL-cholesterol and elevated hsCRP levels remains to be delineated when considering health effects of feeding fructose-rich diets.
Acknowledgements:

This study was funded by a grant from the Hunter Medical Research Institute. The authors declare no conflict of interest.
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
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.
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
<table>
<thead>
<tr>
<th>TABLE 3-1: BASELINE VALUES OF ANTHROPOMETRIC MEASUREMENTS, BLOOD BIOMARKERS &amp; ENERGY AND NUTRIENTS INTAKES OF STUDY PARTICIPANTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>Age (Yrs)</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>SMM (kg)</td>
</tr>
<tr>
<td>FFM (kg)</td>
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<tr>
<td>PBF (%)</td>
</tr>
<tr>
<td>Waist: hip</td>
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<tr>
<td>Glucose (mmol/L)</td>
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<tr>
<td>Cholesterol (mmol/L)</td>
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<tr>
<td>Triglyceride (mmol/L)</td>
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<tr>
<td>LDL-C (mmol/L)</td>
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<tr>
<td>HDL-C (mmol/L)</td>
</tr>
<tr>
<td>Total/HDL ratio</td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
</tr>
</tbody>
</table>

Mean values ± standard error of mean. BMI, Body mass index; SMM, Skeletal muscle mass. FM, Fat Free Mass; PBF, Percentage Body Fat; LDL-C, Low Density Lipoprotein cholesterol; HDL-C, High Density Lipoprotein cholesterol; CRP, c-Reactive Protein

<table>
<thead>
<tr>
<th>TABLE 3-2: DAILY ENERGY AND NUTRIENT INTAKE</th>
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<tr>
<td>Daily intake</td>
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<tr>
<td>Energy No DF (kj)</td>
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<tr>
<td>Energy DF (kj)</td>
</tr>
<tr>
<td>Protein (g)</td>
</tr>
<tr>
<td>Fat (g)</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
</tr>
<tr>
<td>Sugar (g)</td>
</tr>
<tr>
<td>Glucose (g)</td>
</tr>
<tr>
<td>Sucrose (g)</td>
</tr>
<tr>
<td>Bound fructose (g)</td>
</tr>
<tr>
<td>Free fructose (g)</td>
</tr>
<tr>
<td>Total fructose (g)</td>
</tr>
<tr>
<td>Lactose (g)</td>
</tr>
<tr>
<td>Maltose (g)</td>
</tr>
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</table>

Mean values ± standard error of mean; DF: dietary fibre
<table>
<thead>
<tr>
<th></th>
<th>Fructose (F)</th>
<th>Glucose (G)</th>
<th>Sucrose (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.09±1.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.90±1.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-2.41±0.85&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>4.50±2.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-3.22±1.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-3.87±1.25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>6.69±1.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.63±1.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.84±0.60&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>-2.45±2.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.65±3.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.79±2.68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total/HDL ratio</td>
<td>-1.97±0.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.62±0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.57±0.95&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.12±2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.2±6.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.8±3.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
<td>109.4±18.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>610.3±83.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>504.5±172.45&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values± standard error of mean; LDL-C, Low Density Lipoprotein cholesterol; HDL-C, High Density Lipoprotein cholesterol; Values without a common superscript are significantly different, P<0.05
### TABLE 3-4: POST INTERVENTION VALUES OF BLOOD BIOMARKERS OF STUDY PARTICIPANTS

<table>
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<tr>
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<th>Times in minutes</th>
<th></th>
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<tr>
<td></td>
<td></td>
<td>30</td>
<td>60</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>n=14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>5.22±0.20</td>
<td>4.61±0.18</td>
<td>4.29±0.10</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>7.91±0.48</td>
<td>5.41±0.47</td>
<td>4.02±0.21</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>7.48±0.19</td>
<td>4.70±0.32</td>
<td>4.15±0.13</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>4.54±0.17</td>
<td>4.56±0.18</td>
<td>4.41±0.18</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>4.34±0.71</td>
<td>4.38±0.70</td>
<td>4.37±0.72</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>4.16±0.21</td>
<td>4.24±0.21</td>
<td>4.36±0.19</td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>1.09±0.16</td>
<td>1.06±0.15</td>
<td>1.06±0.15</td>
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<tr>
<td>Glucose</td>
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<td>1.20±0.22</td>
<td>1.09±0.24</td>
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<tr>
<td>Sucrose</td>
<td>1.01±0.17</td>
<td>1.07±0.18</td>
<td>1.03±0.19</td>
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</tr>
<tr>
<td>LDL-C (mmol/L)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>2.65±0.20</td>
<td>2.66±0.21</td>
<td>2.56±0.21</td>
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<tr>
<td>Glucose</td>
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<td>2.52±0.21</td>
<td>2.51±0.22</td>
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<tr>
<td>Sucrose</td>
<td>2.38±0.23</td>
<td>2.45±0.23</td>
<td>2.53±0.21</td>
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<tr>
<td>HDL-C (mmol/L)</td>
<td></td>
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<td></td>
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<tr>
<td>Fructose</td>
<td>1.39±0.12</td>
<td>1.42±0.12</td>
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<tr>
<td>Glucose</td>
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<td>Sucrose</td>
<td>1.31±0.13</td>
<td>1.32±0.12</td>
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<tr>
<td>Total/HDL ratio</td>
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<tr>
<td>Fructose</td>
<td>3.62±0.37</td>
<td>3.55±0.35</td>
<td>3.58±0.37</td>
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<tr>
<td>Glucose</td>
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<td>3.61±0.34</td>
<td>3.60±0.36</td>
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<tr>
<td>Sucrose</td>
<td>3.51±0.35</td>
<td>3.52±0.33</td>
<td>3.57±0.35</td>
<td></td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Fructose</td>
<td>21.58±2.64</td>
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<td>9.29±1.38</td>
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<tr>
<td>Glucose</td>
<td>89.03±11.72</td>
<td>59.28±14.76</td>
<td>14.21±3.62</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>67.63±6.99</td>
<td>32.16±7.69</td>
<td>8.76±1.52</td>
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</tr>
<tr>
<td>CRP (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>1.69±0.36</td>
<td>1.72±0.37</td>
<td>1.66±0.36</td>
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</tr>
<tr>
<td>Glucose</td>
<td>1.62±0.35</td>
<td>1.59±0.33</td>
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</tr>
<tr>
<td>Sucrose</td>
<td>1.90±0.38</td>
<td>1.96±0.40</td>
<td>1.94±0.39</td>
<td></td>
</tr>
</tbody>
</table>
4. Chapter 4: Chronic study
4.1 **Thesis aim** addressed in this chapter

4.1.1 **Aim 2** To examine the chronic effects of fructose consumption on glycaemic indices, blood lipids and inflammatory biomarkers in healthy individuals. The study also aimed to examine:

- correlations between baseline fructose intake and body composition, lipid levels and inflammatory biomarkers
- correlations between change in fructose intake and change in body composition, lipid levels and inflammatory biomarkers

4.1.2 **Introduction**

Fructose naturally exists in fruits, honey and some vegetables[320]. It is also a component of sucrose and high fructose corn syrup (HFCS), and is the sweetest sugar, up to 75% (1.15-1.75 times) sweeter than sucrose. Its consumption is significant in western diets [97, 321] and has quadrupled since the beginning of the 20th century[308]. Consumption of fructose in acute and chronic settings raises concern about its adverse effects on human health. An increase in fructose consumption has been associated with an increase in obesity and metabolic disorders [97, 321] and it can potentially increase the risk of developing cardiovascular disease [6, 9-11, 14, 17, 20, 26, 186]. Several previous studies conducted in different chronic settings using varied doses of fructose have shown adverse effects on human health. Fructose supplementation has been shown to increase blood glucose, insulin, triglyceride and fractional DNL [15, 322]. An increase in VLDL-triglyceride and intra-hepatocellular lipids (IHCL) without any change in glycaemia and insulin has been reported after seven days of fructose consumption. Furthermore, after seven days
of fructose consumption, a decrease in insulin binding and sensitivity and increase in IHCL, intra-
myocellular lipids (IMCL) and VLDL-TAG have been reported [11, 323]. Chronic studies involving two weeks of fructose supplementation have been shown to increase triglyceride, insulin and HOMA score with a decrease in HDL and no change in blood glucose levels[143]. Furthermore, following three weeks of fructose consumption, an increase in blood glucose, hs-CRP levels and a reduction in LDL particle size has been reported[20]. A 4-week supplementation of fructose has been shown to increase total and LDL-cholesterol, with no change in blood glucose and triglyceride levels[9]. In another study, with the same duration, an increase in triglyceride, VLDL-triglyceride and blood glucose levels without any significant changes in insulin sensitivity, IHCL, and IMCL have been shown [4]. However, other chronic studies of duration 4 and 6 weeks respectively have been shown to increase triglyceride levels, while no change in total, LDL and HDL cholesterol levels were reported [140, 149]. The mechanisms by which consumption of fructose rich diets may be linked with elevated risk of cardiovascular disease risk factors are debatable. Fructose-induced changes in glycaemia, hyperlipidaemia and inflammation have been thought to mediate the adverse health effects of fructose. In a study conducted in acute settings (chapter 3) we have shown that acute fructose consumption (as a sole source of energy) in a single dose of 50g /day resulted in a significant increase in the plasma levels of total, LDL and HDL cholesterol and acute phase pro-
inflammatory marker hs-CRP as opposed to the effects produced by the same dose of glucose or sucrose dissolved in water Interestingly, no significant change in TG level was observed [324]. This study was aimed to investigate the effects of short-term (4 weeks) dietary supplementation with fructose or glucose on blood lipids and low grade inflammation in healthy subjects.
4.1.3 Methods

4.1.3.1 Study population

Healthy male and female adults (n=36) between the age of 18-60 years were recruited by advertisement and underwent study procedures at the Nutraceuticals Research Group Clinic rooms, University of Newcastle, NSW, Australia. Exclusion criteria were: diagnosed hyperlipidaemia, diabetes, gastrointestinal disorders, currently on fructose/sugar restricted diet, vegan diet or weight loss program, undergone any surgical procedure for obesity, pregnant or lactating mother, taking lipid-lowering or anti-inflammatory drugs and BMI ≥30 kg/m². Approval for the study was granted by the Human Research Ethics Committee of the University of Newcastle, Australia. All participants provided written informed consent and the study was conducted in accordance with The Declaration of Helsinki. The trial was registered with the Australian & New Zealand Clinical Trials Registry (ACTRN12612000172808).

4.1.3.2 Study Design

This was a two-arm, parallel-design, single blind, randomised dietary intervention trial. Participants (n=36) were supplied with sachets of 50g of fructose or glucose per day to supplement their usual diets for a period of 4 weeks. The participant’s adherence to the prescribed fructose or glucose consumption was measured by collecting the empty/unfinished fructose or glucose sachets and weighing them for any remaining sugar in the sachets at the completion of the study. Participants who consumed >80% of the sugar provided were deemed to be compliant. At baseline and post-intervention, participants were asked to complete a medical questionnaire, the International Physical Activity Questionnaire (IPAQ) [310] and a 3 day food record. Subjects were advised not to change their usual eating habits or level of physical activity during the days of recording and intervention. At baseline and post intervention, body composition was assessed.
by BIA using single frequency bioelectrical impedance apparatus (Maltron International, Essex, UK). Measurements were conducted in the supine position, with participants wearing light clothing and without shoes, in the morning after a minimum 10 hour fast. Blood samples were collected at baseline and post intervention for the analysis of blood lipids, glucose, insulin and high sensitivity hs-CRP. During the study, participants were instructed to consume their usual diet, but were asked to refrain from physical exertion and alcohol consumption for 24 hours prior to testing. Participants were asked to bring unused sachets at the last visit to assess adherence to prescribed fructose or glucose consumption.

4.1.3.3 Laboratory methods

Venous blood samples at baseline and post-intervention were collected into tubes pre-coated with EDTA, lithium heparin and sodium fluoride. EDTA blood tubes were centrifuged for 10 minutes at 3000 g at 4°C for separation of plasma and stored at -80°C for further use. The lithium heparin tubes for blood lipids and hs-CRP & sodium fluoride tubes for blood glucose and insulin measurement were analysed by the accredited Hunter New England Area Pathology Service (Newcastle). Three-day food diaries were collected at baseline and post-intervention and analysed using FoodWorks Version 7.0.291 database (Xyris Software Pty Ltd, Queensland, Australia) to determine daily energy and nutrient intake of participants.

4.1.3.4 Statistical analysis

All data are presented as mean ± SEM. Preliminary assumption testing was done to check for normality, linearity, outliers and homogeneity of variance with no serious violations noted for all test variables within the two different groups of fructose and glucose. Comparisons between
different groups were made with an independent t-test. Associations between variables were examined using correlation coefficients. A probability level of p<0.05 was adopted throughout to determine statistical significance unless otherwise mentioned. All statistical analyses were carried out with SPSS software (version 21.0; SPSS Inc., Chicago, IL, USA).

4.1.4 Results

A total of n=36 subjects were enrolled in the trial. A total of thirty two healthy subjects (fructose group n=16 and glucose group n=16; twenty males and twelve females) completed the study, as 3 subjects withdrew from the trial and one subjects was excluded due to non-adherence. Two subjects complained about abdominal discomforts, which was manageable and they continued with participation in the study. Despite randomization, blood total cholesterol levels and dietary fat intakes were significantly higher in the fructose compared to glucose intervention group at baseline. (Participant flow chart 1)
Participant flow chart 1

Assessed for eligibility (n=40)

- Excluded (n=4)
  - Not meeting inclusion criteria (n=3)
  - Declined to participate (n=1)

Randomization (n=36)

- Glucose group (n=18)
  - Received 50g of glucose
  - Excluded (n=2)
    - Withdrew from the trial (n=1)
    - Non-adherence to the protocol (n=1)

- Fructose group (n=18)
  - Received 50g of fructose
  - Excluded (n=2)
    - Withdrew from the trial (n=2)

Enrolment

Allocation of treatment

Analysis

Analysed (n=16)

Follow up

Analysed (n=16)
Table 4-1 & 4-2 represent baseline values for anthropometric measurements, body composition, daily energy and nutrient intake, and blood biomarkers. Anthropometric and body composition measurements were within normal range at baseline. The mean (± SEM) age (years), body weight (kg), BMI (kg/m$^2$), PBF (%), BFM (kg) and WHR for all participants was 39.1±2.4 years, 66.3±3.0kg, 24.1±0.61 kg/m$^2$, 24.2±1.69%, 16.5±1.30 kg and 0.8±0.00 respectively.

When comparing the glucose (n=16) and fructose (n=16) intervention groups at baseline, age: 39.0±3.5 vs 39.3±3.4 years; P=0.10, body weight: 61.7±5.4 vs 71.0±2.4 kg; P=0.12, BMI: 23.3±1.03 kg/m$^2$ vs 24.9±0.61 kg/m$^2$; P=0.18, PBF: 23.6±2.30 vs 24.7±2.56% %; P=0.76, and BFM: 15.7 ±1.84kg vs 17.41±1.84kg; P=0.51 were not different between intervention groups. The WHR was also similar in both the glucose (0.8±0.01) and fructose (0.8±0.01) intervention groups at baseline (Table 4-1).

Lipid levels were within normal range at baseline. The mean (± SEM) cholesterol (mmol/L), triglyceride (mmol/L), LDL-C (mmol/L), HDL-C (mmol/L), and Total/HDL-C ratio for all participants were 5.1±0.14, 1.00±0.07, 3.24±0.14, 1.43±0.07 and 3.8±0.21 respectively (Table 4-1).

When comparing the glucose (n=16) and fructose (n=16) intervention groups at baseline, total cholesterol levels were significantly higher in the fructose compared to glucose group (4.8±0.2 vs 5.4±0.17; P=0.03) respectively. However, triglycerides: 0.87±0.08 vs 1.14±0.11; P=0.07; LDL-C: 2.97 ±0.21 vs 3.51±0.17; P=0.06, HDL-C: 1.45±0.90 vs 1.42±0.12; P=0.83 and Total/HDL-C ratio: 3.5±0.27 vs 4.1±0.28; P=0.10 of fructose were not significantly different between intervention groups at baseline (Table 4-1).
Glucose homeostasis markers were within normal range at baseline. The mean (± SEM) glucose (mmol/L), insulin (m IU/L), and HOMA-IR for all participants were 4.9±0.06, 6.8±0.60, and 1.50±0.14 respectively. When comparing the glucose (n=16) and fructose (n=16) intervention groups at baseline, glucose: 4.9±0.88 vs 4.9±0.09; P=0.96, insulin: 6.1±0.89 vs 7.5±0.82; P=0.27 and HOMA-IR: 1.36±0.20 vs 1.66±0.19; P=0.28 were not different (Table 4-1).

hs-CRP (mg/L) levels were within normal range at baseline. The mean (± SEM) CRP (mg/L) for all participants was 1.5±0.33. When comparing intervention groups at baseline, CRP was not different between the glucose and fructose groups (1.6±0.52 vs 1.5±0.43; P=0.10) respectively (Table 4-1).

Usual daily energy and nutrient intake was not significantly different between the glucose and fructose intervention groups except for total fat intake, which was significantly higher in the fructose group in comparison to the glucose group (65.6±5.9 vs 89.6±6.0; P=0.008) at baseline (Table 4-2).

Correlation analysis of free glucose or free fructose intake with anthropometric measurements, body composition and blood biomarkers showed no significant correlation at baseline.

Following dietary supplementation with fructose or glucose there was no significant difference in fasting blood insulin (8.3±2.45 vs 8.4±1.2; P=0.10), glucose (4.8±0.12 vs 5.0±0.1; P=0.10), HOMA-IR (1.9±0.60 vs 1.9±0.30; P=0.90), triglycerides (0.90±0.08 vs 1.22±0.15; P=0.70), LDL-cholesterol (2.95±0.21 vs 3.48±0.17; P=0.06), HDL-cholesterol (1.44±0.09 vs 1.41±0.08; P=0.84), total/HDL cholesterol ratio (3.49±1.26 vs 4.05±0.27; P=0.14) and hs-CRP (2.55±0.93 vs 1.46±0.38; P=0.32) levels respectively. However, the change in glucose post intervention was
significantly higher in the fructose versus glucose groups (glucose: -0.10±0.09 vs fructose: 0.14±0.06; P=0.03). The post intervention level of total cholesterol in the fructose versus glucose intervention group was higher (glucose: 4.8±0.23 vs fructose: 5.4±0.18; P=0.03). This was due to significantly higher base line values of total cholesterol in fructose group versus glucose group, and remained higher post intervention. The change in cholesterol levels following glucose or fructose supplementation was not significantly different (glucose: -0.02±0.09 vs 0.00± 0.12; P=0.87) (Table 4-3).

The change in blood glucose from baseline to post-intervention was significantly higher in the fructose compared to glucose group (glucose: -0.10±0.09 vs fructose: 0.14±0.06; P<0.05). However, the change in fasting blood insulin, hs-CRP and blood lipids from baseline to post-intervention was similar in the glucose compared to fructose groups (Table 4-3).

Following dietary supplementation with glucose or fructose, there was no significant difference in body weight (66.8±4.3 vs 71.2±2.3; P=0.38), BMI (23.4±0.97 vs 25.0±0.61; P=0.20), FFM (51.1±3.5 vs 54.3±2.7; P=0.12), PBF (23.5±2.3 vs 23.7±2.7; P=0.95), BFM (15.7±1.84 vs 16.8±1.4; P=0.75), and WHR (0.86±0.13 vs 0.89±0.11; P=0.14) levels respectively (Table 4-4). Moreover, the change in anthropometric measurements and body composition from baseline to post-intervention with fructose versus glucose were not significantly different (Table 4-4).

Following dietary supplementation with fructose there was no significant correlation of change in free fructose intake from baseline with changes in anthropometric measurements, body composition and blood biomarkers from baseline. However, following dietary supplementation with glucose there was a negative correlation with change in free glucose intake from baseline with changes in BMI (r=-0.65, P=0.006) and BFM (-0.52, P=0.03) from baseline. Changes in
other anthropometric measurements, body composition and blood biomarkers were not significantly correlated with changes in free glucose intake (Table 4-7).

### 4.1.5 Discussion

Comparison of blood biomarkers pre and post consumption of 50g of fructose or glucose per day for 4 weeks showed an increase in blood glucose concentrations after the fructose compared to glucose intervention. No significant difference in insulin, TG, LDL-C, HDL-C, total cholesterol, Total/HDL-C ratio or hs-CRP levels were noticed.

Changes in blood glucose levels from baseline to post-intervention in the fructose group were significantly higher compared to the glucose supplemented group. This finding is in agreement with previous studies in different chronic settings (6 days-4 weeks) [4, 15, 20, 322] in healthy subjects. Thus, consumption of fructose for 4 weeks or less seems to be sufficient to cause changes in glucose metabolism. Higher blood glucose concentrations as found in our study suggest some degree of impairment in the inhibition of glucose metabolism following fructose consumption [4, 325, 326]. However, not all studies have observed such an effect. One study showed no significant change in blood glucose levels after fructose intervention, but this study was designed to compared fructose with or without allopurinol as their main aim was to test whether features of metabolic syndrome can be protected by allopurinol[143]. Hence a control group comparable to our study was not included. Our finding is also not in accordance with several other studies showing no significant effect or a decrease in fasting blood glucose levels following fructose [11, 142, 147, 327] sucrose or HFCS [142, 147, 328] consumption. The reason for disagreement of previous studies with our finding could be the non-optimal comparison of fructose versus sucrose (sucrose being 50% fructose) or HFCS (55-42% fructose) and short
duration of 7-14 days. This seems to suggest that less than 4 weeks of duration of study is not enough to notice significant changes in blood glucose level. This heterogeneity emanating from comparison of different studies with varied doses (63-150g fructose/day) and duration (2-6 weeks) warrants further long term studies.

Our finding of no change in BMI or body weight following fructose consumption is in agreement with previous studies in healthy individuals [4, 9, 11, 140]. Our finding has been further strengthened by a meta-analysis showing no change in body weight if fructose intake is less than 100g/day [318]. One study has shown an increase in body weight following consumption of a very high fructose dose in healthy subjects (3.5g fructose/kg FFM daily~ 35% of daily energy requirements) [327]. This concurs with results of a systematic review and meta-analysis, which showed that consumption of free fructose at high doses (excess calorie intake) increases body weight modestly [104]. Hence, it appears that body weight increases when calorie intake is in positive balance, regardless of source of the energy.

Our finding of no change in WHR disagrees with a study showing an increase in WHR following fructose consumption [20]. This disagreement could be due to difference in the study design (600ml beverages containing 40-80g fructose with three main meals). If the consumption of fructose was on top of their usual diet (i.e. it did not replace any other food intake), then the increase in WHR could be due to increased caloric intake which is stored abdominally.

Our finding of no change in PBF (%) is in agreement with some previous studies [4, 15, 323] but disagrees with other studies showing an increase in PBF (%) following fructose consumption [20, 327]. This disagreement could be due to the higher amount of fructose used (80g of fructose and 3.5g fructose/kg FFM daily~ 35% of daily energy requirements) and differences in study design.
In our study, participants supplemented their common every day diet with 50g of fructose or glucose per day for 4 weeks. However, previously published studies have compared fructose with sucrose or HFCS [17, 20, 142, 147, 328], have used different fructose doses ranging from 5g to >200g/day for 6days-6weeks and few have compared free fructose versus free glucose, where sugar supplements were taken with meals, used in baking or as beverages[4, 11, 20, 140, 143, 149, 327]. Hence inconsistency in study design limits our ability to compare data.

There was no significant difference in the levels of insulin following fructose consumption in our study and this is in accordance with previous studies in chronic settings conducted using various durations (2 to 14 days) and fructose doses (50-200g/day) [17, 147, 327]. The physiological mechanism by which insulin levels remain stable following fructose consumption, could be the fact that fructose unlike glucose does not stimulate insulin secretion [6].

There was no change in TG level following fructose consumption. This result is in agreement with a previous study comparing a high dose versus low dose of fructose and in a study comparing fructose with sucrose [9, 17, 30]. However, no change in TG is in disagreement with several studies showing increased TGs following fructose consumption [4, 6, 15, 140, 141, 149, 322]. This disagreement may be due to some studies using very high amounts of fructose (>100g/day) in combination with low calorie diet [4, 6, 15, 30, 141, 149, 322] or longer duration of study (6 weeks)[140].

Higher plasma cholesterol levels in the fructose fed group compared to the glucose intervention group at the end of the trial were due to the higher baseline total cholesterol levels in the fructose intervention group that remained higher following the intervention (Table 4-1). No change in total cholesterol level following fructose consumption is in agreement with previous studies [140, 149]. One of these studies showed a transient increase in total cholesterol level at 4 weeks but this
didn’t persist to 6 weeks[140]. Our finding is also in agreement with another study showing no change in total cholesterol level [147] but comparison was made with sucrose. Previous studies that are in agreement with our finding show that consumption of fructose of varied doses (50-150g fructose/day) and duration (4-6 weeks) did not lead to increase in total cholesterol level. However, one previous study showed an increase in total cholesterol level when comparing high doses of fructose (average 88g/day) versus high doses of starch (low dose of fructose, average 5g/day)[9, 30]. The authors suggested that one of the reasons for increase in total cholesterol could be due to high fructose per se[9]. Further, our finding is not in agreement with a study showing a decrease in total cholesterol level from baseline following fructose consumption (63-99g/day) for 2 weeks[142]. The decrease in total cholesterol level in this study comparison could be due to the low level of cholesterol and fat consumed[142]. As mentioned previously, when our finding is compared with previous studies, the heterogeneity in available data is evident, which could be due to differences in study design, type and amount of fructose consumed, as well as duration of the study.

The lack of change in LDL-cholesterol level following fructose consumption that we observed is in accordance with some previous studies [140, 147, 149]. Our finding is in disagreement with two studies showing an increase in LDL-cholesterol following fructose consumption [9, 328]. This disagreement could be due to the different study design which did not include a glucose control group[328] or the high baseline consumption of fructose, which may have masked any potential effects [9].

There was also no significant change in HDL-cholesterol levels following fructose consumption. This result is in agreement with some previous studies which also showed no significant effect on HDL-cholesterol level [140, 147, 149, 328], however, two of the studies provide low quality
evidence, as no control group was used [147, 328]. Conversely, several studies show a decrease in HDL–cholesterol following fructose consumption [142, 143]. This difference could be due to the very high dose of fructose (200g/day) used in one study [143] and the use of sucrose (63-99g) as a comparison diet in another study [142]. Further, there was no change in hs-CRP levels following fructose consumption compared with glucose. This is in disagreement with a study showing an increase in hs-CRP level following fructose consumption, however no control group was used in this study [20].

This study had several limitations that must be taken into account while interpreting the results. One limitation is that participants randomized to consume the fructose supplements had higher baseline values of total cholesterol compared to the glucose group. Whilst we accounted for this in the statistics by analysing the changes compared to baseline, this may have confounded the result post-intervention.

Moreover, 4-week duration of intervention and dose of 50g of fructose may not have been sufficient enough to notice any significant effects of fructose consumption on parameters such as lipoprotein levels. Another limitation to this study could be the fact that the effect of fructose on inducing characteristic features of metabolic syndrome is more pronounced in overweight or hyperinsulinemic subjects [12, 78, 143]. It is therefore possible that testing fructose consumption in a healthy population produced a blunted response.

In addition, participants were given the choice of taking the supplements either as a single dose or in divided doses. The ability to absorb fructose in humans is not completely clear[82]. The absorption of fructose has been suggested to be quite efficient in previous studies, though not as efficient as glucose or sucrose[329]. Indeed, we didn’t collect information on whether fructose
supplement was taken with the meal or on an empty stomach. This is another limitation, as co-consumption of glucose with fructose has been shown to enhance absorption of fructose and may have confounded our outcomes [330]. Variations in frequency and timing of fructose dosing may also have confounded the results.

### 4.1.6 Conclusion

In conclusion, it appears that at the dose and duration used, 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.
**TABLE 4-1: BASELINE VALUES OF ANTHROPOMETRIC MEASUREMENTS, BODY COMPOSITION & BLOOD BIOMARKERS OF STUDY PARTICIPANTS**

Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>ALL</th>
<th>Glucose</th>
<th>Fructose</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=32</td>
<td>n=16</td>
<td>n=16</td>
<td></td>
</tr>
<tr>
<td>Age (Yrs)</td>
<td>39.1 ±2.4</td>
<td>39.0±3.5</td>
<td>39.3 ± 3.4</td>
<td>P=0.10</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.3 ±3.0</td>
<td>61.7 ±5.4</td>
<td>71.0 ± 2.4</td>
<td>P=0.12</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1± 0.61</td>
<td>23.3 ± 1.03</td>
<td>24.9± 0.61</td>
<td>P=0.18</td>
</tr>
<tr>
<td>SMM (kg)</td>
<td>29.3 ± 1.34</td>
<td>28.4± 2.14</td>
<td>30.1 ± 1.68</td>
<td>P=0.53</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>52.2 ± 2.19</td>
<td>50.8 ± 3.49</td>
<td>53.59± 2.72</td>
<td>P=0.54</td>
</tr>
<tr>
<td>PBF (%)</td>
<td>24.2 ± 1.69</td>
<td>23.6 ± 2.30</td>
<td>24.7 ± 2.56</td>
<td>P=0.76</td>
</tr>
<tr>
<td>BFM (kg)</td>
<td>16.5±1.30</td>
<td>15.7±1.84</td>
<td>17.4±1.84</td>
<td>P=0.51</td>
</tr>
<tr>
<td>Waist: hip</td>
<td>0.87 ± 0.01</td>
<td>0.86± 0.13</td>
<td>0.89 ± 0.11</td>
<td>P=0.12</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.9 ± 0.06</td>
<td>4.9 ± 0.88</td>
<td>4.9 ± 0.09</td>
<td>P=0.96</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>5.1 ± 0.14</td>
<td>4.8± 0.22</td>
<td>5.4 ±0.17</td>
<td>P=0.03</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.00 ± 0.07</td>
<td>0.87± 0.08</td>
<td>1.14 ± 0.11</td>
<td>P=0.06</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.24 ± 0.14</td>
<td>2.97 ±0.20</td>
<td>3.51 ± 0.17</td>
<td>P=0.07</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.43 ± 0.07</td>
<td>1.45±0.90</td>
<td>1.42 ± 0.12</td>
<td>P=0.83</td>
</tr>
<tr>
<td>Total/HDL ratio</td>
<td>3.8 ± 0.21</td>
<td>3.5 ±0.27</td>
<td>4.1 ±0.28</td>
<td>P=0.10</td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
<td>6.8 ± 0.60</td>
<td>6.1 ± 0.89</td>
<td>7.5 ±0.82</td>
<td>P=0.27</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.5 ± 0.33</td>
<td>1.6 ± 0.52</td>
<td>1.5 ±0.43</td>
<td>P=0.10</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.50 ± 0.14</td>
<td>1.34±0.20</td>
<td>1.64 ± 0.20</td>
<td>P=0.28</td>
</tr>
</tbody>
</table>

Mean values ± standard error of mean. BMI, Body mass index; SMM, Skeletal muscle mass, FM, Fat Free Mass; PBF, Percentage Body Fat; LDL-C, Low Density Lipoprotein cholesterol; HDL-C, High Density Lipoprotein cholesterol; CRP, c-Reactive Protein; P values are comparing fructose versus glucose group at baseline.
<table>
<thead>
<tr>
<th>Daily intake</th>
<th>ALL (n=32)</th>
<th>Glucose (n=16)</th>
<th>Fructose (n=16)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (MJ)</td>
<td>10.2±2.2</td>
<td>7.6±3.8</td>
<td>12.8±4.4</td>
<td>P=0.24</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>80.6±6.0</td>
<td>77.7±8.6</td>
<td>83.6±8.67</td>
<td>P=0.63</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>77.6±4.7</td>
<td>65.6±5.9</td>
<td>89.6±6.0</td>
<td>P=0.008</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>295.0±57.3</td>
<td>283.2±75.1</td>
<td>306.8±88.9</td>
<td>P=0.84</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>22.2±1.5</td>
<td>21.1±1.9</td>
<td>23.4±2.2</td>
<td>P=0.42</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>213.5±9.2</td>
<td>217.5±15.3</td>
<td>209.6±10.5</td>
<td>P=0.67</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>83.2±7.2</td>
<td>80.5±9.7</td>
<td>84.6±10.7</td>
<td>P=0.72</td>
</tr>
<tr>
<td>Sucrose (g)</td>
<td>27.6±4.7</td>
<td>24.5±6.1</td>
<td>30.7±7.2</td>
<td>P=0.52</td>
</tr>
<tr>
<td>Bound Glucose (g)</td>
<td>13.8±2.3</td>
<td>12.3±3.1</td>
<td>15.3±3.6</td>
<td>P=0.52</td>
</tr>
<tr>
<td>Free Glucose (g)</td>
<td>16.7±3.0</td>
<td>22.3±5.5</td>
<td>11.2±1.7</td>
<td>P=0.07</td>
</tr>
<tr>
<td>Total Glucose (g)</td>
<td>30.5±3.9</td>
<td>34.5±6.2</td>
<td>26.5±4.8</td>
<td>P=0.31</td>
</tr>
<tr>
<td>Bound fructose (g)</td>
<td>13.8±2.3</td>
<td>12.3±3.1</td>
<td>15.3±3.6</td>
<td>P=0.52</td>
</tr>
<tr>
<td>Free fructose (g)</td>
<td>11.4±1.3</td>
<td>10.8±2.0</td>
<td>11.9±1.7</td>
<td>P=0.68</td>
</tr>
<tr>
<td>Total fructose (g)</td>
<td>25.2±3.1</td>
<td>23.1±4.1</td>
<td>27.3±4.9</td>
<td>P=0.52</td>
</tr>
<tr>
<td>Lactose (g)</td>
<td>15.1±1.7</td>
<td>12.2±2.3</td>
<td>17.9±2.3</td>
<td>P=0.09</td>
</tr>
<tr>
<td>Maltose (g)</td>
<td>7.5±1.7</td>
<td>7.1±2.3</td>
<td>8.0±2.5</td>
<td>P=0.80</td>
</tr>
</tbody>
</table>

Mean values ± standard error of mean; values without a common superscript is significantly different; P values are comparing fructose versus glucose group at baseline.
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)

<table>
<thead>
<tr>
<th></th>
<th>Glucose (n=16)</th>
<th>Fructose (n=16)</th>
<th>Δ</th>
<th>Glucose (n=16)</th>
<th>Fructose (n=16)</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post-intervention</td>
<td>Δ</td>
<td>Baseline</td>
<td>Post-intervention</td>
<td>Δ</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.9 ± 0.88</td>
<td>4.8±0.12</td>
<td>-0.10±0.09***</td>
<td>4.9 ± 0.09</td>
<td>5.0±0.08</td>
<td>0.14±0.06***</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.8± 0.22*</td>
<td>4.8±0.23**</td>
<td>-0.02±0.09</td>
<td>5.4±0.17*</td>
<td>5.4±0.18**</td>
<td>0.00±0.12</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.87± 0.08</td>
<td>0.89±0.07</td>
<td>0.02±0.05</td>
<td>1.14 ± 0.11</td>
<td>1.22±0.15</td>
<td>0.09±0.08</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.97 ±0.21</td>
<td>2.95±0.21</td>
<td>-0.01±0.07</td>
<td>3.51 ± 0.17</td>
<td>3.48±0.17</td>
<td>-0.02±0.09</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.45±0.90</td>
<td>1.44±0.09</td>
<td>-0.01±0.04</td>
<td>1.42 ± 0.12</td>
<td>1.41±0.08</td>
<td>-0.01±0.05</td>
</tr>
<tr>
<td>Total/HDL ratio</td>
<td>3.3 ±0.30*</td>
<td>3.49±0.26</td>
<td>0.19±0.17</td>
<td>4.1 ±0.28*</td>
<td>4.05±0.27</td>
<td>-0.09±0.10</td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
<td>6.1 ± 0.89</td>
<td>8.3±2.44</td>
<td>2.20±2.30</td>
<td>7.5 ±0.82</td>
<td>8.4±1.19</td>
<td>0.96±0.73</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.56 ±0.52</td>
<td>2.55±0.93</td>
<td>0.98±0.84</td>
<td>1.53±0.43</td>
<td>1.46±0.38</td>
<td>-0.70±0.12</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.3 ± 0.20</td>
<td>1.9±0.60</td>
<td>0.5±0.55</td>
<td>1.6 ± 0.19</td>
<td>1.9±0.30</td>
<td>0.3±0.18</td>
</tr>
</tbody>
</table>

Mean values ± standard error of mean; Δ, Changes in the levels of blood biomarkers from baseline to post-intervention; LDL-C, Low Density Lipoprotein cholesterol; HDL-C, High Density Lipoprotein cholesterol; CRP, C-Reactive Protein;

* Significantly different at baseline between group; ** Significantly different at post intervention between group;

*** Significantly different change between groups (P<0.05)
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)

<table>
<thead>
<tr>
<th></th>
<th>Glucose (n=16)</th>
<th>Fructose (n=16)</th>
<th>Δ</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post-intervention</td>
<td>Δ</td>
<td>Baseline</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.7 ± 5.4</td>
<td>66.8 ± 4.3</td>
<td>5.1 ± 3.50</td>
<td>71.0 ± 2.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.3 ± 1.03</td>
<td>23.4 ± 0.97</td>
<td>0.08 ± 0.08</td>
<td>24.9 ± 0.61</td>
</tr>
<tr>
<td>SMM (kg)</td>
<td>28.4 ± 2.14</td>
<td>28.8 ± 1.6</td>
<td>0.12 ± 0.0</td>
<td>30.1 ± 1.68</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>50.8 ± 3.49</td>
<td>51.1 ± 3.5</td>
<td>0.21 ± 0.15</td>
<td>53.59 ± 2.72</td>
</tr>
<tr>
<td>PBF (%)</td>
<td>23.6 ± 2.30</td>
<td>23.5 ± 2.32</td>
<td>-0.1 ± 0.37</td>
<td>24.7 ± 2.56</td>
</tr>
<tr>
<td>BFM (kg)</td>
<td>15.7 ± 1.84</td>
<td>15.7 ± 1.95</td>
<td>0.04 ± 0.30</td>
<td>17.4 ± 1.84</td>
</tr>
<tr>
<td>Waist: hip</td>
<td>0.86 ± 0.01</td>
<td>0.86 ± 0.13</td>
<td>-0.00 ± 0.001</td>
<td>0.89 ± 0.01</td>
</tr>
</tbody>
</table>

Mean values ± standard error of mean; Comparison of pre Vs post-intervention; Δ, Changes in the levels of blood biomarkers from baseline to post-intervention; LDL-C, Low Density Lipoprotein cholesterol; HDL-C, High Density Lipoprotein cholesterol; CRP, C-Reactive Protein; no significant differences between groups were observed.
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)

<table>
<thead>
<tr>
<th></th>
<th>Glucose (n=16)</th>
<th></th>
<th>Fructose (n=16)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post-intervention</td>
<td>Δ</td>
<td>Baseline</td>
</tr>
<tr>
<td>Energy (MJ)</td>
<td>7.60±3.82</td>
<td>7.01±1.64</td>
<td>-0.58±0.37</td>
<td>12.80±4.36</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>77.65±8.63</td>
<td>78.24±3.89</td>
<td>0.59±8.53</td>
<td>83.64±8.66</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>65.61±5.90</td>
<td>54.65±3.78</td>
<td>-10.96±6.24</td>
<td>89.55±5.96</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>283.21±75.05</td>
<td>209.29±23.89</td>
<td>-73.93±78.51</td>
<td>306.79±88.94</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>21.05±1.91</td>
<td>19.46±1.34</td>
<td>-1.59±2.33</td>
<td>23.40±2.19</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>217.46±15.33</td>
<td>195.11±10.59</td>
<td>-22.35±14.01</td>
<td>209.58±10.53</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>80.50±9.67</td>
<td>101.54±5.5</td>
<td>21.04±9.97</td>
<td>84.57±10.70</td>
</tr>
<tr>
<td>Sucrose (g)</td>
<td>24.53±6.12</td>
<td>12.23±2.77</td>
<td>-12.31±6.83</td>
<td>30.68±7.22</td>
</tr>
<tr>
<td>Bound Glucose (g)</td>
<td>12.27±3.05</td>
<td>6.11±1.39</td>
<td>-6.15±3.42</td>
<td>15.34±3.61</td>
</tr>
<tr>
<td>Free Glucose (g)</td>
<td>22.26±5.48</td>
<td>58.88±1.62</td>
<td>36.52±5.86</td>
<td>11.17±1.71</td>
</tr>
<tr>
<td>Total Glucose (g)</td>
<td>34.52±6.17</td>
<td>64.90±2.64</td>
<td>30.37±6.58</td>
<td>26.51±4.78</td>
</tr>
<tr>
<td>Bound fructose (g)</td>
<td>12.27±3.05</td>
<td>6.11±1.39</td>
<td>-6.15±3.42</td>
<td>15.34±3.61</td>
</tr>
<tr>
<td>Free fructose (g)</td>
<td>10.84±1.97</td>
<td>10.54±1.81</td>
<td>-0.30±1.64</td>
<td>11.91±1.72</td>
</tr>
<tr>
<td>Total fructose (g)</td>
<td>23.11±4.05</td>
<td>16.66±2.83</td>
<td>-6.45±3.76</td>
<td>27.25±4.87</td>
</tr>
<tr>
<td>Lactose (g)</td>
<td>12.20±2.25</td>
<td>7.78±1.75</td>
<td>-4.41±1.80</td>
<td>17.89±2.28</td>
</tr>
<tr>
<td>Maltose (g)</td>
<td>7.06±2.32</td>
<td>7.84±1.20</td>
<td>0.78±2.75</td>
<td>7.97±2.53</td>
</tr>
</tbody>
</table>

Mean values ± standard error of mean; Comparison of pre Vs post-intervention; Δ, Changes in the levels of blood biomarkers from base line to post-intervention; LDL-C, Low Density Lipoprotein cholesterol; HDL-C, High Density Lipoprotein cholesterol; CRP, c-Reactive Protein; P<0.05.

* Significantly different at baseline between group; ** Significantly different at post intervention between group; *** Significantly different change between groups (P<0.05)
Table 4-6: Correlation coefficients between changes in free fructose and free glucose intake and change in anthropometric, body composition and clinical parameters in fructose and glucose intervention groups respectively

<table>
<thead>
<tr>
<th>Correlation with free fructose or glucose</th>
<th>Fructose n=16</th>
<th>Glucose n=16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>-0.10</td>
<td>0.50</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.16</td>
<td>-0.65</td>
</tr>
<tr>
<td>SMM (kg)</td>
<td>0.31</td>
<td>0.17</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>0.31</td>
<td>0.01</td>
</tr>
<tr>
<td>PBF (%)</td>
<td>-0.17</td>
<td>-0.46</td>
</tr>
<tr>
<td>BFM</td>
<td>-0.19</td>
<td>-0.52</td>
</tr>
<tr>
<td>Waist: hip</td>
<td>0.28</td>
<td>-0.41</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>-0.15</td>
<td>-0.20</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>0.18</td>
<td>-0.17</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>-0.16</td>
<td>-0.18</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>0.06</td>
<td>-0.31</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>0.07</td>
<td>0.11</td>
</tr>
<tr>
<td>Total/HDL ratio</td>
<td>-0.01</td>
<td>-0.25</td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
<td>0.31</td>
<td>-0.06</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.09</td>
<td>-0.15</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.25</td>
<td>-0.16</td>
</tr>
</tbody>
</table>

r, Spearman correlation coefficient; Level of significance given as P ≤ 0.05.

BMI, Body mass index; SMM, Skeletal muscle mass; FM, Fat Free Mass; PBF, Percentage Body Fat; LDL-C, Low Density Lipoprotein cholesterol; HDL-C, High Density Lipoprotein cholesterol; CRP, c- Reactive Protein; HOMA-IR, homeostatic model assessment; N.S., non-significant
<table>
<thead>
<tr>
<th>Author</th>
<th>Population</th>
<th>Age (Y)</th>
<th>Duration of study</th>
<th>Amount of fructose and form</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le et al (2006)</td>
<td>7 M</td>
<td>24.7±1.3</td>
<td>4 weeks</td>
<td>1.5g fructose/kg BW daily (20% solution)=Approx.103.5g/day[30]</td>
<td>↑TG &amp; BG</td>
</tr>
<tr>
<td>Aeberli et al (2011)</td>
<td>29 M</td>
<td>26.3±6.6</td>
<td>3 weeks</td>
<td>600 ml beverages containing 40-80 g fructose</td>
<td>↑BG, ↓LDL particle size ↑hs-CRP</td>
</tr>
<tr>
<td>Perez-Pozo et al (2010)</td>
<td>74 M</td>
<td>51±7.8</td>
<td>2 weeks</td>
<td>200g/day, fructose sweetened beverages</td>
<td>↑TG &amp; insulin, ↓HDL-c</td>
</tr>
<tr>
<td>Silbernagel et al (2011)</td>
<td>20 M &amp; F</td>
<td>32.9±10.5</td>
<td>4 weeks</td>
<td>150 g fructose in 250 ml H2O</td>
<td>=Total cholesterol, LDL-c, HDL-c ↓TG</td>
</tr>
<tr>
<td>Couchepin et al (2008)</td>
<td>16 M &amp; F</td>
<td>22.5±0.9</td>
<td>6 days</td>
<td>3.5g fructose/kg FFM daily (25% of daily energy requirements)=Approx. 146 (F) to 200 g(M) [30]</td>
<td>↑BG, insulin &amp; TG</td>
</tr>
<tr>
<td>Sock et al (2010)</td>
<td>11 M</td>
<td>24.6±0.6</td>
<td>1 week</td>
<td>3.5g fructose/kg FFM daily (35% of daily energy requirements)</td>
<td>=BG &amp; insulin</td>
</tr>
<tr>
<td>Bantle et al (2000)</td>
<td>24 M &amp; F</td>
<td>41.2±14.6</td>
<td>6 weeks</td>
<td>85g crystalline fructose</td>
<td>↑TG (M) =Total cholesterol, HDL-c, LDL-c (M &amp; F both)</td>
</tr>
<tr>
<td>Beck-Nielsen et al (1980)</td>
<td>3 M &amp; 14 F</td>
<td>28±7</td>
<td>1 week</td>
<td>250g in H2O</td>
<td>=BG, ↓ insulin binding and sensitivity</td>
</tr>
<tr>
<td>Faeh et al (2005)</td>
<td>7 M</td>
<td>26.5±4</td>
<td>6 days</td>
<td>20% fructose solution (216.3g)[30]</td>
<td>↑TG &amp; BG</td>
</tr>
<tr>
<td>Swanson et al (1992)</td>
<td>07 M &amp; 07 F</td>
<td>19-60 years</td>
<td>4 weeks</td>
<td>High fructose=88g/day average (range 67-134g/day) Low fructose=5g/day average (range 3.8-7.6g/day)</td>
<td>=BG &amp; TG LDL &amp; Total cholesterol: High fructose&gt; Low fructose</td>
</tr>
<tr>
<td>Crapo et al (1984)</td>
<td>07 F &amp; 4 M</td>
<td>29-62 years</td>
<td>2 weeks</td>
<td>63-99g fructose</td>
<td>=BG</td>
</tr>
</tbody>
</table>

BG, Blood Glucose; LDL-C, Low Density Lipoprotein cholesterol; HDL-C, High Density Lipoprotein cholesterol; ↑, Increase; ↓; Decrease; =: No change, M; Male, F; Female.
5. Chapter 5 - Fructose restricted study
5.1 Thesis aim addressed in this chapter

5.1.1 Aim 3 To examine the effects of restricting fructose intake on glycaemic indices, blood lipids and inflammatory biomarkers in healthy individuals. The study also aimed to see:

- correlations between baseline fructose intake and body composition, lipid levels and inflammatory biomarkers
- correlations between change in fructose intake and change in body comp, lipid levels and inflammatory biomarkers

Information garnered from this study has allowed us to assess the relevance of fructose restricted diets, as a dietary strategy for reducing the risk for development of cardiovascular diseases.

5.1.2 Introduction

Recently updated WHO (draft) recommendations advise that the intake of free sugars should be less than 10% of total energy intake (strong recommendation) with further benefits likely below 5% of energy intake (conditional recommendation), based on clear benefits on body weight[331-333]. Particular concerns have been raised about the adverse health effects of fructose [4, 6, 9, 14, 20, 38, 78, 81, 97, 110, 112-123, 130, 309, 334, 335]. Fructose is a monosaccharide and has a unique absorption and metabolism profile that differentiates it from other sugars. Glucose is absorbed by active transport against the concentration gradient, while fructose is passively absorbed. Glucose, is either processed in the liver or sent to the circulation and further to extra hepatic tissues [82]. In contrast, fructose is metabolised in the liver, with little fructose delivered to systemic circulation or peripheral tissues [82]. The key differences in glucose and fructose metabolism lie in the metabolic pathways that fructose and glucose follow (refer Figure 1.3).

Natural sources of fructose account for approximately 1/3rd of dietary intake, while beverages and other foods constitute 2/3rd of dietary fructose intake [336, 337], mostly in the form of sucrose or
HFCS. Sucrose is made up of one molecule of glucose and one molecule of fructose, bonded together by a 1-4-glycoside bond. HFCS has been named as F55, F42 or F30 containing fructose: glucose ratio of 55:45 or 42:58 or 30:70 respectively [338, 339]. Fructose has been a subject of high level scrutiny in recent years, attracting attention as a unique dietary risk factor and accused of causing detrimental health effects [6, 9, 14, 20, 38, 78, 81, 309, 334, 335]. There is an increasing prevalence of chronic diseases, which corresponds with an increase in fructose consumption [38, 320]. Several epidemiological [14, 38, 78, 81, 334, 335], experimental [4, 97, 110, 112-123, 130] and clinical [6, 9, 14, 20, 309] studies have suggested an undesirable role of fructose consumption in elevating risk factors for the development of cardiovascular disease. In a study conducted in an acute setting of 2 hours (chapter 3), we have shown that fructose, as a sole source of energy, increases plasma lipids (LDL-cholesterol, HDL-cholesterol) and hs-CRP levels in healthy individuals compared to glucose or sucrose [324]. In addition, in a chronic setting of 4 weeks (chapter 4), we have shown that consumption of fructose in comparison to glucose increases fasting blood glucose, but does not modulate components of the lipid profile, insulin or low grade systemic inflammation in healthy individuals.

Reduction in dietary consumption of fructose and its potential effect on the blood lipid profile and low grade systemic inflammation is an important issue because it could potentially provide a strategy for reducing cardiovascular disease risk. While the ability of high fructose intake to increase the risk of developing cardiovascular disease has been shown in literature, there is a limited data available relating dietary fructose restriction and human health. Two studies set out to explore the effect of a low fructose diet on different biological markers in patients with chronic kidney disease or overweight/obese subjects [184, 340]. In addition, one of these studies looked at the effect of low fructose consumption on blood pressure [184] in chronic kidney disease patients. However, to date, the effect of restricting fructose in the diet on cardiovascular risk
factors in healthy individuals has not been tested. This study aimed to investigate the effect of a low fructose diet on glycaemic indices, blood lipids and low grade systemic inflammation in healthy subjects.

5.1.3 Methods

5.1.3.1 Study population

Fifteen (n=15) healthy male and female adults aged between 18-60 years participated in the study. They were recruited by advertisement and underwent study procedures at the Nutraceuticals Research Group Clinic rooms, University of Newcastle, NSW, Australia. Exclusion criteria were: diagnosed hyperlipidaemia, diabetes, gastrointestinal disorders, currently on fructose/sugar restricted diet, vegan diet or weight loss program, undergone any surgical procedure for obesity, pregnant or lactating mother, taking lipid-lowering or anti-inflammatory drugs and BMI >30 kg/m². Participants were asked to complete a medical questionnaire, International Physical Activity Questionnaire (IPAQ) [310] and a 3 day food record pre and post-intervention. Medical questionnaire included questions about any medical conditions, list of all medications including over-the-counter and supplements, consumption of alcohol, smoking and exercise (Appendix 4). The International Physical Activity Questionnaire (IPAQ) includes questions about the kinds of physical activities (vigorous and moderate activities in the last 7 days) that people do as part of their everyday lives (Appendix 7). Three day food diary includes information about details of food and drink consumed for a period of 3 days (Appendix 6). All participants provided written informed consent and the study was conducted in accordance with The Declaration of Helsinki. BIA using single frequency bioelectrical impedance apparatus (Maltron International, Essex, UK) was used to determine body composition of the participants. BIA measurements were taken with subjects in the supine position, wearing light clothing and no
shoes, in the morning after a minimum 10 hour fast. Participants were asked to refrain from physical exertion and consumption of alcohol for 24 hours prior to the testing date.

Participants were provided with a list of foods allowed and not allowed to consume during intervention. Participants who adhered to the recommended food lists was classified as compliant. Compliance was determined with the help of a 3 day food diary. The cut point was 12g of fructose per day as used in a previous study [184]. In addition, subjects who failed to fast on the day of sample collection were excluded.

5.1.3.2 Study Design

This was a randomized, parallel arm, dietary intervention trial in which male and female participants (n=15) were asked to adopt a fructose restricted diet for 1 week. Participants were provided with a list of foods allowed and not allowed to consume during intervention. They were advised not to consume cereals or breads containing fruit, honey roasted nuts, cookies, fruit juices, sugar sweetened soft drinks and beverages, etc. All vegetables were allowed.
List of food allowed and not allowed for the fructose restricted study:

<table>
<thead>
<tr>
<th>FOOD</th>
<th>ALLOWED</th>
<th>NOT ALLOWED</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRUITS</td>
<td>Lemons, Berries</td>
<td>All fruit juices, dried fruits, fruits, foods that contain fruit (e.g. fruited yoghurt)</td>
</tr>
<tr>
<td>Drinks</td>
<td>Milk, Tea, Coffee, diet drinks (Artificial sweeteners are allowed)</td>
<td>Soft drinks, Cordial, Milk shakes, Energy drinks e.g. Gatorade, V drink etc</td>
</tr>
<tr>
<td>Nuts and seeds</td>
<td>Almonds, Cashews, Hazelnuts, Peanuts, Macadamia, Pistachios, Walnuts, Sunflower seeds</td>
<td>Honey roasted nuts</td>
</tr>
<tr>
<td>Meat</td>
<td>Beef, Pork, Fish, Poultry, Eggs</td>
<td>Ham or other meats processed with honey</td>
</tr>
<tr>
<td>Bread/Starch</td>
<td>Oatmeal, Rice, Pasta, Crackers, Puffed rice, Puffed wheat</td>
<td>Cereals or breads containing fruit (e.g. banana bread, Muesli, Cookies)</td>
</tr>
</tbody>
</table>

Suggestion for daily menu:

<table>
<thead>
<tr>
<th>Time</th>
<th>Meal</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td>Cereals, Bread and butter, Eggs, Coffee/Tea, Milk</td>
<td></td>
</tr>
<tr>
<td>Morning Tea</td>
<td>Crackers, Cheese, Coffee/Tea</td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td>Salads, Wraps, Rolls</td>
<td></td>
</tr>
<tr>
<td>Evening Tea</td>
<td>Crackers, Cheese, Coffee/Tea</td>
<td></td>
</tr>
<tr>
<td>Dinner</td>
<td>Meat, Fish and sea foods, Chicken, Vegetables, Pasta, Rice, Potatoes</td>
<td></td>
</tr>
<tr>
<td>Late night Snacks</td>
<td>Crackers</td>
<td></td>
</tr>
</tbody>
</table>
FIGURE 5-1: STUDY DESIGN

Baseline

One Week

Post Intervention

Session 1:
- Anthropometry
- Body composition
- Questionnaires
- Food diary
- Blood test

Session 2:
- Anthropometry
- Body composition
- Questionnaires
- Food diary
- Blood test

Baseline

One Week

Post Intervention
Figure 5-1 describes the study design for this clinical trial and the sequence of events for the participants at each visit. Study duration was limited to one week, in order to maximise compliance with the intervention; a number of previous studies have demonstrated that 7 days duration is sufficient to show effects of fructose or carbohydrate restriction on glucose metabolism and blood lipids [15, 341, 342].

Participants were provided with sachets of glucose to consume ad libitum during the intervention period in case they experienced sugar cravings and asked to record the amount of glucose consumed. Subjects were assessed at baseline and after the 1-week intervention to examine changes in biochemical and anthropometric markers. Each assessment included blood collection for glucose, insulin, lipid and hs-CRP levels, anthropometric measurements for body weight, body composition measurements and collection of information on medical and physical activity. At baseline and end of intervention, daily fructose intake was assessed using a 3-day food diary. On the first visit, participants received dietary education and counselling with the session focussing on fructose containing foods.

Ethics approval

Ethics approval for this trial was granted by Human Research Ethics Committee (HREC) of the University of Newcastle (Approval number H2011-0336).

Clinical trial registration

The trial was registered with the Australia & New Zealand Clinical Trials Registry (ACTRN12612000172808)
Recruitment

Subjects were recruited via advertisement in local newspapers and radio stations. Subjects were also recruited from the Hunter Medical Research Institute (HMRI) register of volunteers and School of Nursing as part of their Research Awareness Exercise. In addition, the study was extensively advertised on the University Campus notice boards.

Dietary intake data

Three day food diaries were collected at baseline and post intervention. Food records collected from participants were entered into FoodWorks Version 7.0.291 database (Xyris Software Pty Ltd, Queensland, Australia) to analyse daily energy and nutrient intake of participants.

5.1.3.3 Laboratory methods

Venous blood samples at baseline and post-intervention were collected into tubes pre-coated with EDTA, lithium heparin and sodium fluoride by venepuncture. EDTA blood tubes were centrifuged for 10 minutes at 3000 g at 4°C for separation of plasma and stored at -80°C for further use. The lithium heparin tubes for blood lipids and hs-CRP & sodium fluoride tubes for blood glucose and insulin measurement were analysed by the accredited Hunter New England Area Pathology Services (Newcastle).

5.1.3.4 Statistical analysis

Sample size

A dietary fructose restriction study has been previously shown to decrease plasma triglyceride level by 9% [343]. To have 80% power to detect a clinically significant decrease in plasma
triglyceride of 0.3 SD, we need to recruit 15 subjects. Allowing for 20% drop outs, we will need to recruit n=20 subjects.

Data analysis

All data are presented as mean ± SEM. The normal distribution of variables was checked using the Kolmogorov-Smirnov test. All non-parametric data was logarithmically transformed to normal before statistical analyses were performed. Comparisons between baseline and post intervention were made with paired, two-tailed t-tests. A probability level of p<0.05 was adopted throughout to determine statistical significance unless otherwise mentioned. The relationships between fructose intake, anthropometric measurements, body composition and clinical parameters were determined using Pearson bivariate correlation test. The relationships between changes in fructose intake, anthropometric, body composition and clinical parameters were also determined using Pearson bivariate correlation test. All statistical analyses were carried out with SPSS software (version 21.0; SPSS Inc., Chicago, IL, USA).

5.2 Results

A total of n=16 subjects commenced the trial. One subject was excluded due to non-fasting prior to blood collection.

A total of n=15 subjects (five males & ten females; all females were premenopausal) completed the trial. Although the mean values for anthropometry, body composition measurements, blood lipids and hs-CRP were within normal range for all participants, some of the participants had higher than normal levels.

Mean anthropometric and body composition measurements were within normal range at baseline (Table 5-1). The mean (± SEM) age (years), body weight (kg), BMI (kg/m2), PBF (%), BFM (kg)
and waist: hip ratio (WHR) for all participants was 29.93±2.20, 65.8±2.40, 23.37±0.60, 27.7±1.74, 17.84±0.86 and 0.8±0.01 respectively.

Mean blood lipid levels were within normal range at baseline. The mean (± SEM) cholesterol (mmol/L), triglyceride (mmol/L), LDL-C (mmol/L), HDL-C (mmol/L), and Total/HDL-C ratio for all participants were 5.0±0.2, 1.09±0.12, 3.15±0.18, 1.38±0.82, 3.8±0.29 respectively.

Mean glucose homeostasis markers were within normal range at baseline. The mean (± SEM) glucose (mmol/L), insulin (m IU/L) and HOMA-IR were 4.6±0.08, 8.4±1.04 and 1.75±0.21 respectively.

Mean hs-CRP (mg/L) concentrations were within normal range at baseline. The mean (± SEM) CRP (mg/L) for all participants was 2.92±0.82.

Daily energy and nutrient intake at baseline (Table 5-2) were: energy = 9071.64±350.06 (kj); protein = 84.70±4.34 (g), carbohydrate = 279.64±10.82 (g), fat = 74.81±6.34 (g), cholesterol = 197.25±30.90 (mg), fibre = 23.10±1.81 (g), sugar = 99.00±7.60 (g), glucose = 30.34±3.40 (g), sucrose = 35.19±4.73 (g), bound fructose = 17.60±2.40 (g), free fructose = 14.90±1.60 (g), total fructose = 32.50±3.35 (g), lactose = 12.21±2.21 (g) and maltose = 19.01±3.86 (g).

Daily energy intake was similar at baseline and post-intervention despite changes in macronutrients (Table 5-2). Protein consumption was increased significantly (P=0.019) during the fructose restriction period from 84.70±4.34 to 92.79±3.72 g/day. As expected, carbohydrate (279.64±10.82 vs 255.15; P=0.006), sugar (99.00±7.60 vs 46.54±3.22; P<0.005), glucose (30.34±3.40 vs 16.43±0.80; P<0.005), sucrose (35.19±4.73 vs 5.18±0.70; P<0.005), bound fructose (17.60±2.40 vs 2.60±0.35;P<0.005), free fructose (14.90±1.60 vs 4.65±0.60; P<0.005), total
fructose (32.50±3.35 vs 7.24±0.70; P<0.005), and lactose (12.21±2.21 vs 8.73±1.15; P=0.04) were all significantly lower as a result of dietary fructose restrictions (Table 5-2). Reduction in fructose intake did not result in any significant changes in anthropometric measurements, body composition and blood biomarkers (Table 5-3 and 5-4).

Consumption of a low fructose diet (<8g of total fructose/day) for one week, resulted in a statistically significant reduction in BMI (23.37±0.60 vs 23.25±0.58; P<0.05) and BFM (17.84±0.86 vs 17.53±0.870; P<0.05) compared with post intervention respectively. However, there was no significant difference in body weight (65.80±2.40 vs 65.49 ±2.36; P=0.055) and percentage body fat (27.73±1.74 vs 27.32±1.71; P=0.07) from baseline to post intervention respectively. Moreover, there was no significant difference in the levels of glucose (4.65±0.08 vs 4.67±0.10; P=0.90), insulin (0.88±0.06 vs 0.83±0.06), total cholesterol (5.02±0.20 vs 4.82±020; P=0.14) , TG (1.09±0.12 vs 0.89±0.08; P=0.08), LDL-cholesterol (3.15±0.18 vs 3.05±0.19; P=0.51), HDL-cholesterol (1.4±0.08 vs 1.4±0.07; P=0.60) and total/HDL ratio (3.83±0.30 vs 3.75±0.32; P=0.35) & low grade systemic inflammatory biomarker hs-CRP (0.13±0.17 vs 0.08±0.14; P=0.45) levels from baseline to post intervention respectively (Table 5-3 & 5-4).

Correlations of bound fructose (fructose bound to glucose in a sucrose molecule) intake with anthropometric measurements, body composition and blood markers showed no significant correlation at baseline. Total fructose and free fructose intake did not show any significant correlation with anthropometric measurements and body compositions at baseline. Following one week of fructose restriction, no significant correlation was found between total, bound and free fructose intake and anthropometric measurements, body composition and blood biomarkers, except for a negative correlation between bound fructose intake and insulin levels post intervention (r= -0.57, P=0.026; Table 5-6).
Correlation between changes in total and bound fructose intake and changes in anthropometric measurements, body composition and blood biomarkers showed no significant correlations.

5.3 Discussion

To the best of our knowledge, this is the first study conducted in healthy individuals looking at the effects of consuming a fructose restricted diet on glycaemic control, blood lipid profiles and low grade systemic inflammation. Consumption of a low fructose diet (< 8g/day, less than 2% energy from fructose) for one week resulted in a statistically significant and potentially beneficial reduction in BMI and BFM. There was no significant difference in other parameters of anthropometric measurements, body composition and blood biomarkers of lipids or systemic inflammation.

To understand if baseline fructose intake is related to the effectiveness of fructose restricted diet, we looked at the baseline fructose consumption in previous fructose restricted studies. Two published studies in chronic kidney disease patients and overweight or obese subjects showing noticeable effects of restricted fructose intake in the diet had a base line fructose intake of 60g/day and 141g/day [184, 340], which is much higher than the current study. (32.50g/day).

Consumption of a low fructose diet showed a statistically significant decrease in 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.

Our finding is in agreement with a study showing a significant decrease in body weight following a low fructose diet (<20g/day) consumption compared with baseline [340]. Our study is also in agreement with a study comparing a fructose diet (17% of energy as fructose) with other a diet sweetened with glucose (3% of energy as fructose) showing significant declines in body
These studies showing greater decrease in body weight were of much longer duration (6 weeks) than our study and were in healthy, overweight or obese subjects [140, 340]. In addition, baseline fructose intake in the low fructose diet (<20g/day) study was reported to be very high (141g/day)[340] compared to our study (32.5g/day), accounting for the inconsistencies in reaching a larger decrease in weight leading to statistical significance.

Our finding is in disagreement with another study showing no change in body weight following a high versus low (20% vs <3%) fructose diet in healthy subjects [9]. Similarly, another study showed no significant change in body weight following low fructose (12g/day) consumption in chronic kidney disease patients [184] with their basal fructose intake of 60g/day. Differences in study design and background diets may contribute to the lack of effect on body weight [9, 184]. Consumption of a low fructose diet resulted in a statistically significant and potentially beneficial reduction in BFM. It may be possible that this statistically significant and potentially beneficial reduction in BMI and BFM reported in this study, may have related to 0.3 kg reduction in weight observed in this study, though it was statistically insignificant.

Consumption of a low fructose diet did not result in any significant change in percent body fat (PBF). Our finding is in disagreement with another study showing a significant decrease in PBF following a low fructose diet (<20g/day)[340] with basal fructose intake of 141g/day. However, the study was in overweight and obese subjects and of longer (6 weeks) duration.

Consumption of a low fructose diet did not result in any significant difference in waist: hip ratio. This differs from a study showing a significant decrease in waist: hip ratio following low fructose diet (<20g/day)[340] with basal fructose intake of 141g/day. However, again, the study was in overweight and obese subjects and of longer (6 weeks) duration. This shows that heterogeneity in
basal fructose intake, duration of study and health status of subjects influences the effect of fructose consumption on anthropometric measurements and body composition and warrants further investigations.

Consumption of a low fructose diet did not result in any significant difference in blood biomarkers. There was no significant increase or decrease in glucose, insulin, triglycerides, LDL, and HDL or hs-CRP levels following low fructose consumption. Our finding is in agreement with studies showing no change in blood glucose level following low fructose (<12g/day, <20g/day, 20% versus <3% and 24% versus 6%) consumption [184, 316, 340, 343]. However, these studies were in chronic kidney disease patients, diabetic subjects, overweight or obese subjects and healthy, obese adolescents; of varied duration of studies (1-6 weeks)[184, 316, 340, 343]. Our finding is in disagreement with a study comparing a diet containing 20% energy from fructose versus <3% energy from fructose which showed increased LDL and total cholesterol at 4 weeks [343]. However, the study was in diabetic subjects. Our finding is in agreement with a study showing no change in insulin levels when a low fructose diet (6% of energy) was compared with a high fructose (24% of energy) diet for 7 days in healthy, obese adolescents[316]. Our finding is in disagreement with a study showing a significant reduction in insulin level following low fructose (<12g/day) consumption in comparison to baseline [184]. However, once again, the study was in chronic kidney disease patients and of longer duration (4 weeks).

Our finding is not in accordance with a study showing a significant reduction in total cholesterol and triglyceride levels following low fructose diet (<20g/day) consumption. However, the study was in overweight and obese subjects and of longer (6 weeks) duration with baseline fructose intake of 141g/day [340]. However, no change in HDL-cholesterol level following low fructose consumption was reported in the same study, which is in accordance with our finding[340].
These studies show that heterogeneity in basal fructose intake, duration and health status of subjects results in varied effects of fructose consumption on blood biomarkers and warrants further investigation.

The duration of this study could be argued as a short term, which is a limitation. A longer study duration was considered but it was deemed to be too difficult for subjects to adhere to the strictly controlled restrictive diet for more than 7 days in an outpatient setting. Nevertheless, a number of previous studies have demonstrated that 7 day duration is sufficient to show dietary effects on glucose metabolism and blood lipids [15, 316, 341, 342]. Furthermore, carbohydrate restriction studies and dietary fructose studies, including a study involving low fructose diet have also demonstrated that 7 days or less duration is sufficient to show dietary fructose effects on glucose metabolism and blood lipids [5, 8, 12, 15, 312, 340, 344]. There were no significant differences in energy intake from baseline to post intervention. Therefore, the results obtained are independent of any effect of restriction of energy.

In summary, in this study, fructose restrictions were not only limited to consumption of foods with added sugar (soft drinks, pastries etc.) but the participants were also advised to restrict natural foods containing fructose including fruits, raisins, honey and dates etc. Since we were particularly interested in fructose restriction, participants were provided with glucose sachets to use as a sweetener to satisfy their sugar cravings. A small but statistically significant reduction in BMI and BFM following short-term (one week) fructose restricted diets is encouraging, suggesting that future studies of longer duration may influence blood biomarkers including blood lipid profile, glycaemic status and inflammation in healthy adults.
Strengths of this study include the use of fructose restriction without restricting other sugars and overall energy intakes. Potential limitations of the study include the short duration of the intervention as well as lack of a parallel control group with no fructose restrictions. Nonetheless, the changes in body composition that we observed (statistically significant and potentially beneficial reduction in BMI and BFM following low fructose diet consumption), suggest that further investigation of fructose restricted diets is warranted.

5.4 Conclusion

In conclusion, consumption of a fructose restricted diet for one week results in small decreases in BMI and BFM. Larger studies with a longer duration of intervention are warranted in healthy individuals, to examine the effect of a low fructose diet on cardiovascular disease risk factors, by evaluating the impact on parameters of anthropometric measurements, body composition and blood biomarkers for lipid status and systemic inflammation.
### TABLE 5-1: BASELINE VALUES OF ANTHROPOMETRIC MEASUREMENTS, BODY COMPOSITION AND BLOOD BIOMARKERS – BASELINE CHARACTERISTICS

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Baseline values</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.8 ±2.40</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>23.37± 0.60</td>
</tr>
<tr>
<td>SMM (kg)</td>
<td>26.70 ± 1.70</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>48.0 ± 2.74</td>
</tr>
<tr>
<td>PBF (%)</td>
<td>27.7 ± 1.74</td>
</tr>
<tr>
<td>BFM (kg)</td>
<td>17.84±0.86</td>
</tr>
<tr>
<td>Waist: hip ratio</td>
<td>0.8 ± 0.01</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.6 ± 0.08</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.09 ± 0.12</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.15 ± 0.18</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.38 ± 0.82</td>
</tr>
<tr>
<td>Total/HDL ratio</td>
<td>3.8 ± 0.29</td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
<td>8.4 ± 1.04</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>2.92 ± 0.82</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.75 ±0.21</td>
</tr>
</tbody>
</table>

Mean values ± standard error of mean. BMI, Body mass index; SMM, Skeletal muscle mass; FM, Fat Free Mass; PBF, Percentage Body Fat; LDL-C, Low Density Lipoprotein cholesterol; HDL-C, High Density Lipoprotein cholesterol; CRP, c-Reactive Protein; HOMA-IR, Homeostasis model assessment-Insulin Resistance; values without a common superscript is significantly different; P<0.05.

### TABLE 5-2: DAILY ENERGY AND NUTRIENT INTAKE AT BASELINE AND POST INTERVENTION

<table>
<thead>
<tr>
<th>Daily intake (n=15)</th>
<th>Baseline values</th>
<th>Post intervention</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kj)</td>
<td>9071±350</td>
<td>8751±322</td>
<td>N.S.</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>84.70±4.34</td>
<td>92.79±3.72</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>74.81±6.34</td>
<td>68.12±6.00</td>
<td>N.S.</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>197.25±30.90</td>
<td>193.74±35.18</td>
<td>N.S.</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>23.10±1.81</td>
<td>23.70±1.65</td>
<td>N.S.</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>279.64±10.82</td>
<td>255.15±8.50</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>99.00±7.60</td>
<td>46.55±3.21</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Glucose (g)</td>
<td>30.34±3.40</td>
<td>16.43±0.80</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Sucrose (g)</td>
<td>35.20±4.73</td>
<td>5.20±0.70</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Bound fructose (g)</td>
<td>17.60±2.36</td>
<td>2.59±0.34</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Free fructose (g)</td>
<td>15.00±1.60</td>
<td>4.64±0.60</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Total fructose (g)</td>
<td>32.50±3.35</td>
<td>7.23±0.70</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Lactose (g)</td>
<td>12.21±2.21</td>
<td>8.73±1.14</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Maltose (g)</td>
<td>19.10±3.85</td>
<td>21.45±3.64</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Mean values ± standard error of mean. N.S: Non significant
### 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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All (n=15)</th>
<th>Post-intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.8 ±2.40</td>
<td>65.5±2.36</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>23.37± 0.60</td>
<td>23.25±0.58*</td>
</tr>
<tr>
<td>SMM (kg)</td>
<td>26.70 ± 1.70</td>
<td>26.73±1.66</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>48.0 ± 2.74</td>
<td>48.0±2.67</td>
</tr>
<tr>
<td>PBF (%)</td>
<td>27.7 ± 1.74</td>
<td>27.3±1.71</td>
</tr>
<tr>
<td>BFM (kg)</td>
<td>17.84±0.86</td>
<td>17.53±0.87*</td>
</tr>
<tr>
<td>Waist: hip</td>
<td>0.8 ± 0.01</td>
<td>0.8±0.01</td>
</tr>
</tbody>
</table>

*P<0.05 (paired t-test) compared to baseline.

BMI, Body mass index; SMM, Skeletal muscle mass; FM, Fat Free Mass; PBF, Percentage Body Fat

### TABLE 5-4: BASELINE AND POST-INTERVENTION VALUES OF BLOOD BIOMARKERS

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All (n=15)</th>
<th>Post-intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.6 ± 0.08</td>
<td>4.7±0.10</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>5.0 ± 0.2</td>
<td>4.8±0.19</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.09 ± 0.12</td>
<td>0.89±0.84</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.15 ± 0.18</td>
<td>3.05±0.19</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.38 ± 0.82</td>
<td>1.37±0.07</td>
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<tr>
<td>Total/HDL ratio</td>
<td>3.8 ± 0.29</td>
<td>3.75±0.32</td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
<td>8.4 ± 1.04</td>
<td>8.2±1.73</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>2.92 ± 0.82</td>
<td>2.20±0.12</td>
</tr>
</tbody>
</table>

There was no difference in baseline and post–intervention values of blood biomarkers (paired t-test).

LDL-C, Low Density Lipoprotein cholesterol; HDL-C, High Density Lipoprotein cholesterol; CRP, C - reactive protein
<table>
<thead>
<tr>
<th>Correlation with free, bound and total fructose</th>
<th>All n=15</th>
<th>All n=15</th>
<th>All n=15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>SMM (kg)</td>
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<tr>
<td>FFM (kg)</td>
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<td></td>
</tr>
<tr>
<td>PBF (%)</td>
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<tr>
<td>BFM</td>
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<td></td>
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<td>Waist: hip</td>
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<tr>
<td>Glucose (mmol/L)</td>
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<td>Triglyceride (mmol/L)</td>
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<td>LDL-C (mmol/L)</td>
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<td>HDL-C (mmol/L)</td>
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<td>Total/HDL ratio</td>
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<td>Insulin (mIU/L)</td>
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<tr>
<td>CRP (mg/L)</td>
<td>-0.52</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

r, Pearson correlation coefficient; Level of significance given as P ≤ 0.05. Mean values ± standard error of mean. BMI, Body mass index; SMM, Skeletal muscle mass, FM, Fat Free Mass; PBF, Percentage Body Fat; LDL-C, Low Density Lipoprotein cholesterol; HDL-C, High Density Lipoprotein cholesterol; CRP, c- Reactive Protein; N.S., non-significant.
TABLE 5-6: CORRELATION COEFFICIENTS BETWEEN FRUCTOSE INTAKE, ANTHROPOMETRIC, BODY COMPOSITION AND CLINICAL PARAMETERS - POST INTERVENTION

<table>
<thead>
<tr>
<th>Correlation with free, bound and total fructose</th>
<th>Free fructose</th>
<th>Bound fructose</th>
<th>Total fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>r</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMM (kg)</td>
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<tr>
<td>FFM (kg)</td>
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<td>PBF (%)</td>
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<tr>
<td>BFM</td>
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<tr>
<td>Waist: hip</td>
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<td></td>
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<tr>
<td>Glucose (mmol/L)</td>
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<td></td>
<td></td>
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<tr>
<td>Cholesterol (mmol/L)</td>
<td></td>
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<tr>
<td>Triglyceride (mmol/L)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
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<td></td>
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<tr>
<td>HDL-C (mmol/L)</td>
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<tr>
<td>Total/HDL ratio</td>
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<tr>
<td>Insulin (mIU/L)</td>
<td></td>
<td>-0.57</td>
<td>0.02</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

r, Pearson correlation coefficient; Level of significance given as P ≤ 0.05. Mean values ± standard error of mean. BMI, Body mass index; SMM, Skeletal muscle mass, FM, Fat Free Mass; PBF, Percentage Body Fat; LDL-C, Low Density Lipoprotein cholesterol; HDL-C, High Density Lipoprotein cholesterol; CRP, c-Reactive Protein; N.S., non-significant.
## Table 5-7: Correlation Coefficients Between Changes in Fructose Intake, Anthropometric, Body Composition and Clinical Parameters

<table>
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<td>Correlation with changes in free, bound and total fructose</td>
<td>ΔFree fructose</td>
<td>ΔBound fructose</td>
<td>ΔTotal fructose</td>
</tr>
<tr>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
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<tr>
<td>ΔWeight (kg)</td>
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<td>ΔBMI (kg/m²)</td>
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<tr>
<td>ΔSMM (kg)</td>
<td>-0.54</td>
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<td>ΔFFM (kg)</td>
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<td>ΔPBF (%)</td>
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<td>ΔBFM</td>
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<td>ΔGlucose (mmol/L)</td>
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<td>ΔCholesterol (mmol/L)</td>
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<tr>
<td>ΔTriglyceride (mmol/L)</td>
<td>-0.53</td>
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<td>ΔLDL-C (mmol/L)</td>
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<td>ΔHDL-C (mmol/L)</td>
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<td>ΔTotal/HDL ratio</td>
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<td>ΔInsulin (mIU/L)</td>
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<td>ΔCRP (mg/L)</td>
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r, Pearson correlation coefficient; Level of significance given as P ≤ 0.05. Mean values ± standard error of mean. BMI, Body mass index; SMM, Skeletal muscle mass; FM, Fat Free Mass; PBF, Percentage Body Fat; LDL-C, Low Density Lipoprotein cholesterol; HDL-C, High Density Lipoprotein cholesterol; CRP, c-Reactive Protein; Δ, Change; N.S., non-significant
6. Chapter 6 - Discussion and conclusion
6.1 Summary

The summary of the main findings are as follows:

1. Acute fructose consumption in a single dose of 50g/day in the form of a beverage resulted in a significant increase in the plasma levels of total, LDL and HDL cholesterol and the acute phase pro-inflammatory marker hs-CRP, compared to the same dose of glucose or sucrose. Interestingly, no significant change in TG levels was observed. The glucose and sucrose supplementation initially resulted in a significant increase in glucose and insulin levels compared to fructose supplementation and returned to near baseline values within two hours.

2. Comparison of blood biomarkers pre and post supplementation with 50g of fructose or glucose per day for 4 weeks showed an increase in blood glucose concentrations following the fructose compared to glucose intervention. No significant difference in insulin, TG, LDL-C, HDL-C, total cholesterol, Total/HDL-C ratio or hs-CRP levels were observed.

3. Consumption of a low (restricted) fructose diet (< 8g/day, less than 2% energy from fructose) for one week resulted in a statistically significant and potentially beneficial reduction in BMI and BFM. There was no significant difference in other parameters of anthropometric measurements, body composition and blood biomarkers of lipids or systemic inflammation.

6.2 Discussion and Limitations

The acute study was designed to examine the metabolic consequences of sugar consumption when it is used as a sole source of energy (no other nutrient to augment postprandial lipemia). There are several possible mechanisms by which acute fructose consumption could lead to an increase in postprandial levels of total, LDL and reduction in HDL cholesterol. Since no
nutrients, other than sugars, were included in the test beverages, the lipoproteins measured were almost exclusively of hepatic origin. Fructose may influence total and LDL-cholesterol levels by blocking LDL-receptors or affect HDL cholesterol via CETP or reverse cholesterol transport and this merits further investigation. In addition, the significance of the increase in HDL-cholesterol that occurs concurrently with the increase in LDL-cholesterol and elevated hsCRP levels remains to be delineated when considering health effects of feeding fructose-rich diets. Our results showed no significant change in postprandial triglyceride levels irrespective of the type of sugar consumed. Due to the absence of other energy yielding nutrients, the clearance rate of triglycerides can be expected to be higher, resulting in overall no change in triglyceride level. 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. Significance of no change in blood glucose and insulin levels following acute fructose consumption also merits further investigations, particularly in view of the satiating effects of insulin.

In the chronic study, 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. Higher blood glucose concentrations as found in our study suggest some degree of impairment in the inhibition of glucose metabolism following fructose consumption [4, 325, 326]. When our finding is compared with previous studies, the heterogeneity in available data is evident, which could be due to differences in study design, type and amount of fructose consumed, as well as duration of the study.
The chronic study has several limitations that must be taken into account while interpreting the results. One limitation is that the participants randomized to consume the fructose supplements had higher baseline values of total cholesterol compared to the glucose group. Whilst we accounted for this in the statistical analyses by analysing the changes compared to baseline, this may have confounded the result post-intervention. Moreover, a 4 week duration of intervention and dose of 50g of fructose may not have been sufficient enough to notice any significant effects of fructose consumption on parameters such as lipoprotein levels. Another limitation to this study could be the fact that the effect of fructose on inducing characteristic features of metabolic syndrome is more pronounced in overweight or hyperinsulinemic subjects [12, 78, 143]. It is therefore possible that testing fructose consumption in a healthy population produced a blunted response.

In addition, in the chronic study participants were given the choice of taking the supplements either as a single dose or in divided doses. Variations in frequency and timing of fructose dosing may also have confounded the results [17, 318, 320, 336]. For example, in our study, the fructose was not consumed in excess or energy requirements, which may have avoided potential harmful effects. Another potential confounder is that we didn’t collect information on whether the fructose supplement was taken with meals or on an empty stomach. This is another potential limitation, as co-consumption of glucose with fructose has been shown to enhance absorption of fructose and may have confounded our outcomes [330].

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.

The fructose restriction study 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. It appears that statistically significant and potentially beneficial reduction in BMI and BFM reported in this study, relates to the 0.3 kg reduction in weight observed in this study, though reduction in weight was not statistically significant. There was no significant difference in other parameters of anthropometric measurements, body composition and blood biomarkers of lipids or systemic inflammation.

The duration of fructose restricted study could be argued as a short term, which is a limitation. Further, Base level fructose intake was low, therefore further fructose restriction may not have caused any significant effects on glucose or lipid metabolism. A longer study duration was considered, but deemed to be too difficult for subjects to adhere to for more than 7 days in an outpatient setting. Nevertheless, a number of previous studies have demonstrated that 7 day duration is sufficient to show dietary effects on glucose metabolism and blood lipids [15, 316, 341, 342]. Furthermore, carbohydrate restriction studies and dietary fructose studies, including a study involving low fructose diet have also demonstrated that 7 days or less duration is sufficient to show dietary fructose effects on glucose metabolism and blood lipids [5, 8, 12, 15, 312, 340, 344].
Strengths of this study include the use of fructose restriction without restricting other sugars and overall energy intakes. Potential limitations of the study include the short duration of the intervention as well as lack of a parallel control group with no fructose restrictions. Nonetheless, the changes in body composition that we observed (statistically significant and potentially beneficial reduction in BMI and BFM following low fructose diet consumption), suggest that further investigation of fructose restricted diets is warranted to examine the effect of a low fructose diet on cardiovascular disease risk factors.
6.3 Conclusion

In conclusion, acute consumption of fructose (as sole source of energy) modulates plasma lipids and increases hsCRP levels in healthy individuals. However, it appears that under the conditions that we used; dose (50g) and duration (4 weeks), chronic consumption of fructose does not modulate CVD risk factors in healthy individuals. Furthermore, a small but statistically significant reduction in BMI and BFM following short-term (one week) fructose restriction suggests that future studies of longer duration may have a positive influence on body composition in healthy adults. This is an important area of future research. As fructose is metabolised differently from 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.
References


80. Tappy, L. and K.A. Le, *Does fructose consumption contribute to non-alcoholic fatty liver disease?* Clinics and research in hepatology and gastroenterology, 2012.


133. Tsai, J., R. Zhang, W. Qiu, Q. Su, M. Naples, and K. Adeli, Inflammatory NF-kappaB activation promotes hepatic apolipoprotein B100 secretion: evidence for a link between hepatic inflammation


<table>
<thead>
<tr>
<th>Reference</th>
<th>Title</th>
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Information Statement
Dietary fructose consumption and cardio-metabolic health
Investigators: Professor Manohar Garg, Dr. Lisa Wood & Mr. Faizan Jameel
Version 2.4; 05/07/2012

Thank you for your interest in our research on dietary fructose consumption and plasma lipids, satiety hormones, insulin resistance and inflammation. This study is being carried out by researchers from the School of Biomedical Sciences & Pharmacy at the University of Newcastle, as part of the PhD studies of Faizan Jameel under the supervision of Prof. Manohar Garg and Dr Lisa Wood.

Why is the research being done?

There is evidence in the published literature that consumption of a high fructose diet is detrimental to cardio-metabolic health. The purpose of this research is to determine the effect of dietary fructose consumption on plasma lipids, satiety hormones, insulin resistance and inflammation. If our hypothesis is validated, dietary strategies can be designed to reduce body weight and associated risk for the development of diabetes and cardiovascular disease.

Fructose – commonly known as fruit sugar, is a component of many fruits, honey, table sugar, high fructose corn syrup etc and forms a significant part of our daily food consumption.

Who can participate?
To take part in this study you must be:

- Male or female aged 18 – 65 years at initial assessment.

You are NOT eligible to participate if you:

i. Are currently on cholesterol lowering drugs e.g. Statins
ii. Are diabetic
iii. Are pregnant or lactating mother
iv. Are currently on anti-inflammatory drugs e.g. NSAIDs
v. Are currently on fructose/sugar restricted diets
vi. Have been diagnosed with any gastrointestinal disorders
vii. Are currently on a weight loss program
viii. Have undergone any surgical procedures for obesity
ix. Are on Vegan diet

Participation in this research is voluntary. Only those people who have given their written informed consent will be included in this project. If you do decide to participate you may withdraw your consent for participation in this research project at any time without giving a reason and have the right to withdraw data you have provided (up until the point where your data is de-identified).

What would you be asked to do?
If you agree to participate in this study, you will be asked to consume a single dose of either a) lemon-flavoured drink containing 25-30g of fructose, or b) lemon-flavoured drink containing 25-30 g of glucose or c) lemon flavoured drink containing 25-30g of sucrose. This will be followed by collection of blood samples at 0, 20, 40, 60, 90, 120 minutes.

Throughout the study, you will need to visit the University on 3 occasions; at week 1, 2 and 3. You will give body weight, height and body composition measurements at week 1 only.
At each of the visits, we will be asking you to do the following;

- Complete a brief medical questionnaire.
- Complete a physical activity questionnaire.
- Donate blood (42 mL), after an overnight fast

Each visit will take approximately 150 minutes to obtain information and collect blood samples.

You will also be asked to complete a 24 hours food record on 3 occasions: during week 0-3. This involves recording all the food and beverages that you consume over a 3 day period. You will be given instructions on how to record this information. You will be provided with a light breakfast following donation of the blood sample.

What are the risks and benefits of participating?
There are some risks of having blood collected, including bruising or bleeding from the collection site, as well as fainting and dizziness. However, these risks are minimal and a qualified and experienced phlebotomist will take your blood in order to minimise these risks.

You may request information about your health from any examinations and laboratory tests that are undertaken in this study. Feedback of results from the investigation and other information regarding to the outcome of the research will be made available to you and will be posted as an individual letter at the completion of the study.
How will the information collected be used?
Results of this research will be published in scientific journals and be published in Faizan Jameel’s PhD thesis. Individual participants will not be identified in any reports arising from the project.

How will your privacy be protected?
Your information will be treated with the same respect for privacy and confidentiality as is undertaken for all medical information collected about you during your visits to your local doctor. Access to the collected information will be limited to the named investigators only.

All collected information on data sheets will be physically stored in a locked filing cabinet in the Medical Science Building accessible to the investigators at the University of Newcastle. All data will only be identifiable by a participant identification number with no other identifying details on file. During statistical data analysis the database will be stored in a password protected computer file on a computer that is kept in a locked room. After completion of computer data analysis, the information will be transferred onto a CD before it will be removed and permanently deleted from the computer file. The CD will also be stored in a locked cabinet in a locked room of the principle investigator.

All data for the study will be kept by the Chief Investigator for the period of 15 years following the completion of the study. Only staff of the University of Newcastle conducting this research will have access to this information.

What do you need to do to participate?
Please read this Information Statement and be sure you understand its contents before you consent to participating. If there is anything you do not understand, or you have questions please contact the study principle investigator.

If you would like to participate, please complete the attached Consent Form and return it to the investigators at the University of Newcastle.

Further information
Further information about this or any other research project undertaken in the department at the University of Newcastle can be obtained from Principle Investigators of the study, Professor Manohar Garg and Dr. Lisa Wood or Mr. Faizan Jameel (4921 5638), Email: faizan.jameel@uon.edu.au

Thank you for considering this invitation to participate in research undertaken at the University of Newcastle.

______________________________  ________________________________
Professor Manohar Garg            Dr. Lisa Wood
**Professor Manohar Garg**
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**Mr. Faizan Jameel**
Principal Investigator 3,
University of Newcastle

**Ms. Melissa Fry**
Principal Investigator 4,
University of Newcastle
Research Assistant
melissa.fry@newcastle.edu.au

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**Complaints about this research**
This project has been approved by the University’s Human Research Ethics Committee, Approval No. H-2011-0336

Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, Australia, Telephone (02) 4921 6333, Email: Human-Ethics@newcastle.edu.au.
I give my consent to participate in the above research project designed to test the effect of dietary fructose consumption on plasma lipids, satiety hormones and inflammation. I understand that the following information has been provided to me in the Information Statement, a copy of which I have retained;

- I understand that my participation is voluntary and entirely my choice.
- I understand I can withdraw my consent for participation at any time and do not have to give any reason for withdrawing.
- I understand that I will need to visit the University on 3 occasions over 3 weeks.
- I understand that at each visit, I will be required to donate blood (42 mL) after an overnight fast, complete a medical and a physical activity questionnaire.
- I understand that I will also be required to complete a 24 hours food diary on 3 occasions.
- I understand that my personal information will remain confidential to the researchers.
- Feedback of results from the investigation and other information regarding to the outcome of the research will be made available to me and will be posted as an individual letter at the completion of the study.

Signed by Participant
Print Name: 
Phone: 
Email: 
Date: 

Dietary fructose consumption and cardio-metabolic health
Appendix Two: Study 2 Information Statement & Consent Form

Information Statement

Dietary fructose restriction improves cardio-metabolic health
Investigators: Professor Manohar Garg, Dr. Lisa Wood & Mr. Faizan Jameel
Version 2.5: 09/08/13

Thank you for your interest in our research on dietary fructose restriction and plasma lipids, satiety hormones, insulin resistance and inflammation. This study is being carried out by researchers from the School of Biomedical Sciences & Pharmacy at the University of Newcastle, as part of the PhD studies of Faizan Jameel under the supervision of Prof. Manohar Garg and A/Prof Lisa Wood.

Why is the research being done?
There is evidence in the published literature that consumption of a high fructose diet is detrimental to cardio metabolic health. The purpose of this research is to determine the effect of dietary fructose restriction on plasma lipids, satiety hormones, insulin resistance and inflammation. If our hypothesis is validated, dietary strategies can be designed to reduce body weight and associated risk for the development of diabetes and cardiovascular disease.

Fructose – commonly known as fruit sugar, is a component of many fruits, honey, table sugar, high fructose corn syrup etc. and forms a significant part of our daily food consumption.

Who can participate?
To take part in this study you must be:
- Males or female with BMI<30 [BMI = weight (kg)/ height (m)^2]
- Aged between 18 – 65 years at initial assessment.

You are NOT eligible to participate if you:
- Are currently on cholesterol lowering drugs e.g. Statins
- Are diabetic
- Are pregnant or lactating mother
- Are currently on anti-inflammatory drugs e.g. NSAIDs
- Are currently on fructose/sugar restricted diets
- Have been diagnosed with any gastrointestinal disorders
- Are currently on a weight loss program
- Have undergone any surgical procedures for obesity
- Are on Vegan diet
Participation in this research is voluntary. Only those people who have given their written informed consent will be included in this project. If you do decide to participate you may withdraw your consent for participation in this research project at any time without giving a reason and have the right to withdraw data you have provided (up until the point where your data is de-identified). You will receive $20 as reimbursement to cover for travel and parking costs.

**What would you be asked to do?**

If you agree to participate in this study, you will be asked to keep a 3 day food record to determine how much sugar you usually consume. If your fructose intake comes out to be <60 grams/day, you will be asked to supplement your diet with either 50g of fructose or 50g of glucose per day for a period of 4 weeks. On the other hand, if your fructose intake is >60 grams/day, you will be asked to consume a fructose restricted diet for a period of 4 weeks. Consuming a fructose restricted diet involves reducing your intake of foods such as fruit, fruit juices and sweetened beverages such as soft drinks. You will be given detailed advice on the types of foods that you need to avoid.

Throughout the study, you will need to visit the University on 2 occasions; at week 0 and 4. At each of the visits, we will be asking you to do the following:

- Complete a brief medical questionnaire.
- Complete a physical activity questionnaire.
- Give body weight, height and body composition measurements
- Donate blood (25 mL), after an overnight fast

Each visit will take approximately 30 minutes to obtain information and collect blood sample. You will also be asked to complete a 3 day food record on 2 occasions: for screening and 4 weeks post-intervention. This involves recording all the food and beverages that you consume over a 3 day period. You will be given instructions on how to record this information. You will be provided with a light breakfast following donation of the blood sample.

**What are the risks and benefits of participating?**

There are some risks of having blood collected, including bruising or bleeding from the collection site, as well as fainting and dizziness. However, these risks are minimal and a qualified and experienced phlebotomist will take your blood in order to minimise these risks.

You may request information about your health from any examinations and laboratory tests that are undertaken in this study. Feedback of results from the investigation and other information regarding to the outcome of the research will be made available to you and will be posted as an individual letter at the completion of the study.

**How will the information collected be used?**

Results of this research will be published in scientific journals and be published in Faizan Jameel’s PhD thesis. **Individual participants will not be identified** in any reports arising from the project.
How will your privacy be protected?

Your information will be treated with the same respect for privacy and confidentiality as is undertaken for all medical information collected about you during your visits to your local doctor. Access to the collected information will be limited to the named investigators only.

All collected information on data sheets will be physically stored in a locked filing cabinet in the Medical Science Building accessible to the investigators at the University of Newcastle. All data will only be identifiable by a participant identification number with no other identifying details on file. During statistical data analysis the database will be stored in a password protected computer file on a computer that is kept in a locked room. After completion of computer data analysis, the information will be transferred onto a CD before it will be removed and permanently deleted from the computer file. The CD will also be stored in a locked cabinet in a locked room of the principal investigator.

All data for the study will be kept by the Chief Investigator for the period of 15 years following the completion of the study. Only staff of the University of Newcastle conducting this research will have access to this information.

What do you need to do to participate?

Please read this Information Statement and be sure you understand its contents before you consent to participating. If there is anything you do not understand, or you have questions please contact the study principal investigator.

If you would like to participate, please complete the attached Consent Form and return it to the investigators at the University of Newcastle.

Further information

Further information about this or any other research project undertaken in the department at the University of Newcastle can be obtained from Principal Investigators of the study, Professor Manohar Garg and A/Prof Lisa Wood or Mr. Faizan Jameel (4921 5638), Email: faizan.jameel@uon.edu.au

Thank you for considering this invitation to participate in research undertaken at the University of Newcastle.

______________________________  ________________________________
Professor Manohar Garg         A/Prof Lisa Wood
Principal Investigator 1,       Principal Investigator 2,
University of Newcastle         University of Newcastle

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Complaints about this research
This project has been approved by the University’s Human Research Ethics Committee, Approval No.H-2011-0336

Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, Australia, Telephone (02) 4921 6333, Email: Human-Ethics@newcastle.edu.au.
Appendix Three: Study 3 Information Statement & Consent Form

Information Statement

Dietary fructose restriction improves cardio-metabolic health
Investigators: Professor Manohar Garg, A/Prof Lisa Wood & Mr. Faizan Jameel
Version 2.9; 07/08/14

Thank you for your interest in our research on dietary fructose restriction and plasma lipids, satiety hormones, insulin resistance and inflammation. This study is being carried out by researchers from the School of Biomedical Sciences & Pharmacy at the University of Newcastle, as part of the PhD studies of Faizan Jameel under the supervision of Prof. Manohar Garg and A/Prof Lisa Wood.

Why is the research being done?

There is evidence in the published literature that consumption of a high fructose diet is detrimental to cardio metabolic health. The purpose of this research is to determine the effect of dietary fructose restriction on plasma lipids, satiety hormones, insulin resistance and inflammation. If our hypothesis is validated, dietary strategies can be designed to reduce body weight and associated risk for the development of diabetes and cardiovascular disease.

Fructose – commonly known as fruit sugar, is a component of many fruits, honey, table sugar, high fructose corn syrup etc. and forms a significant part of our daily food consumption.

Who can participate?

To take part in this study you must be:
- Males or female with BMI<30 [BMI = weight (kg)/ height (m)^2]
- Aged between 18 – 65 years at initial assessment.

You are NOT eligible to participate if you:
- Are currently on cholesterol lowering drugs e.g. Statins
- Are diabetic
- Are pregnant or lactating mother
- Are currently on anti-inflammatory drugs e.g. NSAIDs
- Are currently on fructose/sugar restricted diets
- Have been diagnosed with any gastrointestinal disorders
- Are currently on a weight loss program
- Have undergone any surgical procedures for obesity
ix. Are on Vegan diet

Participation in this research is voluntary. Only those people who have given their written informed consent will be included in this project. If you do decide to participate you may withdraw your consent for participation in this research project at any time without giving a reason and have the right to withdraw data you have provided (up until the point where your data is de-identified). You will receive $20 as reimbursement to cover for travel and parking costs.

**What would you be asked to do?**

If you agree to participate in this study, you will be asked to keep a 3 day food record to determine how much sugar you usually consume. If your fructose intake comes out to be <60 grams/day, you will be asked to supplement your diet with either 50g of fructose or 50g of glucose per day for a period of 4 weeks. On the other hand, if your fructose intake is >60 grams/day, you will be asked to consume a fructose restricted diet for a period of 1 week. Consuming a fructose restricted diet involves reducing your intake of foods such as fruit, fruit juices and sweetened beverages such as soft drinks. You will be given detailed advice on the types of foods that you need to avoid and will be provided with small sachets of glucose for ad libitum consumption during the week.

Throughout the study, you will need to visit the University on 2 occasions; at week 0, 1 or 4. At each of the visits, we will be asking you to do the following:

- Complete a brief medical questionnaire.
- Complete a physical activity questionnaire.
- Give body weight, height and body composition measurements
- Donate blood (25 mL), after an overnight fast

Each visit will take approximately 30 minutes to obtain information and collect blood sample. You will also be asked to complete a 3 day food record on 2 occasions: for screening and 1 or 4 weeks post-intervention. This involves recording all the food and beverages that you consume over a 3 day period. You will be given instructions on how to record this information. You will be provided with a light breakfast following donation of the blood sample.

**What are the risks and benefits of participating?**

There are some risks of having blood collected, including bruising or bleeding from the collection site, as well as fainting and dizziness. However, these risks are minimal and a qualified and experienced phlebotomist will take your blood in order to minimise these risks.

You may request information about your health from any examinations and laboratory tests that are undertaken in this study. Feedback of results from the investigation and other information regarding to the outcome of the research will be made available to you and will be posted as an individual letter at the completion of the study.

**How will the information collected be used?**

Results of this research will be published in scientific journals and be published in Faizan Jameel’s PhD thesis. **Individual participants will not be identified** in any reports arising from the project.
How will your privacy be protected?

Your information will be treated with the same respect for privacy and confidentiality as is undertaken for all medical information collected about you during your visits to your local doctor. Access to the collected information will be limited to the named investigators only.

All collected information on data sheets will be physically stored in a locked filing cabinet in the Medical Science Building accessible to the investigators at the University of Newcastle. All data will only be identifiable by a participant identification number with no other identifying details on file. During statistical data analysis the database will be stored in a password protected computer file on a computer that is kept in a locked room. After completion of computer data analysis, the information will be transferred onto a CD before it will be removed and permanently deleted from the computer file. The CD will also be stored in a locked cabinet in a locked room of the principal investigator.

All data for the study will be kept by the Chief Investigator for the period of 15 years following the completion of the study. Only staff of the University of Newcastle conducting this research will have access to this information.

What do you need to do to participate?

Please read this Information Statement and be sure you understand its contents before you consent to participating. If there is anything you do not understand, or you have questions please contact the study principal investigator.

If you would like to participate, please complete the attached Consent Form and return it to the investigators at the University of Newcastle.

Further information

Further information about this or any other research project undertaken in the department at the University of Newcastle can be obtained from Principal Investigators of the study, Professor Manohar Garg and A/Prof Lisa Wood or Mr. Faizan Jameel (4921 5638), Email: faizan.jameel@uon.edu.au

Thank you for considering this invitation to participate in research undertaken at the University of Newcastle.

Professor Manohar Garg  
Principal Investigator 1,  
University of Newcastle

A/Prof Lisa Wood  
Principal Investigator 2,  
University of Newcastle
Complaints about this research
This project has been approved by the University’s Human Research Ethics Committee, Approval No.H-2011-0336

Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, Australia, Telephone (02) 4921 6333, Email: Human-Ethics@newcastle.edu.au.
Appendix Four: Pre-Trial Medical Questionnaire

Pre-trial Medical Questionnaire

Participant code: _______________

Date of birth: ___/___/___        Sex: _________        Height: ______cm        Weight: ______kg

1. Please list all current medical conditions for which you are presently receiving treatment:

   Condition:_________________________    Condition:_________________________

   Year diagnosed:__________    Year diagnosed:__________    Year diagnosed:__________

   Condition:_________________________    Condition:_________________________

   Year diagnosed:__________    Year diagnosed:__________    Year diagnosed:__________

2. Have you ever had:

   A stroke?        Yes___        Angioplasty?        Yes___        No___

   No___        By-pass surgery?        Yes___        No___

   A heart attack?        Yes___        Other heart/vascular surgery    Yes___

   No___        No___

   Angina?        Yes___

   No___

   Other heart-related problems?        Yes___

   No___
3. Please list all medications that you take as prescribed by a doctor:

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Dose</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

4. Please list all over-the-counter medications you take regularly

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Dose</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

5. Please list all vitamin, mineral, and/or herbal supplements you take regularly

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Dose</th>
<th>Frequency</th>
</tr>
</thead>
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</tbody>
</table>

6. How many alcoholic beverages do you normally consume per week?

0-3 ________  4-7 ________  8-10 ________  More than 10 ________

7. Do you smoke?

Yes ________  No ________
8. How often do you currently exercise, and what types of exercise do you do?

<table>
<thead>
<tr>
<th>Exercise</th>
<th>Hours per day</th>
<th>Hours per week</th>
<th>Hours per month</th>
<th>per</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walking, medium pace</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking, briskly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Running/jogging</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bike riding</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Gym</td>
<td></td>
<td></td>
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<tr>
<td>Sport (specify):</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Other physical activities</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>(specify):</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Appendix Five: 24 Hour Food recall Form

Food Diary

Instructions for recording food and drink consumption
You are being asked to record everything you eat and drink for the last 24 hours. The information you record is very important to the success of this study. These instructions will help you to fill in your food and drink consumption in the most accurate manner.

If you have any questions, please do not hesitate to contact Faizan Jameel on 4921 5638.

Instructions:

- Write down everything that you eat and drink (including water) for the last 24 hours
- Complete the record in the time frame you are given by Faizan Jameel
- List the food and give a description, including method of cooking (e.g. grilled rump steak or fried rump steak). An example of how to record the information is given below.
- State the weight of the food or use standard household measure, such as a cup, a tablespoon, a teaspoon.
- Specify brand names of products for processed foods (e.g. Nestle diet yoghurt)
- Include supplements, and any condiments such as sauce or salad dressing
- Try to record each item when you eat or drink so that nothing is forgotten.

Example:

<table>
<thead>
<tr>
<th>Time/Meal (Breakfast/Lunch/Dinner)</th>
<th>Food/Drink + description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 am (Breakfast)</td>
<td>Kellogg’s corn flakes</td>
<td>1 cup</td>
</tr>
<tr>
<td></td>
<td>Dairy Farmer’s lite milk</td>
<td>100 mL</td>
</tr>
<tr>
<td></td>
<td>Boiled egg</td>
<td>2 medium</td>
</tr>
<tr>
<td></td>
<td>Black coffee – Nescafe instant</td>
<td>1 cup</td>
</tr>
<tr>
<td>12 noon (Lunch)</td>
<td>Salad sandwich – wholegrain bread</td>
<td>2 slices</td>
</tr>
<tr>
<td></td>
<td>Baby spinach leaves</td>
<td>1 cup</td>
</tr>
<tr>
<td></td>
<td>Tomato slices</td>
<td>3 slices</td>
</tr>
<tr>
<td></td>
<td>Edgell’s tinned beetroot</td>
<td>30 grams</td>
</tr>
<tr>
<td></td>
<td>Grated carrot</td>
<td>1 tablespoon</td>
</tr>
<tr>
<td></td>
<td>Red delicious apple</td>
<td>1 large</td>
</tr>
<tr>
<td></td>
<td>Nestle tropical fruit diet yoghurt</td>
<td>125 mL</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>500 mL</td>
</tr>
<tr>
<td>Time</td>
<td>Item</td>
<td>Quantity</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------</td>
<td>----------</td>
</tr>
<tr>
<td>3pm (Dinner)</td>
<td>Diet coke</td>
<td>500 mL</td>
</tr>
</tbody>
</table>

Etc.

<table>
<thead>
<tr>
<th>Participant code</th>
<th>Day</th>
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<tbody>
<tr>
<td></td>
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</table>
Appendix Six: 3 days food recall form

Food Diary

Instructions for recording food and drink consumption
You are being asked to record everything you eat and drink for a period of 3 days. Choose consecutive days made up of 2 week days and 1 weekend day. The information you record is very important to the success of this study. These instructions will help you to fill in your food and drink consumption in the most accurate manner.

If you have any questions, please do not hesitate to contact Faizan Jameel on 4921 5638.

Instructions:

☐ Write down everything that you eat and drink (including water) for n=3 consecutive days (2 week days and one weekend day).
☐ Complete the record in the time frame you are given by Faizan Jameel
☐ Start a new page each day (pages are provided):
  o List the food and give a description, including method of cooking (e.g. grilled rump steak or fried rump steak). An example of how to record the information is given below.
  o State the weight of the food or use standard household measure, such as a cup, a tablespoon, a teaspoon.
  o Specify brand names of products for processed foods (e.g. Nestle diet yoghurt)
  o Include supplements, and any condiments such as sauce or salad dressing
☐ Try to record each item when you eat or drink so that nothing is forgotten.

Example:

<table>
<thead>
<tr>
<th>Time/Meal (Breakfast/Lunch/Dinner)</th>
<th>Food/Drink + description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 am (Breakfast)</td>
<td>Kellogg’s corn flakes</td>
<td>1 cup</td>
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<tr>
<td></td>
<td>Dairy Farmer’s lite milk</td>
<td>100 mL</td>
</tr>
<tr>
<td></td>
<td>Boiled egg</td>
<td>2 medium</td>
</tr>
<tr>
<td></td>
<td>Black coffee – Nescafe instant</td>
<td>1 cup</td>
</tr>
<tr>
<td>12 noon (Lunch)</td>
<td>Salad sandwich – wholegrain bread</td>
<td>2 slices</td>
</tr>
<tr>
<td></td>
<td>Baby spinach leaves</td>
<td>1 cup</td>
</tr>
<tr>
<td></td>
<td>Tomato slices</td>
<td>3 slices</td>
</tr>
<tr>
<td></td>
<td>Edgell’s tinned beetroot</td>
<td>30 grams</td>
</tr>
<tr>
<td></td>
<td>Grated carrot</td>
<td>1 tablespoon</td>
</tr>
<tr>
<td>Time/Meal (Breakfast/Lunch/Dinner)</td>
<td>Food/Drink + description</td>
<td>Quantity</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>--------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Red delicious apple</td>
<td>1 large</td>
<td></td>
</tr>
<tr>
<td>Nestle tropical fruit diet yoghurt</td>
<td>125 mL</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>500 mL</td>
<td></td>
</tr>
<tr>
<td>3pm (Dinner)</td>
<td>Diet coke</td>
<td>500 mL</td>
</tr>
<tr>
<td></td>
<td>Etc.</td>
<td></td>
</tr>
</tbody>
</table>

Participant code_______________  Day______________
INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE
(October 2002)
LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ
The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ
Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation
Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ
International collaboration on IPAQ is on-going and an International Physical Activity Prevalence Study is in progress. For further information see the IPAQ website.

More Information
INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the vigorous and moderate activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?
   - Yes
   - No  Skip to PART 2: TRANSPORTATION

The next questions are about all the physical activity you did in the last 7 days as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, heavy construction, or climbing up stairs as part of your work?

Think about only those physical activities that you did for at least 10 minutes at a time.
   ______ days per week

   - No vigorous job-related physical activity  Skip to question 4

3. How much time did you usually spend on one of those days doing vigorous physical activities as part of your work?

   ______ hours per day
   ______ minutes per day

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads as part of your work? Please do not include walking.

   ______ days per week

   - No moderate job-related physical activity  Skip to question 6

5. How much time did you usually spend on one of those days doing moderate physical activities as part of your work?

   ______ hours per day
   ______ minutes per day
6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time as **part of your work**? Please do not count any walking you did to travel to or from work.

____ days per week

☐ No job-related walking *Skip to PART 2: TRANSPORTATION*

7. How much time did you usually spend on one of those days **walking** as part of your work?

____ hours per day
____ minutes per day

**PART 2: TRANSPORTATION PHYSICAL ACTIVITY**

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

____ days per week

☐ No traveling in a motor vehicle *Skip to question 10*

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

____ hours per day
____ minutes per day

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

____ days per week

☐ No bicycling from place to place *Skip to question 12*

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

____ hours per day
____ minutes per day

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

____ days per week

☐ No walking from place to place *Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY*

13. How much time did you usually spend on one of those days **walking** from place to place?
PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY
This section is about some of the physical activities you might have done in the last 7 days in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, chopping wood, shoveling snow, or digging in the garden or yard?

_____ days per week

☐ No vigorous activity in garden or yard  
   Skip to question 16

15. How much time did you usually spend on one of those days doing vigorous physical activities in the garden or yard?

_____ hours per day
_____ minutes per day

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, sweeping, washing windows, and raking in the garden or yard?

_____ days per week

☐ No moderate activity in garden or yard  
   Skip to question 18

17. How much time did you usually spend on one of those days doing moderate physical activities in the garden or yard?

_____ hours per day
_____ minutes per day

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, washing windows, scrubbing floors and sweeping inside your home?

_____ days per week

☐ No moderate activity inside home  
   Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY

19. How much time did you usually spend on one of those days doing moderate physical activities inside your home?

_____ hours per day
_____ minutes per day

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY
This section is about all the physical activities that you did in the last 7 days solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.
20. Not counting any walking you have already mentioned, during the last 7 days, on how many days did you walk for at least 10 minutes at a time in your leisure time?

____ days per week

☐ No walking in leisure time Skip to question 22

21. How much time did you usually spend on one of those days walking in your leisure time?

____ hours per day

____ minutes per day

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do vigorous physical activities like aerobics, running, fast bicycling, or fast swimming in your leisure time?

____ days per week

☐ No vigorous activity in leisure time Skip to question 24

23. How much time did you usually spend on one of those days doing vigorous physical activities in your leisure time?

____ hours per day

____ minutes per day

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis in your leisure time?

____ days per week

☐ No moderate activity in leisure time Skip to PART 5: TIME SPENT SITTING

25. How much time did you usually spend on one of those days doing moderate physical activities in your leisure time?

____ hours per day

____ minutes per day

PART 5: TIME SPENT SITTING
The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the last 7 days, how much time did you usually spend sitting on a weekday?

____ hours per day

____ minutes per day

27. During the last 7 days, how much time did you usually spend sitting on a weekend day?
_____ hours per day
_____ minutes per day

THIS IS THE END OF THE QUESTIONNAIRE, THANK YOU FOR PARTICIPATING.