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Investigating the potential of whole grain sorghum as an ingredient in foods to assist in the prevention of chronic disease

Anita Stefoska-Needham
University of Wollongong

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**UNIVERSITY OF
WOLLONGONG**



**Faculty of Science, Medicine and Health
School of Medicine**

**Investigating the potential of whole grain
sorghum as an ingredient in foods to assist in
the prevention of chronic disease**

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This thesis is presented as part of the requirement for the
award of the degree of

DOCTOR OF PHILOSOPHY

University of Wollongong

2016

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ABSTRACT

Background: Whole grain intake is associated with reduced risk of chronic diseases, such as cardiovascular disease and Type 2 diabetes. Unlike more commonly recognised whole grains, sorghum is a so-called “ancient”, gluten-free whole grain food that is gaining attention from food manufacturers, consumers and researchers as a beneficial ingredient in novel product development. Sorghum is widely cultivated globally due to its adaptability to semi-arid/arid conditions and high temperatures, and has significant potential for sustainable grain production in harsh climates. It is a dietary staple in some communities of Asia, Africa and Central America, however in countries such as the United States and Australia, sorghum is predominantly used as an animal feed, with only small quantities utilised for the manufacture of human foods. If sorghum is to be accepted as a common food, attention to food formulations and processing methods that produce desirable sorghum-based cereal products, whilst preserving the grain’s beneficial nutritional and functional attributes, is required.

Sorghum whole grain has been shown to have lower starch and protein digestibility *in vitro*, resulting in reduced metabolisable energy and hence smaller weight gains in animals, and some cultivars are high in dietary fibre and rich in polyphenolic compounds. Based on their purported mechanisms, these attributes may contribute to positive effects on metabolic markers and body weight and therefore may more broadly influence chronic disease prevention in humans when they consume sorghum-based foods regularly. However, there is a paucity of human studies to date.

Research Hypothesis and Study Aims: This thesis proposes that sorghum grain consumption may assist in chronic disease prevention via weight management, especially in populations where food is ubiquitous and obesity-related chronic diseases are prevalent. Research examining the effects of sorghum intake on health outcomes, such as weight loss and disease biomarkers, requires clinical studies. These studies may also expose potential protective roles of the sorghum grain as part of a whole diet. The key hypothesis of this thesis is that sorghum is a viable alternative to more commonly consumed whole grain cereals in the human diet and may have positive benefits on factors associated with metabolic health including weight management. A review of the literature described sorghum grain structure and composition which relate to mechanisms by which sorghum grain components may influence weight management and chronic disease risk; and summarised the evidence on effects of sorghum consumption on health outcomes related to chronic disease prevention. This review provided insights into the knowledge gaps and study imperatives that informed the experimental components of the thesis.

In order to conduct human trials successfully, particularly with participants who do not typically consume traditional sorghum foods, suitable ready-to-eat sorghum grained cereal foods in the form of flaked breakfast cereal biscuits were formulated and tested prior to intervention trials commencing. A randomised, crossover, feeding study using the flaked sorghum biscuits was conducted to investigate mechanisms related to general metabolic markers and antioxidant status, as well as acute satiety. A randomised controlled trial (RCT) was then conducted with the aim to test the weight-loss effects of longer-term whole grain sorghum flaked biscuits consumption

incorporated into an energy-restricted diet plan. This study also examined the effects on key biomarkers of metabolic health, inflammation and oxidative stress.

Methods:

Gaps in the knowledge of effects of sorghum consumption on indicators of metabolic disease, including weight, were identified and elements of study designs were incorporated into the experimental components of the thesis. Whole grain flaked cereal biscuits were formulated on a pilot line by our industry partner (Sanitarium Health and Wellness) using a multistep process involving steaming, drying, rolling and baking. For the acute satiety study, forty subjects (20 males and 20 females) were tested on four occasions after a 12-hour fast. At baseline, they consumed 50 grams (or 3 biscuits) of one of four treatment meals: white, red or brown whole grain sorghum flaked biscuits or a whole grain flaked wheat biscuit control. Subjective satiety was measured at 8 time-points over four hours and food intake at the subsequent meal and for the rest of the day recorded. In a subset of 20 subjects, plasma glucose, insulin, and four appetite-related gut peptides (GIP, GLP-1, PYY and ghrelin) were also measured. In the subsequent RCT investigating chronic sorghum consumption, sixty subjects (14 males and 46 females) were randomised to either a sorghum (intervention) or wheat (control) group. Both groups received advice on an energy-restricted diet from an Accredited Practising Dietitian and were provided with 45 g of cereal products to include daily in their prescribed diets for 12 weeks. The primary outcome was weight loss. Secondary outcomes included: plasma glucose, HbA1c, insulin, total cholesterol, HDL-c, LDL-c, TAG, and various markers of inflammation and oxidative stress (IL1 β , IL-6, IL-8, TNF α , hsCRP, HPX

and TAC), measured at 0 and 12 weeks. Subjective satiety ratings were assessed at 0, 6 and 12 weeks.

Results: A vast array of nutritional and bioactive sorghum grain components, such as slowly digestible starches, polyphenols including anthocyanins in red sorghum, unsaturated fatty acids, and dietary fibre (including resistant starch), were identified in the literature, with potential actions in health protective metabolic processes. There was a paucity of human studies that are necessary to better understand effects of sorghum consumption (as a whole food), especially those related to health outcomes and indicators of disease. These findings justified the human studies described. During the acute meal test study, subjects reported significantly lower satiety ratings after consuming wheat compared to sorghum biscuits. Incremental AUC of postprandial GLP-1, GIP and in males, PYY, were significantly higher ($p=0.018$, $p=0.031$, $p=0.036$ respectively) for sorghum breakfasts compared to wheat. Energy intake at a subsequent meal did not differ between treatments, nor did glucose, ghrelin and TAC responses. The red sorghum biscuit showed the greatest alteration in appetite hormones, suggesting that the specific combination of its components (such as unique anthocyanin flavonoids) requires further investigation. Hence, these results informed the choice of a red sorghum-based biscuit to test in the subsequent 3 month RCT. This trial did not identify any significant differences in weight loss or any clinical variables between a sorghum cereal group and a wheat control in an energy-restricted diet. For both groups, the majority of clinical indices changed significantly over time ($p<0.05$) and equivalent amounts of weight were lost ($p=0.369$). These results did not concur with the initial acute satiety study and there

was no translation to differences in weight reduction effects for sorghum and wheat cereals.

Conclusions: Research presented in this thesis provides human evidence for acute satiety effects from whole grain sorghum ingestion in the form of flaked cereal biscuits. However, it could not be concluded that whole grain sorghum assists specifically with weight management, or is superior to wheat (another whole grain product). Further clinical trials are necessary to establish an evidence base for weight loss effects from chronic sorghum consumption. In the longer term, this research would help to further evaluate the weight loss potential of sorghum consumption, especially red sorghum grown in abundance locally in Australia. Weight loss effects would be considered to assist in the prevention of chronic diseases, particularly those associated with obesity. Nevertheless, new knowledge was generated from these studies demonstrating the potential of sorghum consumption to enhance acute satiety. Sorghum is not only a gluten-free whole grain cereal, but also a promising ingredient in cereal foods targeting appetite control. Commercially, the results of the feeding trials may contribute to the development of the sorghum industry globally by adding to the evidence base on human health effects. This thesis also showcases the collaboration between food industry and science to progress sorghum, a relatively unknown human food in Australia, through the food product innovation pipeline with positive outcomes for all stakeholders including researchers, food manufacturers and also consumers.

DECLARATION

I, Anita Stefoska-Needham, hereby declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Medicine, University of Wollongong, is my own work unless otherwise referenced or acknowledged. This document has not been submitted in whole, or in part, for qualifications at any other academic institution.

Wollongong, Australia

6 May 2016

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“The credit belongs to the man who is actually in the arena, whose face is marred by dust and sweat and blood; who strives valiantly . . . who at best knows the triumph of high achievement and who at the worst, if he fails, at least fails while daring greatly”

~ Theodore Roosevelt, 1910 ~

Daring best describes my decision to venture into higher degree research in 2012. Leaving a successful clinical career and with a young family, I soon felt very exposed and vulnerable in this new territory. But with every uneasy step, I became more determined and more confident. By the end, I too had triumphed in the academic arena mostly through hard work, commitment and tenacity. Many people have supported the completion of my PhD, including my academic supervisors and my devoted family. Firstly though, I would like to acknowledge my family for enabling me to commit almost four years of my life (and theirs) to this endeavour.

Every step of this journey has been taken with my husband, Dr Scott Needham, by my side. His outpouring of love, care and encouragement has only been surpassed by his incredible intellect and great insights into my research and the academic process. This experience has unified us and reinforced the special bond we have shared for 25 years. I am immensely grateful and deeply touched. My precious sons, Benjamin and Oliver, have equally been my strength. Their unconditional love and belief in my ability to do this, has given me the motivation to keep trying. Their constant distractions have provided perspective – this has been their greatest gift to me. In return, I hope to have encouraged them to ‘have a go’ and reassured them not to hold back for fear of failure. I love them dearly. I also want to make special mention of my mother, Rajna, who lived with us during this time – a live-in nanny who assisted with the logistics that enabled this adventure and most importantly, a caring Baba who helped to weather the storms.

When in the clutches of a *melt-down*, I had a great team of supporters willing to put a smile back on my face: Jack (with whom coffee often turned to vino), Tony, Billy, Monika, Corinne, Marselle, Tristen, Michele, Nat, Thomas and my Warehouse gym crew. However, the most special comfort came from my big brother, Rob, whose almost daily calls and humourous texts were a welcome reminder to ‘take a breath’. Thank you also to my father, Done, for instilling in me the virtues of hard work and tenacity, which fuelled my drive to keep going ‘till the job was done’; to my beloved grandparents, my guardian angels, Evra, Kare, Deska, Spase and Enid, who soothed me in my most defeated moments; and to the Needhams and my extended family for their ongoing support. I hope to have made everyone proud.

Now I would like to acknowledge the important role of my academic supervisors in the completion of this thesis. Associate Professor Eleanor Beck – always fair, logical and intelligent with a unique ability to simplify the most complex of problems – thesis-related or otherwise! Our time working together saw the genesis of a special and life-long friendship, one which I will cherish forever. Equally, it was an honour to be under the wing of Professor Linda Tapsell (OAM), a true academic visionary who inspires and guides with years of knowledge, experience and nurturing. I am thankful to the insightful advice offered by Associate Professor Stuart Johnson, Chief Investigator of the ARC Linkage Project which funded my stipend; and to Professor John Ashton for advocating for my work and securing industry support for both clinical trials. I am also very grateful to Professor Georgius Adam, who encouraged me from the outset to do my PhD; that it would be *good for me*. Thank you also to the support staff in IHMRI’s CRTU and especially to Rebecca Thorne, Research Dietitian and phlebotomist extraordinaire.

Finally, I would like to extend a heart-felt thank you to all the participants in my trials – their commitment and belief in my research was touching and made the long hours of testing a real pleasure.

DEDICATION

For my beloved family,

Benjamin, Oliver and Scott,

&

Mum, Dad and Rob,

with love.

TABLE OF CONTENTS

ABSTRACT	i
DECLARATION	vi
ACKNOWLEDGEMENTS	vii
DEDICATION	ix
TABLE OF CONTENTS	x
LIST OF FIGURES	xiv
LIST OF TABLES	xvi
LIST OF PUBLICATIONS	xix
LIST OF ABBREVIATIONS (A-Z)	xxiii
CHAPTER 1:	1
Introduction and Thesis Overview	1
1.1 Introduction	2
1.2 Thesis Overview and Structure	9
CHAPTER 2:	11
Nutritional composition of sorghum and evidence of its effects on health	11
2.1 Introduction	12
2.2 Literature Review Method	15
2.3 Results	16
2.2.1 Structure of the sorghum plant	16
2.2.2 Sorghum: potential as a environmentally-sustainable, commercial crop for human food production	17
2.2.3 Nutritional and chemical composition of the sorghum grain	18
2.2.4 Processing of sorghum grain to produce foods for human consumption	24
2.2.5 Sorghum grain components with the potential for functional properties.....	25
2.2.6 Sorghum as a whole food: the case for “food synergy”	39
2.2.7 Experimental research testing effects of sorghum consumption on human health	40
2.4 Conclusions	57

CHAPTER 3:	60
Methodology	60
3.1 Overview of methodological framework	61
3.2 Key hypothesis and study aims	64
3.3 Measurement Issues	65
3.3.1 Measuring Satiety, Satiation and Appetite.....	66
3.3.2 Measurement of Dietary Intake.....	81
3.3.3 Measurement of Relevant Markers of Health/Disease Risk	85
3.4 Overview of thesis research design	93
CHAPTER 4:	94
Formulation of Ready-To-Eat Sorghum Flaked Breakfast Biscuits	94
4.1 Introduction	95
4.2 The opportunity for sorghum-based foods with satiety attributes	96
4.3 Developing an optimised sorghum-based breakfast cereal	97
4.3.1 Processing Methods	100
4.3.2 Analyses of Biscuit Contents	101
4.4 Results and Discussion	104
4.5 Conclusions	107
CHAPTER 5:	108
Acute meal test study investigating satiety-related effects of flaked sorghum biscuit intake	108
5.1 Introduction	109
5.2 Study Aims and Hypothesis	110
5.3 Materials and Methods	110
5.3.1 Subjects	110
5.3.2 Test Protocols.....	111
5.3.3 Test Foods	112
5.3.4 Appetite Markers and Measures.....	114
5.3.5 Statistical Analysis	115
5.4 Results	117
5.4.1 Subjects	117
5.4.2 Test Food Analysis.....	117
5.4.3 Baseline Measurements.....	118
5.4.4 Subjective Satiety.....	119

5.4.5 Biochemical Indices	121
5.4.6 Energy intake at ad-libitum lunch meal and for the rest of the day	122
5.5 Discussion.....	126
5.6 Conclusions	129
CHAPTER 6:	131
Examining the longer-term effects of chronic sorghum consumption: a randomised controlled trial.....	131
6.1 Introduction	132
6.2 Study Aims and Hypothesis	132
6.3 Materials and Methods	133
6.3.1 Subjects and Recruitment.....	133
6.3.2 Dietary Intervention	137
6.3.3 Test Food Products.....	139
6.3.4 Clinical Indices.....	141
6.3.5 Statistical Analysis	141
6.6 Results	142
6.6.1 Baseline Data	142
6.6.2 Dietary Intervention	143
6.6.3 Clinical Indices.....	144
6.6.4 Subjective Satiety.....	147
6.7 Discussion.....	147
6.8 Conclusions	153
CHAPTER 7:.....	154
Conclusions and Recommendations	154
7.1 Summary of Findings.....	155
7.2 Limitations and Considerations for Further Research	160
7.3 Key Recommendations for Future Studies.....	164
7.4 Concluding Remarks	166
7.5 Post-Script.....	167

REFERENCES	168
APPENDICES	195
APPENDIX 1	196
Appetite-Related Visual Analogue Scales (VAS).....	196
APPENDIX 2	198
Product Evaluation Questionnaire: Flaked Cereal Biscuits	198

LIST OF FIGURES

Figure 1.1	
Key factors in the innovation of sorghum-based food products	7
Figure 2.1	
Field of red sorghum plants depicting maize-like leaves and compact panicles.....	16
Figure 2.2	
Example of different sorghum-containing food products currently on supermarket shelves	17
Figure 2.3	
Sorghum Caryopsis	19
Figure 2.4	
Grain colour variation in seed heads of different sorghum cultivars	23
Figure 2.5	
Classification of dietary phytochemicals	33
Figure 2.6	
Antioxidant activity in sorghum bran fractions (dry basis) relative to other cereals and common fruits	34
Figure 2.7	
Procyanidin B-1, a type of condensed tannin, isolated in sorghum	37
Figure 3.1	
Conceptualisation of thesis framework	62
Figure 4.1 (a)	
Red sorghum flaked breakfast biscuits	98
Figure 4.1 (b)	
Brown sorghum flaked breakfast biscuits	98
Figure 4.1 (c)	
White sorghum flaked breakfast biscuits	99

Figure 4.1 (d)	
Wheat (control) flaked breakfast biscuits	99
Figure 4.2	
Scanning electron micrographs of (a) white sorghum flaked biscuit (b) red sorghum flaked biscuit (c) brown sorghum flaked biscuit depicting gelatinised starch granules.	107
Figure 5.1	
Participant engagement before randomisation into study and prior to first testing visit	111
Figure 5.2	
Overview of protocol for acute meal test study	113
Figure 5.3	
Buffet (ad-libitum) lunch	115
Figure 5.4	
RMANOVA of mean satiety ratings (mm) for all 4 questions (Q1, Q2, Q3, Q4) after intake of different breakfast cereal biscuits at different time points over 4 h.....	120
Figure 5.5	
Changes in mean plasma concentrations of (a) glucose, (b) insulin, (c) GLP-1 over time.....	124
Figure 5.5	
Changes in mean plasma concentrations of (d) GIP, (e) PYY and (f) ghrelin over time	125
Figure 6.1	
Overview of protocol for RCT.....	134
Figure 6.2	
Flow of participants through the trial.....	136

LIST OF TABLES

Table 1.1	
NHMRC Hierarchy of Evidence.....	8
Table 2.1	
Sorghum’s nutritional and functional attributes associated with metabolic disease effects.....	13
Table 2.2	
Nutritional Composition of Sorghum, Wheat and Corn	22
Table 2.3	
Classification of sorghum according to appearance and extractable phenols	23
Table 2.4	
Human studies incorporating sorghum-based test meals	50
Table 2.4	
Human studies incorporating sorghum-based test meals (continued...)	51
Table 2.4	
Human studies incorporating sorghum-based test meals (continued...)	52
Table 2.4	
Human studies incorporating sorghum-based test meals (continued...)	53
Table 2.4	
Human studies incorporating sorghum-based test meals (continued...)	54
Table 2.4	
Human studies incorporating sorghum-based test meals (continued...)	55
Table 2.4	
Human studies incorporating sorghum-based test meals (continued...)	56
Table 3.1	
Visual Analogue Scale Questions	80

Table 3.2	
Summary of some limitations associated with RCTs	82
Table 3.3 (a)	
Summary of disease markers evaluated in the experimental studies in this thesis.....	87
Table 3.3 (b)	
Summary of disease markers evaluated in the experimental studies in this thesis (continued...)	88
Table 3.3 (c)	
Summary of disease markers evaluated in the experimental studies in this thesis (continued...)	89
Table 4.1	
Proximate and dietary fibre composition and energy content of flaked breakfast cereals	105
Table 4.2	
Free, bound and total phenolic content and antioxidant capacity of flaked breakfast cereals	106
Table 4.3	
Starch properties of flaked breakfast cereals	106
Table 5.1	
Subject characteristics at baseline (mean \pm standard deviation).....	117
Table 5.2	
Correlation between polyphenol content of standardised dinner meal and baseline plasma TAC	119
Table 5.3	
Mean VAS ratings (mm) for all 4 questions after intake of different breakfast cereal biscuits	120
Table 5.4	
Postprandial responses of glucose, insulin and various gut hormones over 4h (expressed as mean net iAUC \pm standard deviation)	123

Table 6.1	
Dietary prescription (totaling 5, 500kJ) for a typical participant.....	138
Table 6.2	
Proximate and dietary fibre composition and energy content of flaked breakfast cereals	140
Table 6.3	
Free, bound and total phenolic content and antioxidant capacity of flaked breakfast cereals	140
Table 6.4	
Starch properties of flaked breakfast cereals	141
Table 6.5	
Baseline characteristics of study subjects (means and standard deviations).....	143
Table 6.6	
Energy and macronutrient intakes reported by subjects at baseline, mid- point and 3 months, with p-values for repeated measures ANOVA between control and sorghum groups	145
Table 6.7	
Changes in anthropometric and biochemical indices over time between control and sorghum groups.....	146

LIST OF PUBLICATIONS

Peer reviewed journal publications in support of this thesis

Published

- Stefoska-Needham, A., Beck, E., Johnson, S., Tapsell, L. (2015) Sorghum: an underutilized whole grain cereal with the potential to assist in the prevention of chronic disease. *Food Reviews International*. 31:401-437.
- Stefoska-Needham, A., Beck, E., Johnson, S., Tapsell, L. (2016) Sorghum flaked breakfast biscuits increase postprandial GLP-1 and GIP levels and extend subjective satiety in healthy subjects. *Molecular Nutrition and Food Research*. 60:1118-1128.
- Stefoska-Needham, A., Beck, E., Tapsell, L. (2016) Perspective: The path to confirming and exploiting potential satiety-enhancing effects of sorghum-based foods for human diets. *Quality Assurance and Safety of Crops and Food*. DOI:10.3920/QAS2015.0695
- Stefoska-Needham, A., Beck, E., Batterham, M., Johnson, S.K., Grant, R., Ashton, J., Tapsell, L. (2016) A sorghum-enriched diet does not enhance the effectiveness of an energy-restricted meal plan in mildly obese adults over 3 months. *Journal of the American College of Nutrition*. (Accepted)

Submitted for Publication

- Stefoska-Needham, A. (2016) Progressing the position of sorghum, a potentially sustainable cereal crop, through the food product innovation pipeline: an Australian perspective. *Agroecology and Sustainable Food Systems*. (Under Review)

In preparation

- Price, W., Kang, J., Ashton, J., Fang, Z., Stefoska-Needham, A., Tapsell, L., Johnson, S. (2016) Nutritional and phytochemical properties of flaked sorghum breakfast cereals. *Food Research International*.

Conference presentations in support of this thesis

Oral and scientific poster presentations

- Stefoska-Needham, A., Beck, E., Tapsell, L. (2016) A sorghum-enriched diet does not enhance the effectiveness of an energy-restricted meal plan in overweight subjects over 3 months. *International Congress of Dietetics 2016*, Granada, Spain. (Scientific Poster).
- Stefoska-Needham, A., Beck, E.J, Johnson, S.K., Tapsell, L.C (2015) Sorghum flaked breakfast biscuits increase postprandial GLP-1 levels and extend subjective satiety in healthy subjects. *International Dietary Fibre Conference 2015*, Paris, France. (Scientific Poster).
- Needham, A., Beck, E., Tapsell, L. (2014) Does a standardised dinner meal consumed the evening prior to testing add methodological integrity to an acute meal test study design? *Nutrition and Dietetics, 71 (Suppl. S1), 26. DAA Conference*, Brisbane, Australia. (Oral Presentation).
- Stefoska-Needham, A., Beck, E., Tapsell, L. (2014) Acute effects of consuming sorghum-based breakfast meals on objective and subjective measures of satiety. *Cereal Foods World. 59, No.6 (Suppl):A29. AACCI Annual Meeting*, Rhode Island, U.S. (Oral Presentation).
- Needham, A., Beck, E., Tapsell, L. (2013) SORGHUM: a novel high value food ingredient in the Australian food supply chain. *International Cereal Chemists (ICC) Conference*, Fremantle, Australia. (Scientific Poster).

Invited Speaker

- AusSORGM Annual Meeting: July 2015, Lamington Park, QLD.
- Australasian Grain Science Conference: September 2015, Sydney, NSW.
- Australian Summer Grains Conference: March 2016, Gold Coast, QLD.
- Home Economics Institute of Australia Annual Conference: March 2016, Sydney, NSW.

Non Peer-reviewed publications in support of this thesis

- The Land (August 2014):
Value adding sorghum
- Grains and Legumes Nutrition Council Newsletter (August 2014):
Sorghum: the new whole grain on the block

Other Publications (2012-2016)

Patents

- Adams, G.A. and Needham, A. (2013) Edible Plasticizers for Food and Food Packaging Films. PCT/US2013/048604
- Adams, G.A. and Needham, A. (2014) Food Additive, Food Packaging Additives, and Uses Thereof. PCT/US2013/066343
- Adams, G.A. and Needham, A. (2015) Humic Acid Derivatives and Methods of Preparation and Use. PCT/US2013/042814

AWARDS

Scholarships

- Australian Postgraduate Industry-Sponsored Award, 2012-2016.
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Conference Awards

- Mondelez/International Cereal Chemists (ICC):
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Travel Grants

- Grains Research and Development Corporation, Australia.
To: AACCI Conference, Rhode Island, 2014.
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To: AACCI Conference, Rhode Island, 2014.
- Grains Research and Development Corporation, Australia.
To: ICC Conference, Freemantle, 2013.

LIST OF ABBREVIATIONS (A-Z)

3-DA	3-deoxyanthocyanidins
3DFR	Three day food record
ABARES	Australian Bureau of Agricultural and Resource Economics and Science
ABS	Australian Bureau of Statistics
AGEs	Advanced glycation end products
ANOVA	Analysis of variance
AOAC	Method of the Association of Analytical Communities
BCE	Before Common Era
°C	Degrees Celsius
DAA	Dietitians Association of Australia
DH	Diet history
FAO	Food and Agricultural Organisation of the United Nations
g	Gram
GAE	Gallic acid equivalents
GAX	Glucoarabinoxylans
GDP	Gross domestic product
GI	Glycaemic Index
GIP	Glucose inhibitory peptide
GLHC	General level health claim
GLP-1	Glucagon-like peptide-1
Grp	Group
HbA1c	Glycosylated haemoglobin
HCl	Hydrochloric acid
HDL	High density lipoprotein
HLHC	High level health claim
HPX	Hydroperoxide
Hr	Hour
iAUC	Incremental area under the time curve
IL1β	Interleukin 1 β
IL-6	Interleukin 6
IL-8	Interleukin 8
IU	International units

keV	Kilo electron volts
kg	Kilogram
kJ	Kilojoule
LDL	Low density lipoprotein
LC-MS	Liquid chromatography - mass spectrometry
MetS	Metabolic Syndrome
Min	Minutes
µm	Micrometers
mm	Millimeters
MUFA	Monounsaturated fatty acids
NSP	Non-starch polysaccharides
ORAC	Oxygen radical absorbance capacity
PP	Polyphenols
PYY	Peptide tyrosine tyrosine
R²	Coefficient of determination
RMANOVA	Repeated measures analysis of variance
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RS	Resistant starch
RTE	Ready-to-eat
SCFA	Short chain fatty acids
SD	Standard deviation
SE	Standard error
SEM	Scanning electron microscopy
TAC	Total antioxidant capacity
tbsp	Tablespoon
TE	Trolox equivalents
TFEQ	Three Factor Eating Questionnaire
TNFα	Tumour necrosis factor alpha
Total chol	Total cholesterol
TS	Total starch
tsp	Teaspoon
VAS	Visual analogue scales
Vs	Versus
Wt	Weight

CHAPTER 1:

Introduction and Thesis Overview

A component of this chapter is the substantive content of the following article:

Stefoska-Needham, A. (2016) Progressing the position of sorghum, a potentially sustainable cereal crop, through the food product innovation pipeline: an Australian perspective. *Agroecology and Sustainable Food Systems*. (Under Review)

1.1 Introduction

Human health, food and the national economy are interlinked. The current Australian economy is undergoing change that reflects shifts within primary industries. With the decline of mining, other sectors such as manufacturing, services, education and agriculture have become increasingly important in stimulating economic growth. Of these five economic pillars, agriculture has been a consistent and important component, despite contributing less than 3% of gross domestic product (GDP) in 2014⁽¹⁾. Australia's farmers produce enough food to feed 80 million people⁽²⁾, providing not only 93% of the domestic food supply⁽³⁾, but also supporting an export market valued at more than A\$41 billion per annum or more than 13% of export revenue^(1;2). The major commodities are grains and oilseeds, followed by meat, then industrial crops (sugar, cotton and wine), wool, dairy and horticulture^(1;2).

In fact, the demand for Australian-produced food is increasing, especially from Asian markets such as China, driven by their large populations and a growing middle class. Furthermore, frequent food contamination incidents in Asia have resulted in a general mistrust of food produced in that area and a growing demand for Australia's "green and clean" produce. The problem was seen in China, in 2008, when melamine (a toxic polymer) was added to infant formulae, resulting in 6 infant deaths and 52,000 hospitalisations⁽⁴⁾. As a consequence, the demand for Australian-made infant formulae, perceived to be safe, surged, stripping local supplies⁽⁴⁾. These types of incidents have reinforced Australia's reputation as a producer of the highest food quality, with exceptional food safety and biosecurity standards.

Australian agriculture faces major challenges in supplying the increasing demand for food in the local and Asia-Pacific region, one of which is climate change. Climate change has resulted in increased global temperatures and reduced rainfall, which in turn have impacted on the usability of land for farming through loss of biodiversity, dry-land salinity, acid soils, pests and weeds⁽⁵⁾. Hence, it has become critical to engage in sustainable agricultural practices in order to protect farming land whilst meeting demands for greater food production. In turn, cultivating more environmentally sustainable crops, suitable to Australia's often-harsh climate, is important, especially since grains are the nation's leading commodity⁽¹⁾.

Sorghum (*Sorghum bicolor* (L.) Moench) is a good example of a sustainable crop that requires fewer nutrient inputs and significantly less water than other crops, such as corn⁽⁶⁾. In many countries of Africa, Asia and Central America, sorghum is widely cultivated due to its adaptability to semi-arid and arid conditions and high temperatures, and is a major contributor to the staple diets of local populations⁽⁷⁾. Globally it ranks fifth in cereal production and in Australia it ranks third, with the 2014-2015 sorghum harvest producing 2.2 million tonnes of grain across 730,000 hectares of total land area^(7; 8). This was a significant increase on previous years, predominantly in response to above-average rainfalls and a higher demand from China. In Australia, the primary use of sorghum has been as low-value livestock feed and more recently in biofuel production⁽⁹⁾, while demand for use in human food manufacturing has been negligible. Sorghum has therefore remained both under-utilised and less-recognised compared to other cereal crops, such as wheat.

The commercialisation of sorghum for human use in Australia has faced significant challenges, limited mainly by (a) a reluctance from farmers, in the absence of reliable markets, to invest in crop management necessary to improve the volume and consistency of production, and (b) the lack of incentives for grain processors to invest in market development, given the availability of alternative grains with better profit margins. Despite these limitations, a small number of food products have emerged on supermarket shelves over recent years that contain sorghum as a key or component ingredient, suggesting that demand for sorghum in human food production is increasing. For example, the Sanitarium Health and Wellbeing Company, a leading cereal food manufacturer with a strong ethos for healthy eating and wholesome foods, engaged in food innovation and launched a gluten-free breakfast cereal made exclusively from sorghum in 2014⁽¹⁰⁾. The launch of this product aligned with the broader expansion of the health food sector seen in Australia⁽¹¹⁾, suggesting that the relationship between food and health is having an increasing impact on food innovations by food producers. These types of food product developments and investments are what is required to further stimulate consumer interest in sorghum as a human food ingredient, and encourage growers and food product manufacturers to participate. Furthermore, the lure of a growing consumer movement dedicated to "healthy living" and "naturally functional" foods⁽¹¹⁾ and the ability of food companies to reformulate rather than to always innovate, often helps to overcome initial corporate resistance.

To successfully launch new products in the marketplace and promote them to consumers, marketing and branding become critical components. However, in Australia, the marketing of novel food products made from ingredients with potential

benefits for human health, such as sorghum-based foods, must be conducted within a strict regulatory environment. Moreover, any food companies wishing to make a health claim must comply with “Standard 1.2.7 - Nutrition, Health and Related Claims”, regulated by Food Standards Australia New Zealand (FSANZ)⁽¹²⁾. This standard regulates claims appearing on food labels and in advertisements, in the form of *nutrition content claims* and *health claims*. These claims are voluntary statements made by food companies and may refer to the nutritional content of the food (nutrition content claims) or they may refer to a relationship between food, or a property of food, and a health effect (health claims). Health claims are further categorised as “general level health claims” (GLHC) or “high level health claims” (HLHC). GLHCs refer to a component or nutrient in a food and how it may affect health. For example, ‘Fibre helps to keep you regular’⁽¹³⁾. These types of claims cannot be used in association with serious diseases, such as heart disease, or indicators of disease, such as cholesterol. HLHCs refer to a component or nutrient in a food and how it may affect a serious disease or biomarker (indicator) of a serious disease. An example of this type of claim is: ‘This food is low in sodium (salt). A diet low in sodium may help reduce blood pressure’⁽¹³⁾. Overall, health claims must be based on food-health relationships that have been substantiated according to Standard 1.2.7 and manufacturers need to provide supporting scientific evidence to demonstrate that their product contains the described ingredient/s⁽¹²⁾. Health claims related to weight control and satiety effects of foods are currently not permitted. HLHCs must also meet the Nutrient Profiling Scoring Criterion (NPSC), a point system broadly ranking the nutritional quality of a food⁽¹²⁾. For example, foods high in saturated fat and sugar are not allowed to carry a health claim, even if low in salt, because they would not meet the NPSC.

Scientific evidence therefore underpins the substantiation of health claims for food. However, not all scientific evidence is equivalent. The National Health and Medical Research Council (NHMRC) classifies the quality of scientific evidence according to a hierarchy which assigns “levels of evidence” based on the type of research question asked and its associated research design (Table 1.1)⁽¹⁴⁾. FSANZ uses these “levels of evidence” in its validation of health claims for food. Thus, for novel sorghum-based foods, substantiating potentially beneficial effects on disease risk factors will require rigorous scientific investigation in human studies. In addition, consistent results from randomised controlled trial (RCTs) are needed to build the *highest level of evidence* for practice. Specifically, investigating the long-term effects of consuming sorghum as part of a healthy diet is necessary in order to build on the small number of promising human studies, as well as other encouraging *in vitro* and animal research.

To this end, the research presented in this thesis is a contribution to the level of evidence necessary to substantiate potential future health claims related to sorghum-based foods. The results may also guide other researchers, consumers, the food industry, growers, health professionals, and government-based grain advocacy organisations on the general benefits of sorghum consumption for humans, and the process for research to investigate health effects from particular food ingredients. Findings from the body of work presented in the thesis collectively add to the knowledge base for sorghum as a promising whole grain cereal with the potential to assist in the prevention of chronic disease, particularly in developed countries such as Australia. The relevant thesis components are: (1) a critical review of the existing literature on sorghum, (2) research underpinning the innovative development of a sorghum-based food product, (3) a study of the mechanisms of sorghum grain action,

and (4) a randomised controlled trial testing the effects of chronic consumption of a novel sorghum based product.

Moreover, this thesis represents more than just a contribution of evidence in support of a scientific question. It showcases the collaboration between food industry and science to progress the position of sorghum, a relatively unknown human food in Australia, through the food product innovation pipeline (Figure 1.1). In doing so, our knowledge is increased about many facets of sorghum - from plant biology through to environmental and agronomic considerations necessary for plant growth, to the nutritional and chemical composition of sorghum grain, and then finally the potential mechanisms of action that may be behind observed longer-term effects of chronic sorghum consumption. In addition, by developing a food product suitable for the modern Australian palate, the impact of grain processing and food format selection on clinical effects was elucidated.

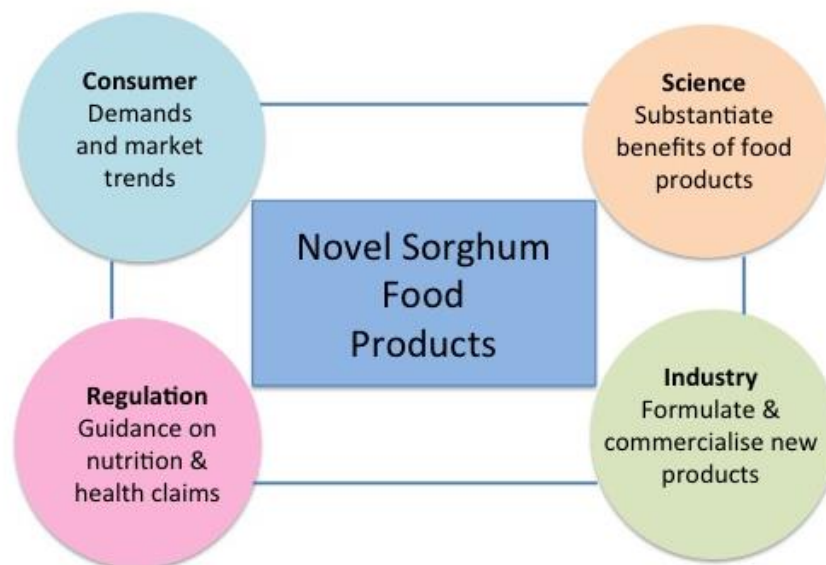


Figure 1.1 Key factors in the innovation of sorghum-based food products

Table 1.1 NHMRC Hierarchy of Evidence⁽¹⁵⁾

Level	Intervention	Diagnostic Accuracy	Prognosis	Aetiology	Screening Intervention
I	A systematic review of level II studies	A systematic review of level II studies	A systematic review of level II studies	A systematic review of level II studies	A systematic review of level II studies
II	A randomised controlled trial	A study of test accuracy with an independent, blinded comparison with a valid reference standard, among consecutive persons with a defined clinical presentation	A prospective cohort study	A prospective cohort study	A randomised controlled trial
III-1	A pseudorandomised controlled trial (i.e. alternate allocation or some other method)	A study of test accuracy with an independent, blinded comparison with a valid reference standard, among non-consecutive persons with a defined clinical presentation	All or none	All or none	A pseudorandomised controlled trial (i.e. alternate allocation or some other method)
III-2	A comparative study with concurrent controls: <ul style="list-style-type: none"> • Non-randomised experimental trial • Cohort study • Case-control study • Interrupted time series with a control group 	A comparison with reference standard that does not meet the criteria required for Level II and III-1 evidence	Analysis of prognostic factors amongst persons in a single arm of a randomised controlled trial	A retrospective cohort study	A comparative study with concurrent controls: <ul style="list-style-type: none"> • Non-randomised, experimental trial • Cohort study • Case-control study
III-3	A comparative study without concurrent controls: <ul style="list-style-type: none"> • Historical control study • Two or more single arm study • Interrupted time series without a parallel control group 	Diagnostic case-control study	A retrospective cohort study	A case-control study	A comparative study with concurrent controls: <ul style="list-style-type: none"> • Historical control study • Two or more single arm study
IV	Case series with either post-test or pre-test/post-test outcomes	Study of diagnostic yield (no reference standard)	Case series, or cohort study of persons at different stages of disease	A cross-sectional study or case series	Case series

1.2 Thesis Overview and Structure

This thesis begins with a summary of the existing evidence on whether sorghum consumption assists in the prevention of chronic disease (Chapter 2). Gaps in the literature were identified, in particular a paucity of reports on research involving human subjects. These observations were used to guide the development of the human clinical trials designed for the experimental components of the thesis.

A methodological framework underpinning the research undertaken for the thesis is presented in Chapter 3. This includes overviews of experimental study designs and a discussion of the different experimental methods utilised in the clinical trials within the thesis, giving insights into concepts of validity, strengths and limitations. Importantly, reviews of specific biomarkers, both as indicators of health status and appetite regulation, are also provided.

In order to conduct human trials testing sorghum-based foods in the Australian context, product development is important as such foods are not commonly available. Chapter 4 describes the overall processing protocols used to formulate a ready-to-eat sorghum grain breakfast cereal for use as a suitable test food in human trials. Details are provided on the nutritional, chemical and physicochemical properties of the end-products to enable more accurate interpretation of clinical effects observed in the clinical trials conducted.

To examine some of the potential mechanisms of action of sorghum consumption on health, an acute study was conducted (Chapter 5). This trial investigated not only subjective satiety, but also alterations to post-prandial responses in glucose, insulin

and a number of appetite-regulating gut hormones. In addition, antioxidant status was measured. Results confirmed that sorghum-grained breakfast cereal enhances acute subjective satiety and increases satiety-enhancing hormone levels acutely after ingestion. The cereal food made from red sorghum grain, a variety rich in anthocyanin flavonoids, elicited the greatest overall acute satiety-enhancing responses.

While these initial acute satiety results indicated that satiety effects might translate to longer-term effects on food consumption and thereby weight, this required testing on chronic disease biomarkers or health outcomes from a sorghum-enriched diet (containing red sorghum flaked biscuits) using a “gold-standard” study design, the randomised controlled trial. Chapter 6 presents results from this trial, demonstrating that equivalent amounts of weight were lost in the sorghum intervention group and the wheat control group, and for both groups the majority of clinical indices showed significant beneficial changes over time.

Chapter 7 summarises the results and conclusions of the four tenets of this thesis (the current scientific evidence of effects, the product formulation and the two experimental studies), and uses these to address the central aim and hypothesis. Limitations of the study designs are discussed and key recommendations for future research are provided.

CHAPTER 2:

Nutritional composition of sorghum and evidence of its effects on health

A major component of this chapter is the substantive content of the published article:

Stefoska-Needham, A., Beck, E.J., Johnson, S.K., Tapsell, L.C. (2015) Sorghum: An Underutilized Cereal Whole Grain with the Potential to Assist in the Prevention of Chronic Disease. *Food Reviews International*, 31:401-437.

2.1 Introduction

Cereal whole grains are significant contributors to energy, nutrients and dietary fibre in the human diet and are important for health. Numerous prospective studies demonstrate that regular consumption of whole grains lowers the risk of heart disease and diabetes by 20-30%^(16; 17), improves blood glucose regulation⁽¹⁸⁾, achieves better weight management over time^(19; 20), and lowers the risk of certain types of cancer⁽²¹⁾. A critical appraisal of the body of evidence is reflected in multiple national dietary guidelines that inform the community to eat more “grain foods particularly whole grain cereals” and to reduce consumption of refined grains^(17; 22).

Sorghum (*Sorghum bicolor* (L.) Moench) is a gluten-free whole grain cereal that is better known to Western societies as an animal feed rather than a human food source. Sorghum is grown around the world and ranks fifth in global cereal production after maize, rice, wheat and barley⁽⁷⁾. Sorghum is widely cultivated due to its adaptability to semi-arid and arid conditions and high temperatures. In these regions it is a major contributor to the staple diets of local populations⁽⁹⁾. In countries such as Australia and the United States, the primary use of sorghum has been as livestock feed and more recently in biofuel production⁽⁹⁾. Increasingly, the nutritional and agronomic advantages of sorghum, combined with a growing consumer movement dedicated to “healthy living”⁽¹¹⁾, has peaked commercial interests in developed economies on how to make sorghum-based food products more accessible to consumers who remain largely unaware of their potential health benefits.

The starting point for exposing health benefits of foods is often their nutritional properties. In the case of sorghum, as with plant foods generally, the phytochemical

component is of particular interest and this reflects recent developments in the nutritional sciences. The type of phytochemicals, namely phenolic acids, flavonoids and condensed tannins, in some sorghum varieties have been purported to reduce the risk of certain types of cancer, cardiovascular disease, obesity and diabetes⁽²³⁾. Sorghum also has decreased starch and protein digestibility *in vitro*, and is high in dietary fibre and resistant starch and this array of qualities may play a role in mechanisms that reduce disease risk (Table 2.1). Not least, is the fact that sorghum is gluten-free and is suitable for people with coeliac disease and gluten intolerances⁽²⁴⁾.

Table 2.1 Sorghum’s nutritional and functional attributes associated with metabolic disease effects

Component/Property	Proposed benefits
Slow starch digestibility ^(25; 26) (slowly digestible starches; interactions with endosperm & polyphenolic compounds that reduce starch hydrolysis)	Potential to attenuate blood glucose & insulin responses & increase satiety through reduction of glycaemic index of sorghum-based foods. This has relevance in appetite regulation, weight management & risk reduction of obesity-related diseases such as diabetes
High antioxidant activity ⁽²³⁾ (phenolic acids, monomeric polyphenolic flavonoids, polymeric polyphenolic condensed tannins)	Potential to reduce oxidative stress that plays an important role in the pathogenesis of many chronic diseases such as diabetes, atherosclerosis, some cancers, aging, arthritis & neurological diseases
High fibre ⁽²⁷⁾ (including resistant starch, ranging from 2.2 g ⁽²⁸⁾ – 6.5 g ⁽²⁹⁾ / 100 g dry matter)	Offers benefits to gut microbiome, metabolic disease risk & gastrointestinal health
High unsaturated fatty acid content of lipid ^(30; 31) (oleic acid, linoleic acid, linolenic acid and policosanols in wax ⁽¹⁸⁾)	Improving dyslipidemia & thus promoting heart health

In a traditional nutrition sense, the value of sorghum grain has been considered to be slightly inferior compared to other cereal grains on the basis of lower protein and starch digestibility and consequently, reduced metabolisable energy. This consideration is especially relevant to many of the world's poorest and most food-insecure communities where sorghum is a core food. In these cases sorghum is combined with legumes and other cereals to increase macro- and micro- nutrient density of sorghum-based foods and diets^(32; 33), and to this end, bio-fortified transgenic sorghum lines have been developed⁽³⁴⁾. Paradoxically, these properties of lower digestibility and reduced available energy may prove to be better suited in populations where over-weight and obesity related chronic diseases, such as metabolic syndrome, diabetes, heart disease and cancer are major public health issues⁽³⁵⁾.

Over the past decade, key researchers have exposed the potential role of sorghum in human health and in disease prevention^(23; 36; 37). They have argued for a paradigm shift from perceiving sorghum as a low-value cereal grain to a health-promoting, environmentally sustainable food for inclusion in the global human diet. In order to achieve commercial adoption of this position, food innovation is required that would extend the range of sorghum-based products available to consumers. At the same time, quality human clinical trials are required to provide evidence of health effects.

The research needs to be conducted in a food-health paradigm that considers not only the effects of individual grain constituents and their involvement in physiological processes, but also the effects of consuming sorghum-based foods within the broader context of whole diets. Because sorghum has been largely used as an animal feed in

Western societies, much of the research has been done on livestock, but a wide range of studies have emerged that provide the basis for moving into human clinical studies. To guide valid hypotheses for clinical human research, a summary of the existing evidence and knowledge base is required.

2.2 Literature review method

A comprehensive, narrative literature review was determined most appropriate in light of the complex amalgamation of research topics considered (plant anatomy, food chemistry, food processing, nutrition, and evidence for health effects). Search terms included “sorghum”, “sorghum grain”, “composition”, “nutrient”, “chemical”, “human”, “health”, “chronic disease”, “diet”, “benefit”, “weight”, “subject”, “intervention”, “clinical”, “trial”, and combinations thereof. The following databases were utilised to identify relevant literature; Cochrane Library, CINAHL, MEDLINE, PubMed, Science Direct, Scopus and Web of Science.

Aims of the review were to:

- 1) describe sorghum grain structure and the specific chemical, nutritional and functional attributes that relate to mechanisms by which sorghum grain components may influence weight management and chronic disease risk;
- 2) summarise the evidence reported in the scientific literature on effects of sorghum consumption on health outcomes related to chronic disease prevention.

2.3 Results

2.2.1 Structure of the Sorghum Plant

Sorghum is a self-pollinating, summer annual belonging to the grass family of Poaceae. The sorghum plant resembles a cane-like grass, ranging in height from 60cm to 460cm. It mostly has a single stem and produces a deep taproot under favourable soil conditions⁽³⁸⁾. The leaves look similar to maize leaves, varying from 7 to 24 leaves per plant depending on the cultivar (Figure 2.1). The flower head (or seed head) is usually a compact panicle, of approximately 25-36cm, that sits on top of the stalk of a mature plant and carries two types of flowers.



Source: iStock by Getty Images (www.istockphoto.com)

Figure 2.1 Field of red sorghum plants depicting maize-like leaves and compact panicles (grain seed heads)

2.2.2 Sorghum: potential as an environmentally-sustainable, commercial crop for human food production

Sorghum is a dependable crop that requires fewer nutrient inputs and significantly less water than other crops⁽⁶⁾. Largely due to its remarkable natural diversity, sorghum can be grown on over 80% of the world's agricultural land, including marginal land not suited to cultivation of other crops⁽³⁹⁾. As global demand increases for more productivity from low-quality land with fewer resource inputs, sorghum is positioned well to meet those needs in a sustainable way. However, historically the utilisation of sorghum for human use in Western societies has been grossly limited mainly due to its reputation as a livestock feed, the lack of potential markets and the paucity of human dietary studies, as discussed in Chapter 1. Having said this, during the course of this thesis, a growing number of food products have emerged on supermarket shelves that contain sorghum as a component ingredient, suggesting that demand for sorghum for use in human food production is increasing (Figure 2.2). In the majority of instances, these food products have been marketed for their gluten-free attribute rather than other potential nutritional attributes of the sorghum grain.



Figure 2.2 Example of different sorghum-containing food products currently on supermarket shelves (May 2016)

2.2.3 Nutritional and chemical composition of the sorghum grain

Sorghum grain is similar to maize with respect to chemical composition, with its key components being: starch, proteins, lipids, non-starch polysaccharides and phytochemicals such as phenolic compounds, phytosterols and policosanols. Sorghum grain also contains dietary fibre, including resistant starch, and micronutrients including vitamins and minerals, oil bodies, waxes and ash.

The grain is spherical with a slightly flattened germ end, reaching approximately 4mm in length⁽⁴⁰⁾ (Figure 2.3). Like other cereal grains, the sorghum caryopsis is divided into three distinct anatomical parts: the *pericarp* (outer layer or seed coat), *endosperm* (storage tissue) and *germ* (embryo). Genes referred to as the R and Y genes control pericarp colour. The testa is a sub-coat located between the pericarp and endosperm, and is also controlled by genes (B1 and B2 genes)⁽⁴¹⁾. When both genes are dominant, the testa is pigmented. In general, sorghum grains are smaller than those of maize but have a similar starchy endosperm that is usually white and floury. Most sorghum grains are partially covered by husks (glumes) that are removed from the grain during threshing. Consequently, there is no husk to remove during milling⁽⁴²⁾. The pericarp is made of 3 segments - epicarp, mesocarp and endocarp⁽⁴³⁾. The epicarp is the outermost layer and is usually covered with a thin waxy film. It contains most of the sorghum pigments that strongly influence the grain colour. The mesocarp, the middle structure, contains varying amounts of starch granules⁽⁴⁰⁾, a feature unique to sorghum and pearl millet.

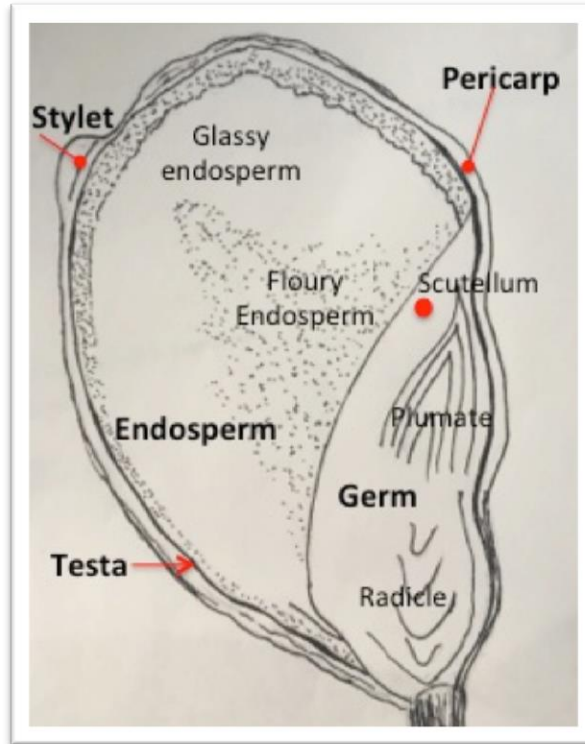


Figure 2.3 Sorghum Caryopsis
(© Anita Stefoska-Needham adapted from Sautier and O'Deyes⁽⁴⁴⁾)

The endosperm is the largest part of the caryopsis, accounting for 82-87% of the grain weight⁽⁴³⁾ in the form of mostly starch and protein. It is comprised of the aleurone layer, peripheral layer, and corneous and floury areas. The aleurone contains proteins (protein bodies, enzymes), ash (phytin bodies) and oil (spherosomes). The germ is comprised of the scutellum, the embryonic axis and embryonic disc. The germ is very rich in lipids and contains a large proportion of protein. The protein of the germ is mainly albumins and globulins, which are rich in lysine, tryptophan and other essential amino acids⁽⁴⁵⁾.

The proximate nutritional composition of sorghum whole grain is similar to wheat whole grain; energy density is 1377 vs 1418 kJ/100 g dry weight, total carbohydrate 74.6 vs 71.1, fat 3.3 vs 2.5 and protein 11.3 vs 13.7 g/100 g dry weight, respectively (Table 2.2). However, sorghum has lower starch digestibility relative to other grains such as maize, rice, wheat and barley, although the degree of digestibility depends on the method of processing^(25; 46). The nutritional quality of sorghum proteins is diminished because they are more resistant to digestion⁽⁴⁶⁾ and have low levels of essential amino acids such as lysine, tryptophan and threonine⁽⁴⁷⁾. In contrast, there are high levels of leucine that were previously implicated, but now not accepted, as a cause of niacin deficiency and consequently endemic pellagra in some sorghum-eating populations⁽⁴⁸⁻⁵¹⁾.

As with cereals more broadly, sorghum is a source of B-complex vitamins such as thiamin, riboflavin, vitamin B6, biotin, and niacin, but levels are diminished with grain refining processes including decortication⁽⁵²⁾. The mineral composition in sorghum is similar to millet and is predominantly composed of potassium and phosphorus, with low levels of calcium⁽⁵³⁾. Sorghum-based foods are a good source of both iron and zinc, although anti-nutrients such as phytates may diminish bioavailability^(54; 55). A complete nutrient analysis of sorghum is detailed in Table 2.2.

Sorghum grain is generally rich in health-promoting phytochemicals, especially polyphenols. Importantly, sorghum varieties are classified largely according to their total extractable phenols, in combination with grain appearance (Table 2.3). White sorghums have no detectable tannins or anthocyanins and have very low total

extractable phenol levels. Red sorghums have no tannins but have a red pericarp with significant levels of extractable phenols, namely anthocyanins. Brown sorghums have a pigmented testa and contain significant levels of tannins, with varying degrees of pericarp pigmentation. Black sorghums are a special variety of red sorghum that turns black in sunlight during maturation and they contain very high levels of 3-deoxyanthocyanidins (3-DAs), which are located in the pericarp^(57; 58).

The colour of the sorghum grain, the naturally occurring variations of which are shown in Figure 2.4, significantly influences the colour of the flour and thus the appearance of end food products.

Table 2.2 Nutritional Composition of Sorghum, Wheat and Corn
(per 100g dry weight, edible portion)^(40; 56)

Proximates	Sorghum, White, Whole	Wheat, Durum, Whole	Corn, Yellow
Energy (kcal / kJ)	329/1377	339/1418	365/1527
Protein (g)	10.62	13.68	9.42
Total lipid (fat) (g)	3.46	2.47	4.74
Carbohydrate, by difference (g)	72.09	71.13	74.26
Fibre (g)	6.7 [#]	10.7*	7.3
Lipids			
Fatty acids, total saturated (g)	0.610	0.454	0.667
Fatty acids, total monounsaturated (g)	1.131	0.344	1.251
Fatty acids, total polyunsaturated (g)	1.558	0.978	2.163
Cholesterol (mg)	0	0	0
Minerals			
Calcium, Ca (mg)	13	34	7
Iron, Fe (mg)	3.36	3.52	2.71
Magnesium, Mg (mg)	165	144	127
Phosphorus, P (mg)	289	508	210
Potassium, K (mg)	363	431	287
Sodium, Na (mg)	2	2	35
Zinc, Zn (mg)	1.67	4.16	2.21
Copper, Cu (mg)	1.080	0.553	0.314
Manganese, Mn (mg)	1.630	3.012	0.485
Selenium, Se (mg)	12.2	89.4	15.5
Vitamins			
Vitamin C, total ascorbic acid (mg)	0	0	0
Thiamin, B1 (mg)	0.332	0.419	0.385
Riboflavin, B2 (mg)	0.096	0.121	0.201
Niacin, B3 (mg)	3.688	6.738	3.627
Pantothenic acid, B5 (mg)	1.250	0.935	0.424
Vitamin B6 (mg)	0.440	0.419	0.622
Folate, DFE (µg)	20	43	19
Vitamin B-12 (µg)	0	0	0
Vitamin A, RAE (µg)	0	0	11
Vitamin D (IU)	0	0	0
Vitamin E (alpha-tocopherol) (mg)	0.50	0.71*	0.49
Amino Acids			
Tryptophan (g)	0.124	0.176	0.067
Threonine (g)	0.346	0.366	0.354
Isoleucine (g)	0.433	0.533	0.337
Leucine (g)	1.491	0.934	1.155
Lysine (g)	0.229	0.303	0.265
Methionine (g)	0.169	0.221	0.197
Cystine (g)	0.127	0.286	0.170
Phenylalanine	0.546	0.681	0.463
Tyrosine (g)	0.321	0.357	0.383
Valine (g)	0.561	0.594	0.477
Arginine (g)	0.355	0.483	0.470
Histidine (g)	0.246	0.322	0.287
Alanine (g)	1.033	0.427	0.705
Aspartic acid (g)	0.743	0.617	0.655
Glutamic acid (g)	2.439	4.743	1.768
Glycine (g)	0.346	0.495	0.386
Proline (g)	0.852	1.459	0.822
Serine (g)	0.462	0.667	0.447

*Whole grain wheat flour #White sorghum (fibre in other sorghum types ranges between 8.8-11.1g / 100 g).

Table 2.3 Classification of sorghum according to appearance and extractable phenols

	Tannins	Anthocyanins	Pericarp	Testa
White or “Food-type” Sorghum	Nil	Nil	Not pigmented	Absent
Red Sorghum	Nil	High	Red	Not pigmented
Black Sorghum	Nil	Very High	Black	Not pigmented
Brown Sorghum	Very high	High	Pigmentation variable	Pigmented



Figure 2.4 Grain colour variation in seed heads of different sorghum cultivars
(Image source: © Anita Stefoska-Needham)

2.2.4 Processing of sorghum grain to produce foods for human consumption

Cereal-based food products, including those made from sorghum grain, require processing⁽⁵⁹⁾. The aim of processing is to transform raw grains into edible food products that possess good sensory qualities and retain beneficial nutritional and functional properties⁽⁶⁰⁾. In Western countries, cereal grains are most commonly utilised in the production of breads, breakfast cereals and cereal-based snack foods. For breads and other baked goods, grains are generally milled to produce flour, which in turn is subjected to treatment with water and heat⁽⁵⁹⁾. Commercial breakfast cereals may be extruded, puffed, baked and flaked. Feasibly, sorghum grain can be treated using the same methods, in addition to being popped, in a similar manner to corn⁽⁶⁰⁾. However, processing affects the chemical composition and physical properties of foods, either positively or negatively, thereby impacting on the potential health benefits that can be delivered by the end food product⁽⁶⁰⁾. For example, processing may affect starch gelatinisation and digestibility and therefore impact on the glycaemic index (GI) of a food⁽⁶¹⁾, with implications for blood glucose regulation⁽⁶²⁾. Also, the levels of phytochemicals tend to be lower in processed sorghum foods than in the native grains, lowering the food's total antioxidant capacity and thereby sorghum's potential to mitigate oxidative stress processes⁽⁶⁰⁾. Overall, research on the effects of different processing methods on sorghum grain is limited, in particular in relation to the flaking of sorghum grain, which was the method used to manufacture a food product for testing in the experimental components of this thesis (full method described in Chapter 4). Only one study was identified in the literature⁽³⁴⁵⁾, showing that flaking sorghum grains reduces soluble, bound and total polyphenol content, however because this was not a clinical study extrapolation to the human condition is not possible.

Sorghum constituents isolated from the native grain may have different effects after processing/treatment compared to those arising from the natural, unprocessed grain. Moreover, the effects elicited by the whole food may be greater or different from the corresponding actions of the individual food constituents, through synergistic interactions between components within the food matrix (described by the concept of food synergy: Section 2.2.6)⁽⁶³⁾. Thus, it is essential to examine sorghum as a whole food, *as is*, after processing. To assist with our understanding of the whole food effects, it is important to firstly recognise the array of sorghum components, as reviewed in the following section.

2.2.5 Sorghum grain components with the potential for functional properties

Starches

Sorghum grain is a good source of starch, containing approximately 71% of dry whole grain weight⁽⁴⁰⁾. The starch is encapsulated in granules that are located predominantly in the endosperm (storage tissue), though uniquely some are present in the pericarp (outer layer of grain)⁽⁴⁰⁾. Sorghum starch is comprised of both amylose and amylopectin polysaccharides (branched polymers of glucose) with very low percentages of amylose present in the starch of waxy sorghum varieties compared to 24-33% in non-waxy sorghum starch⁽³⁷⁾. Sorghum starch granules are densely packed and enclosed by protein bodies embedded in a protein matrix, a unique structural aspect of sorghum grain⁽⁴⁶⁾. Disulphide-bond cross-linking involving kafarins in the protein matrix forms a protective network around the starch granules reducing starch digestibility⁽²⁶⁾.

The lower starch digestibility reported for sorghum foods is not an intrinsic property of the sorghum starch granules themselves, but appears mainly to be a consequence of the interactions of the starch with the endosperm protein matrix, as well as with cell wall material and polyphenolic compounds, such as condensed tannins and flavonoids^(62; 64-67). These interactions impede carbohydrate-hydrolyzing enzymes, such as α -glucosidase and α -amylase, thereby lowering starch digestibility⁽⁶⁸⁾. The presence of the protein matrix has also been associated with reduced starch gelatinisation during cooking resulting in partially-gelatinised sorghum starch granules that may resist enzymatic degradation⁽⁴⁶⁾. Sorghum starch has amongst the highest gelatinisation temperatures, ranging from 66-81 °C, depending upon cultivars, and is higher than that of maize, wheat and barley^(37; 69). However, the extent of gelatinisation of starch granules as a result of processing cannot easily predict digestibility and physiological effects such as glycaemic responses. Factors such as the precise ratios of amylose to amylopectin, their arrangement within the starch granule, further degradation of other polymer molecules and post-processing conditions also influence the postprandial effects of a starchy food⁽⁷⁰⁾.

Recent publications report on the *in vitro* starch digestibility of different sorghum foods, including sorghum-refined maize snack-like extrudates⁽⁷¹⁾, whole grain sorghum-refined wheat flour flat bread⁽⁷²⁾ and whole grain sorghum-durum semolina pasta⁽²⁸⁾. These *in vitro* studies confirm that sorghum foods can be formulated and processed to deliver slowly digested starch (SDS), with the potential to assist in improving blood glucose control, however these predicted positive results require rigorous testing in humans.

Resistant Starch and Non-Starch Polysaccharides

Sorghum foods also contain varying amounts of resistant starch (RS) depending on factors such as processing, cooking, cooling, food storage, gelatinisation, and cultivar^(28; 29; 62; 72). Physiologically, RS resists hydrolysis by enzymatic digestion in the small intestine⁽⁷³⁾ and enters the colon where it is partially or completely fermented to produce beneficial short-chain fatty acids (SCFA)⁽⁷⁴⁾. Here, the RS can act as a prebiotic^(75; 76) by stimulating the proliferation of beneficial bacteria already in residence in the gastrointestinal tract (GIT). To date these effects have not been widely researched with respect to sorghum foods and sorghum-based diets.

Non-starch polysaccharides (NSPs) have been associated with lower blood plasma cholesterol levels, reduced small intestine transit time, and improved bowel function^(77; 78). NSPs are the major component of dietary fibre in sorghum grain and are mainly located in the pericarp and endosperm cell walls, constituting 2-7% of the total weight of the grain depending upon cultivar^(27; 79). Sorghum NSPs are both cellulose and non-cellulosic consisting of arabinose, xylose, mannose, galactose, glucose, and uronic acid monomers^(37; 80). The non-cellulosic polysaccharides are primarily water-insoluble glucuronoarabinoxylans (GAX) along with β -glucans⁽⁸¹⁾, although naturally occurring β -glucans in sorghum are lower than that of barley and oats^(29; 82). The GAX in sorghum are very abundant and are highly substituted with glucuronic acid residues, and acetyl and feruloyl compounds. Sorghum contains other non-carbohydrate cell-wall components that form part of the dietary fibre fraction such as lignins, at levels up to 20% of the total cell wall contents by dry weight⁽⁸³⁾. The total dietary fibre content of different sorghum cultivars ranges from 7.6% in low-tannin sorghums to 9.2% in high tannin varieties⁽²⁷⁾, and its level in

sorghum-based meals can be manipulated by cooking and fermentation⁽⁸⁴⁾. The effect of consumption of sorghum NSPs has not been investigated in humans.

Proteins

Protein is the second largest constituent of sorghum grain (6-18%) after starch⁽⁴²⁾. Sorghum endosperm proteins are found in both a matrix and as protein bodies that are enveloped by the matrix. Sorghum proteins are classified as albumins, globulins, kafirins, cross-linked kafirins and glutelins⁽⁸⁵⁾. Of these, kafirins are the main protein⁽⁸⁶⁾, comprising 50-70% of total protein content⁽⁴⁶⁾. The kafirins are prolamin storage proteins with limiting levels of some amino acids, in particular lysine⁽⁴⁵⁾, a disadvantage not unique among cereal grains. The kafirins differ in structure from the gliadin and glutenin storage proteins in wheat. They do not elicit damage to the mucosa of the small intestine of people with coeliac disease⁽²⁴⁾, making sorghum a viable ingredient for gluten-free foods such as bread. However, the inability of sorghum kafirins to make elastic dough and the difficulty in making bread of high consumer acceptability presents challenges and has driven research into the manufacture of quality sorghum-based gluten-free food products^(72; 87).

Sorghum kafirins are poorly digested due to the formation of cross-linking especially when moist cooked, resulting in protease resistance⁽⁴⁶⁾. *In vitro* and animal studies have also shown that sorghum protein digestibility may be reduced by other protein-protein, protein-phenol and carbohydrate-phenol complexes that have been identified^(80; 88-90). Cornu and Delpeuch⁽⁹¹⁾ reported that the nitrogen digestibility in humans on a diet of 80% sorghum decreased from 65.4% to 60.5% when the decorticated sorghum in the diet was replaced by whole grain sorghum, suggesting

that higher fibre sorghum varieties may have lower protein digestibility. Rather than a fibre effect per se, this more likely relates to the higher polyphenol content that naturally occurs in whole grain sorghum and the resultant binding of phenols to dietary protein⁽⁸⁰⁾. In sorghum-consuming communities, where protein malnutrition is an issue, efforts to increase protein digestibility are imperative and lactic acid fermentation, decortication and extrusion have been shown to improve digestibility and consequent amino acid availability^(71; 90; 92).

Sorghum grain also contains a broad range of bioactive peptides, recently reviewed by Lin et al.⁽⁹³⁾ These are of current interest to researchers due to their potential biological role in human physiological processes including pathogenesis. The peptide bioactivities include antioxidant, antihypertensive, anticancer, antimicrobial, and opioid activities as well as immunomodulatory and cholesterol-lowering effects. The specific bioactive peptides isolated in sorghum include but are not limited to: amylase inhibitors⁽⁹⁴⁾, proteinase inhibitors⁽⁹⁵⁾, cationic peroxidase⁽⁹⁶⁾, 2-kDa antiviral peptide⁽⁹⁷⁾ and xylanase inhibitors⁽⁹³⁾. To date, research linking cereal grains with potential bioactive peptide activity has been limited, however there are more sorghum studies appearing in the literature^(93; 98). Overall, there is much to consider in translating this knowledge to human clinical trials, in particular the study populations of interest and the health/disease outcomes that might be researched.

Lipids

Sorghum grain contains approximately 3-4% lipids, the majority of which are neutral triglycerides, rich in unsaturated fatty acids and mostly present in the germ⁽³⁰⁾. The predominant fatty acids are oleic acid (31.1–48.9%), linoleic acids

(27.6–50.7%), linolenic acid (1.7–3.9%), stearic acid (1.1–2.6%), palmitic acid (11.7–20.2%) and palmitoleic acid (0.4–0.6%)^(40; 99; 100). Two less common saturated fatty acids, octanedioic (C8:0) and azelaic acid (C9:0), have been identified in some sorghum varieties⁽⁹⁹⁾. This lipid composition has generated interest in sorghum as a source of edible oil, representing a potentially valuable dietary source of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), with higher PUFA levels than MUFA⁽⁹⁹⁾. This desirable lipid composition is conducive to mechanisms that lower lipid levels in humans and therefore to potentially lower risk factors associated with heart disease.

Based on research in hamsters, Carr et al.⁽¹⁰¹⁾ suggested that the primary cholesterol-lowering mechanism of sorghum lipid extracts appears to be a reduction in cholesterol absorption with a concomitant increase in faecal sterol excretion. Specifically, policosanols (a mixture of long-chained primary alcohols) in the sorghum lipid extracts appear to inhibit endogenous cholesterol synthesis⁽⁸⁴⁾. Sorghum also contains plant sterols that may reduce cholesterol absorption to collectively lower plasma and liver cholesterol concentrations⁽¹⁰¹⁾. These results were recently supported by Lee et al.⁽¹⁰²⁾ in a hamster model of hypercholesterolemia, investigating the effects of whole kernel grain sorghum oil (rich in plant sterols) and wax (high in policosanols). The authors report that the sorghum oil played a more significant role in modulating cholesterol, most likely by inhibiting absorption, however subtle interactions by the wax may have contributed to the effect⁽¹⁰²⁾.

The policosanols in sorghum wax (found on the surface of the grain kernel) are comprised of mainly docosanol (C22), tetracosanol (C24), hexacosanol (C26),

octacosanol (C28), triacontanol (C30), and dotriacontanol (C32)⁽¹⁰³⁾. In sorghum, C28 and C30 are the most abundant policosanols⁽³¹⁾. A mixture of C28 and C30 from sugar cane wax has been shown to improve blood lipid levels⁽¹⁰⁴⁾, however reports on the human effects of sorghum-derived policosanols have not been published to date.

Martinez et al.⁽¹⁰⁵⁾ reported an alternative mechanism for the cholesterol lowering effect of sorghum lipid extract from research done with hamsters. They reported that sorghum lipid extract acts as a “prebiotic” to improve the host cholesterol metabolism through effects on gut microbiota. *Bifidobacteria* significantly increased in the hamsters fed grain sorghum lipid extract and was positively associated with HDL plasma cholesterol levels⁽¹⁰⁵⁾. In humans, this shift in *bifidobacteria* is associated with improved overall health, including reduced gut infections and suppression of colon cancer initiation⁽¹⁰⁶⁻¹⁰⁸⁾.

Finally, sorghum lipids may also possess antiproliferation properties. Zbasnik et al.⁽¹⁰⁹⁾ extracted lipids from sorghum dry distiller's grain (a by-product of the ethanol industry) and observed an anti-proliferative effect on human colon carcinoma cells. They suggested that the effect may have been a result of synergistic interactions of vitamin E (predominantly gamma-tocopherol), triacylglycerides, free fatty acids (predominantly linoleic acid), policosanols, aldehydes, and sterols (predominantly campesterol and stigmasterol) that were identified in the extracts⁽¹⁰⁹⁾. Although sorghum dry distiller's grain is primarily used for animal feed, it is chemically and microbiologically safe as a human food ingredient, therefore further research in humans is relevant.

Phytochemicals

Most sorghum varieties, except white sorghums, have a high concentration of phytochemicals (Figure 2.5), particularly phenolic compounds, which exhibit high antioxidant activity and are linked to health benefits^(23; 57; 110). In fact, bran of some sorghum grain varieties reportedly has the highest antioxidant activity of all cereal crop fractions, even higher than many fruits and vegetables⁽²³⁾. Specifically, sorghum bran has up to two orders of magnitude higher antioxidant activity than oat bran and wheat cereal, and an order of magnitude higher than rice bran although the precise amount is highly dependent on the variety of sorghum (Figure 2.6).

Phenolic compounds

The phenolic compounds in some sorghum grain varieties are more abundant and diverse than in any other cereal grain⁽⁵⁷⁾. Sorghum grain varieties that have a pigmented testa and thick pericarps have the highest levels⁽¹¹¹⁾. The phenolic compounds are concentrated in the bran component of the grain (in particular the testa and pericarp) and can be categorised into three main groups; 1) phenolic acids (hydrobenzoic acids and hydrocinnamic acids), 2) monomeric polyphenolic flavonoids (flavanols, flavanones, flavones, flavan-4-ols and anthocyanins), and 3) polymeric polyphenolic condensed tannins (also known as proanthocyanidins or procyanidins). Refer to Figure 2.5.

The phenolic compounds in sorghum grain exhibit high antioxidant activity through their ability to scavenge free radicals⁽⁵⁷⁾. The degree of antioxidant activity is correlated to the content of phenolic compounds in a specific sorghum cultivar and

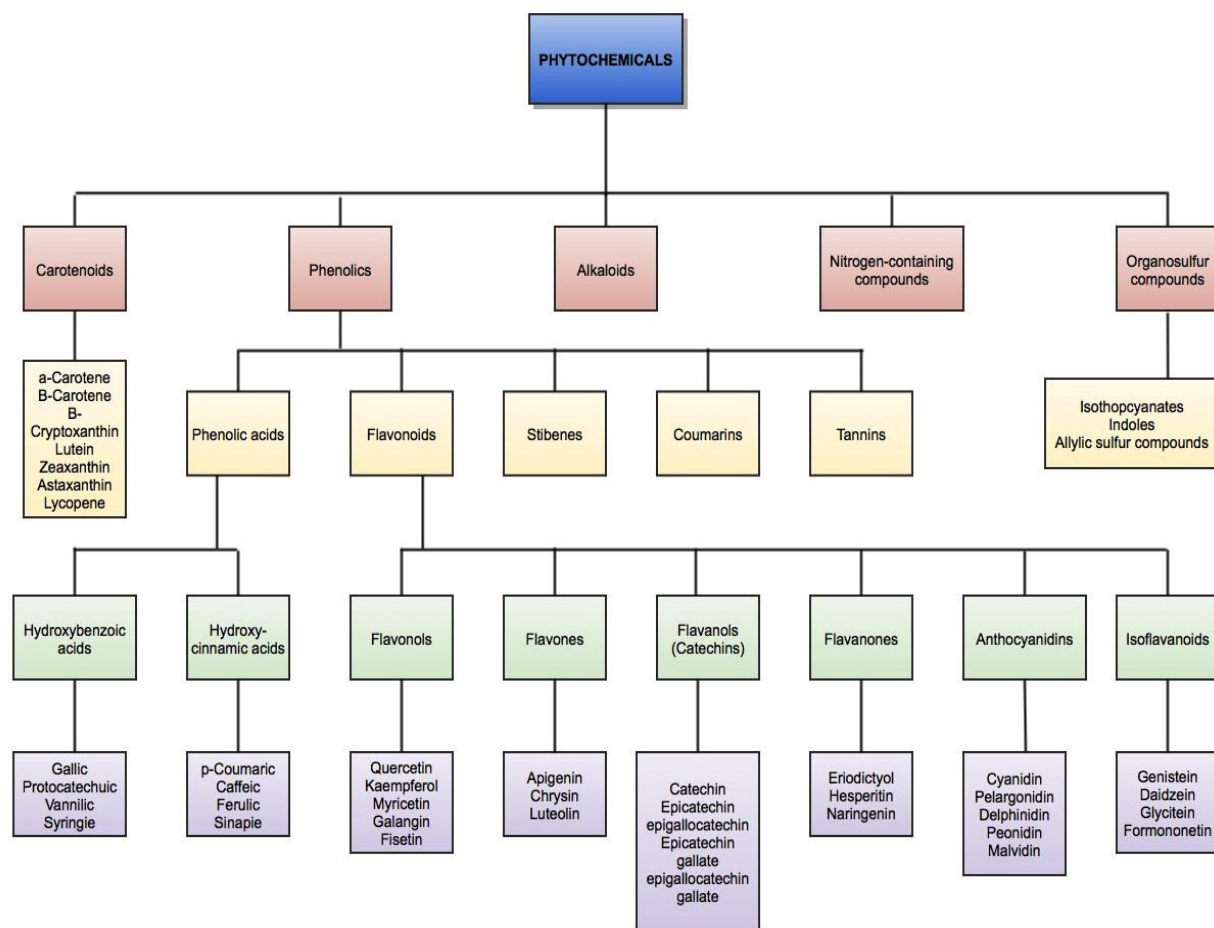


Figure 2.5 Classification of dietary phytochemicals (adapted from Liu, 2004)⁽¹²¹⁾

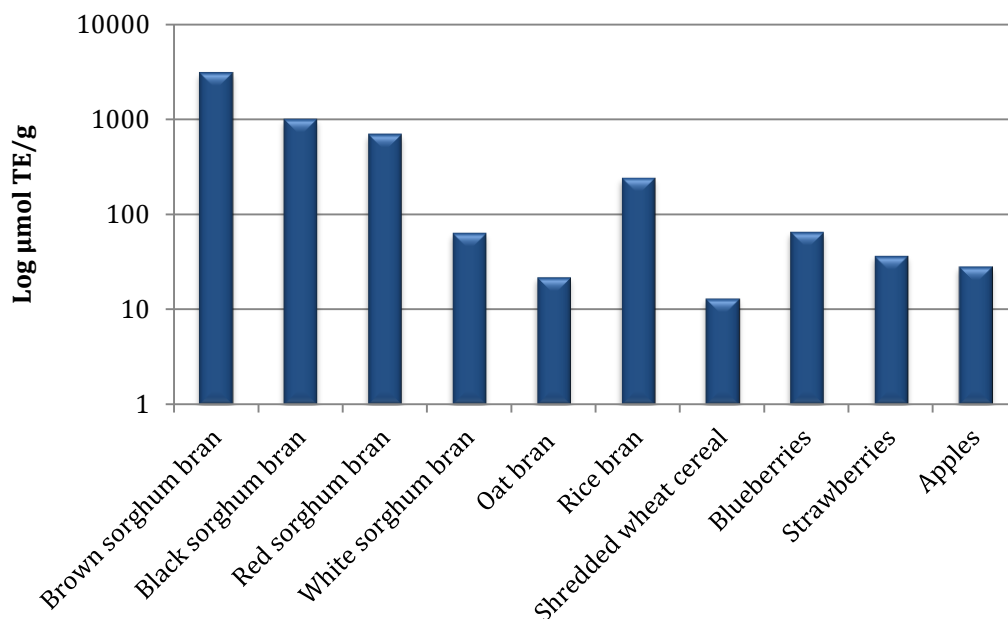


Figure 2.6 Antioxidant activity in sorghum bran fractions (dry basis) relative to other cereals and common fruits measured by Oxygen Radical Absorbance Capacity (ORAC) and expressed as μmol Tocopherol Equivalents (TE)

(Compiled from previously reported data^(23; 56))

this in turn is influenced by its genotype and growing environment⁽¹¹¹⁾. Levels of phenolic compounds and the activity of enzymes, which synthesize or catabolize phenols in sorghum grain, strongly influence food product properties such as flavor and color, and are therefore important determinants of sorghum for food use⁽³⁶⁾. In general, sorghum processing decreases antioxidant activity mainly as a result of reducing levels of measurable phenolic compounds⁽¹¹²⁻¹¹⁴⁾. This may be as a result of thermal degradation or lowered extractability during the analytical procedures used for their measurement⁽¹¹³⁾. However, some processes including steeping, germination, fermentation⁽¹¹⁵⁾ and roasting of steamed grain⁽¹¹⁶⁾ have been reported to increase the level of polyphenolics. These may be related to improved extractability through breakdown of the food matrix, which might also result in higher bioavailability.

It has been postulated that sorghum grain phytochemicals may provide overall disease protection through not only antioxidative but also hypoglycaemic, and hypolipidaemic mechanisms^(117; 118). However, the extent of these health beneficial effects is unclear since only limited clinical research has been reported. Reduction in oxidative stress is implicated in these protective processes⁽¹¹⁹⁾, therefore sorghum polyphenolic compounds may be relevant in disrupting the cascade of pathophysiological changes that lead to metabolic disease. *In vitro*, sorghum bran extracts with a high phenolic content and thus high antioxidant properties were shown to inhibit albumin glycation, whereas wheat, rice, oat and low-phenolic sorghum bran extracts (such as white sorghum) did not⁽¹²⁰⁾. Albumin glycation is the non-enzymatic process that results in formation of advanced glycation end-products (AGEs). AGEs have been associated with metabolic diseases such as diabetes and atherosclerosis. Human clinical investigations are warranted to further test these effects, especially since sorghum bran extracts have been suggested for use in food ingredients, food supplements or nutraceutical products.

Flavonoids

The anthocyanin flavonoids found in pigmented sorghums, but not in white sorghums, are of particular interest to researchers since some are unique to sorghum grain and they have potent antioxidant properties⁽¹²²⁾. The 3-deoxyanthocyanins (3-DA and derivatives) are the major class of flavonoid and are located in the pericarp^(57; 58). 3-DAs lack the hydroxyl group in the 3-position of the C-ring and include the apigenidin and luteolinidin that are largely responsible for the pigmentation of certain sorghum grain varieties, namely red and black sorghums⁽¹²³⁾. A recent *in vitro* analysis of red sorghum flour extracts showed strong free radical

scavenging activity as measured by an oxygen radical absorbance capacity (ORAC) assay and protection against LDL-oxidation, contributing to the evidence base for the potential of red sorghum as a valuable health-promoting food grain⁽¹²⁴⁾. However, understanding the bioavailability of sorghum anthocyanins for the putative health-promoting effects in humans is a much-needed focus of future research.

In vitro research investigating effects of specific sorghum anthocyanins is emerging. A study utilising the human epithelial larynx carcinoma cell line (Hep-2) by Devi et al.⁽¹²⁵⁾ demonstrated that anthocyanins extracted from red sorghum bran, specifically luteolinidin and apigenidin, induced significant anti-proliferative activity. Powerful anti-proliferative effects were also observed against colon cancer cells when black, red, and white sorghum extracts, rich in 3-DA were tested^(126; 127). Yang et al.⁽¹²⁸⁾ proposed that these protective effects result from estrogen-induced apoptosis of the non-malignant colonocytes that were strongly influenced by the flavones, apigenin and luteolin. In breast cancer cell lines, 3-DA isolated from red sorghum bran have been shown to have strong anti-proliferative properties and to be cytotoxic⁽¹²⁹⁾. Sorghum chloroform extracts have particularly strong anti-inflammatory effects *in vitro* (in both cell-free and cell-mediated experimental systems) through almost complete suppression of lipopolysaccharide-mediated production of nitric oxide, tumor-necrosis factor- α , and interleukin-6. These effects are correlated to flavonoid concentration in the extracts⁽¹³⁰⁾.

Tannins

Tannin sorghums contain high molecular weight *condensed tannins* that are oligomers or polymers composed of flavan-3-ol nuclei, found in the pigmented testa

of the sorghum grain⁽⁴¹⁾. They are formed by B-type (dimeric) procyanidins (3,5,7,4'-OH), namely procyanidin B-1 as represented in Figure 2.7. Condensed tannins exhibit strong antioxidant activity *in vitro* via free radical scavenging activity, chelation of transition metals and inhibition of pro-oxidative enzymes. The antioxidant activity of sorghum tannins is higher than that of tannins extracted from any other crop^(122; 131; 132). In animal studies, sorghum tannins have been shown to be 15-30 fold more effective at quenching peroxy radicals than simple phenolics⁽¹³³⁾.

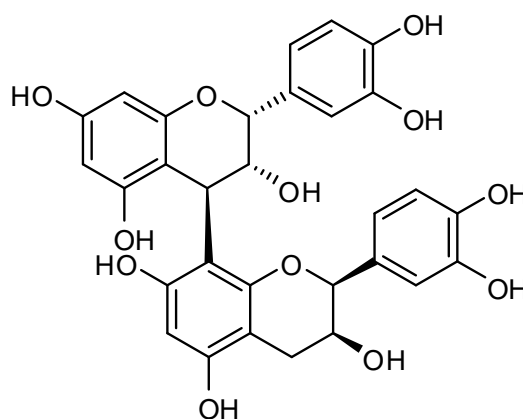


Figure 2.7 Procyanidin B-1, a type of condensed tannin, isolated in sorghum
(Image source: ©Anita Stefoska-Needham, adapted from Koleckar et al.⁽¹³⁴⁾)

The presence of tannins in sorghum grain may reduce the nutritive value and lower metabolisable energy of the grain. Several mechanisms have been proposed to explain this “anti-nutritional” effect as reviewed by Awika and Rooney⁽²³⁾. These include: binding of proteins and carbohydrates into insoluble complexes that resist digestive enzyme breakdown⁽¹³⁵⁻¹³⁸⁾; binding of digestive enzymes directly, inhibiting their enzymatic activity^(139; 140); and inhibition of intestinal brush border bound amino acid transporters⁽¹⁴¹⁾, particularly by tannin sorghums with higher degrees of polymerization⁽¹⁴²⁾, resulting in reduced digestive enzyme activity. These effects were also reflected in animal feeding trials that demonstrated the feeding efficiency of tannin sorghums was 5 to 10% lower than non-tannin sorghums,

depending on the animal species, the method of grain processing and diet type. In general, animals consumed more feed yet experienced the same or slightly less weight gain when tannin sorghum formed the basis of their diets^(139; 140; 143). Such effects in a Western diet, where food is ubiquitous, may be beneficial if these results are to be translatable to humans.

Antioxidant tannins may be key protective components in sorghum foods for the mitigation of oxidative stress-induced diseases, with anti-proliferative and anti-inflammatory effects as their key mechanisms of action. For example, brans from tannin sorghum varieties (naturally high in tannins, such as brown sorghum) and non-tannin sorghum varieties (black, red and white-grained) have demonstrated significant anti-inflammatory potential *in vitro* on the basis of strong inhibition of hyaluronidase activity (enzymes involved in cancer metastasis, osteoarthritis and skin aging)⁽¹²⁹⁾. The inhibition of the hyaluronidases correlated positively with total phenolic content and antioxidant capacity of the extracts, with greater effects observed in sorghum bran extracts than those of wheat and rice bran. In two experimental inflammatory systems using blood cells and a mouse-model, Burdette et al.⁽¹⁴⁴⁾ also demonstrated that the anti-inflammatory activity of ethanolic extracts of different sorghum brans correlated with their phenolic content and antioxidant activity. At present, it is not possible to extrapolate these *in vitro* effects to *in vivo* effects after realistic consumption of sorghum by humans. However, the research provides mechanistic models for further investigation.

Grimmer et al.⁽¹⁴⁵⁾ demonstrated the potent anti-mutagenic activity of higher molecular weight compared to lower molecular weight tannins isolated in sorghum-

derived polyphenol extracts. Gomez-Cordoves et al.⁽¹⁴⁶⁾ also demonstrated that sorghum tannins induce anti-carcinogenic effects against human melanoma cells *in vitro* through increased melanogenic activity (a protective effect against UV irradiation damage to human skin) and therefore reduced formation of human melanoma cells. Collectively, these cell line studies demonstrate the bioactive potency of sorghum grain constituents, in particular the tannins, although *in vivo* studies have not occurred.

2.2.6 Sorghum as a whole food: the case for “food synergy”

All of these components represent the lifecycle and survival of the sorghum plant, and may in part explain the evolutionary success of sorghum, whose origins date back to 8000 BCE⁽¹⁴⁷⁾. When the sorghum plant is eaten, as for all foods, there is an impact on human biological systems, including processes that lead to chronic disease. However, information about nutrients and bioactive components in isolation is incomplete in explaining the health effects of sorghum foods because food is what people eat, not individual constituents. Therefore, food is central to the relationship between nutrition and health, underpinned by the food synergy concept, which views food as a whole integrated system, and not merely as a collection of individual nutrients and other bioactive substances⁽¹⁴⁸⁾. Hence, evidence from studies of whole food sorghum foods will provide more useful information about the sorghum-diet-health relationship, examples of which are reviewed in the subsequent sections of this review.

2.2.7 Experimental research testing effects of sorghum consumption on human health

There is extensive scientific literature identifying a diverse range of nutrients and bioactive components in sorghum grain, with potential actions in numerous biological systems in the human body. However, a key question remains: *what happens when people actually eat sorghum?* To understand this, human studies are needed, specifically designed to elucidate the effects of sorghum consumption on protective health benefits. To date, these types of human investigations are severely limited and there is an obvious paucity in the literature. Although numerous studies have been conducted with human subjects (Table 2.4), the majority have addressed micronutrient metabolism (such as interactions between leucine, molybdenum, and niacin)⁽¹⁴⁹⁾, iron absorption from cereals⁽⁵⁴⁾, dietary fibre effects⁽¹⁵⁰⁾, protein digestibility⁽¹⁵¹⁾ and oral rehydration therapies⁽¹⁵²⁾. Overall, these studies contribute to the knowledge base on sorghum, however they do not address the central question associated with this thesis: *what is sorghum's potential to assist in chronic disease prevention?* The next section of this review therefore summarises the limited, existing experimental evidence that suggests protective health effects of sorghum consumption (for example, studies that investigate effects on energy balance, glycaemic control, lipids, oxidative stress and cell-mediated responses).

Effects on energy balance

Energy balance is pivotal in body weight regulation. Changes in body weight are associated with an imbalance between the energy content of food eaten and energy expended by the body to maintain life and to perform physical work⁽¹⁵³⁾. Sorghum may be a valuable lower-energy grain alternative in Western diets where overweight

and obesity rates continue to rise and represent major public health burdens⁽³⁵⁾. Sorghum's energy value is approximately 1377 kJ/100 g however the available energy for human metabolism may be lower than this estimate due to the described low starch and protein digestibility rates. This postulation is partly based on evidence from numerous feeding studies that show animals (from rodents to livestock species) fed whole grain sorghum, in particular the slowly digested high tannin sorghum varieties, have reduced weight gain^(139; 140; 143; 154; 155).

In general, dietary fibre and whole grain intakes have been associated with reduced risks of obesity, overweight and with lowered waist-to-hip ratio^(156; 157). Effects of dietary fibre on appetite and satiety have been proposed as major mechanisms for these reductions. Whole grain sorghum, with high fibre and slowly digestible starches, may increase satiety in humans due in part, to effects on GI of foods. It is believed that many communities in Africa who prefer to eat foods made from tannin sorghums do so because they impart stronger feelings of satiety and satiation compared to other cereals⁽²³⁾. Sorghum's satiety effects in humans have not yet been investigated through controlled dietary trials.

Sorghum contains resistant starch (RS) and fermentation of RS in the colon is linked to a number of positive effects including those on the gut microbiome^(76; 158). Studies specific to energy control with sorghum intake are limited however a recent study by Shen et al.⁽¹⁵⁹⁾, evaluated the effects of sorghum RS on changes to body weight, blood lipids and intestinal flora in 60 overweight and obese rats receiving treatment for 8 weeks. Results demonstrated that overweight rats fed a high-fat diet containing 30% sorghum RS gained less weight than rats fed a comparator diet devoid of

sorghum RS ($p < 0.05$). However, there was no significant difference in weight measures in the obese rats who were administered the same test diets. Thus sorghum RS did not overcome weight gain caused by high fat diets, but it did have an ameliorating effect. Statistically significant changes to the synthesis and secretion of serum leptin and adiponectin, two adipose-derived hormones that are involved in the regulation of food intake and body weight, were also reported in the sorghum RS groups, as were improvements to the intestinal flora (as measured by increased populations of *Bifidobacterium* and *Lactobacillus* and reduced populations of *Enterobacteriaceae*). This is an important study that demonstrates mechanisms by which sorghum RS may assist in the prevention and treatment of obesity. The study also identified positive lipid changes. Triglycerides, total cholesterol and LDL-cholesterol in both the overweight and obese rats consuming sorghum RS-enriched diets were significantly lower than the control groups ($p < 0.05$). HDL-cholesterol levels were significantly higher in the sorghum RS groups ($p < 0.05$). It remains to be seen whether these effects can be translated to the human condition. At this stage, the human studies demonstrate only possible mechanisms but the positive results in animal models identify that whole grain sorghum may be useful in managing energy balance to assist with control of over-weight and obesity.

Effects on glycaemic control

Good glycaemic control is associated with reduced post-prandial glucose peaks and is important in diabetes management⁽¹⁶⁰⁾. Sorghum foods have demonstrated slow starch digestibility *in vitro* and in animal feeding trials, suggesting favorable effects on post-prandial glycaemic and insulinaemic responses in humans. Numerous animal feeding studies have shown that sorghum in the diet effectively improves glucose

metabolism compared to sorghum-free diets^(133; 161-163). A limitation in some of these studies is that the specific type of sorghum extract is not defined, thus it cannot be determined whether effects are linked to phenolic, fibre or macronutrient contents. Furthermore, whether the concentrations of sorghum extracts are physiologically relevant, that is, capable of eliciting these blood glucose attenuation effects in humans after a realistic dose, is not yet clear.

A recent study by Cervantes-Pahm et al.⁽¹⁶⁴⁾ reported on the use of a pig model to investigate the comparative nutrient and energy digestibility of a range of grains widely used for human consumption, including whole grain sorghum. In this study, the apparent ileal digestibility of sorghum starch was lower than for corn⁽¹⁶⁴⁾. The authors attributed this to the high level of resistant starch in sorghum, which appeared to be fully fermented in the pig hindgut since ~100% starch disappearance was reported. The low apparent ileal digestibility of its starch in pigs suggests that sorghum may be of value for reducing the GI of human foods⁽¹⁶⁴⁾. Caution in extrapolating these pig trials to human health is needed, since in this study raw grains were used, whereas in human food the grains are invariably cooked, changing the structure and digestion properties of the starch.

Dixit et al.⁽¹⁶⁵⁾ have even gone as far as to specifically recommend sorghum grain is regularly consumed in the modern Indian diet to assist in the reduction of Type 2 diabetes and cardiovascular disease in this population. Despite the positive recommendation, only four *in vivo* human studies exploring these effects have been reported in the literature, each with limitations and inconsistencies⁽¹⁶⁶⁻¹⁶⁹⁾. The most recent of these human studies investigated the effects of consuming muffins made

from grain sorghum on plasma glucose and insulin levels⁽¹⁶⁹⁾. In a randomised-crossover design, 10 male subjects consumed muffins containing 50 g of total starch (TS) from either grain sorghum flour or whole wheat flour (although the available carbohydrate was not reported), with all additional ingredients the same across both treatments. Glucose and insulin levels were measured at baseline (15 minutes prior to consumption), time-point 0 (onset of consumption) and 15, 30, 45, 60, 75, 90, 120, and 180 minutes after consumption. Additionally, levels of rapidly digestible starch (RDS), SDS, RS and TS in muffins were analyzed. Results indicated that RDS, SDS and RS contents were significantly higher in sorghum muffins compared to wheat muffins ($p < 0.05$). Plasma glucose incremental area under the curve (iAUC) reduced by ~26% and glucose measures at the 45 to 120 minute intervals were significantly lower for the sorghum muffin ($p < 0.05$). Also, plasma insulin iAUC reduced significantly and insulin measures at the 15 to 90 minute intervals were significantly lower for the sorghum muffins ($p < 0.05$), reducing by ~55%. Lack of information on the available carbohydrate in each test muffin is a limitation but this study shows the potential of sorghum-based foods to attenuate blood glucose and insulin responses.

In a similar study, Lakshmi and Vimala⁽¹⁶⁷⁾ also demonstrated that the consumption of whole grain sorghum meals compared with consumption of the same meals based on dehulled sorghum and other recipes prepared with wheat and rice (as controls), resulted in significantly lower glycaemic responses ($P < 0.05$) in 6 subjects with Type 2 diabetes mellitus⁽¹⁶⁷⁾. These observations may have been in part due to the difference in fibre content of the meals that ranged from 2.2 g to 4.8 g in whole grain sorghum treatment meals and 1.8 g to 2.7 g in dehulled sorghum treatment meals. Also, the different cooking methods utilised in the treatment meal recipes (pan-fried,

boiled, fermented-steamed) may have had an effect on starch digestibility and therefore carbohydrate metabolism.

Further glucose control studies by Mani et al.⁽¹⁶⁶⁾ evaluated the GI of six traditional Indian meals, one of which was based on sorghum. The test meals were consumed as baked bread (prepared from flours of sorghum or finger millet or pearl millet) or as pressure-cooked meals (based on kodo millet, consumed *as is* or with added whole mung beans or with added mung bean dal). No fats were added in the preparation of the test meals. Testing was undertaken in 36 subjects with Type 2 diabetes mellitus. Glucose responses were measured one and two hours after consumption of the test foods (50 g available carbohydrate) and compared to a 50 g glucose load. The mean GI of the sorghum bread was relatively high at 77% +/-8 (SE), but not as high as the finger millet bread which had a GI of 104% +/-13 (SE), equivalent to the glucose load. The pearl millet bread had the lowest GI of all six test meals, producing a GI of 55% +/-13 (SE). No significant difference was observed in blood glucose level after each of the test foods at the 1 hour and 2 hour time-points when compared with the corresponding blood glucose response to the 50 g glucose load. The study identifies sorghum's digestibility in this meal format (baked bread) may not be as slow as *in vitro* studies may suggest.

Abdelgadir et al.⁽¹⁶⁸⁾ investigated the influence of six traditional Sudanese carbohydrate-rich meals (prepared from wheat, sorghum, millet and maize flours) on glucose and insulin responses in a randomised crossover design with 10 subjects with Type 2 diabetes mellitus (6 males and 4 females). Millet porridge had the most favorable (lowest) post-prandial glucose and insulin responses followed by wheat

pancakes, then sorghum porridge and sorghum flat bread, whereas maize porridge induced higher glucose and insulin responses (as measured by mean iAUCs). Consideration of the method and time of preparation, particularly the duration of fermentation and the degree of milling, as well as the nature of starch and fibre content is important when interpreting these findings. That is, inadequate reporting of the precise physicochemical properties of the final products limits generalisability in food studies. Overall, the glucose and insulin response studies using sorghum in humans have used small sample sizes (with low statistical power), reducing the likelihood that a statistically significant result reflects a true effect. Hence results are ambiguous and the evidence base is inconclusive.

Effects on serum lipids

Measurement of a standard lipid profile (including total cholesterol, LDL (low-density lipoprotein) cholesterol, HDL (high-density lipoprotein) cholesterol, and triglycerides) is recommended in the prediction of cardiovascular risk⁽¹⁷⁰⁾. Mechanisms for the role of sorghum grain components in cardiovascular protection have been investigated⁽²³⁾, however only one study has been conducted with human subjects. In this study, using 10 males and 6 females, a significant reduction ($p < 0.05$) in total cholesterol, triglycerides and HDL cholesterol was observed after daily consumption of 100 g of unrefined sorghum in the form of pancakes over 3 weeks⁽¹⁷¹⁾. However, the content of subjects' background diets was not adequately reported, making assessment of dietary confounders difficult.

Most of the research identifying beneficial effects of sorghum consumption still lies with animal models^(133; 159; 172-174). Klopfenstein et al.⁽¹⁷⁵⁾ concluded that sorghum

bran was effective in lowering serum and liver cholesterol levels in hamsters. This effect was repeated in another hamster model of hypercholesterolemia, when grain sorghum lipid extract included in the diet significantly reduced plasma non-HDL and liver esterified cholesterol levels while increasing HDL levels⁽¹⁰¹⁾. In all these studies, total cholesterol levels were reduced in animals consuming sorghum-based diets compared to sorghum-free control diets. In a single negative finding, Lee et al.⁽¹⁷⁶⁾ found that while sorghum consumption increased HDL-cholesterol levels, total cholesterol and LDL-cholesterol levels were increased in a rat model. However, the lack of a control group in this research makes conclusions difficult.

Sorghum tannins have not been broadly investigated in relation to their effects on cardiovascular disease risk factors, unlike tannins from some other foods and beverages such as red wine and tea. There may be an anticoagulant effect of sorghum tannins, yet to be tested in humans, as evidenced in cultured mullet fish that were fed tannin-containing sorghum distillery residues⁽¹⁷⁷⁾. The sorghum residue significantly improved blood thinning and erythrocyte membrane integrity of the fish blood cells in cooler water temperatures over the winter months, enabling normal blood viscosity and prevention of red blood cell hemolysis induced by typical oxidation processes. The authors suggest that the antioxidant activity of the tannins and polyphenols present in the sorghum residue contributed to the prevention of red blood cell hemolysis. A translation to human studies has yet to be conducted but overall the experimental evidence suggests that sorghum grain may induce beneficial lipid-lowering effects.

Effects on oxidative stress biomarkers and plasma antioxidant capacity

Oxidative stress is a condition in which an imbalance results between the production and inactivation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the body that contributes to cellular dysfunction and pathogenesis of a variety of human diseases (including atherosclerosis, diabetes, hypertension, aging, Alzheimer's disease, kidney disease and cancer)⁽¹⁷⁸⁾. Oxidative stress is also linked to metabolic syndrome (MetS)⁽¹⁷⁹⁾. A randomised, controlled, crossover human study, involving 22 healthy adults, was conducted to assess the acute effects of consuming pasta containing red or white whole grain sorghum flour (30% sorghum, 70% semolina) on plasma total polyphenols, antioxidant capacity and oxidative stress markers compared to a wheat control made from 100% semolina⁽¹⁸⁰⁾. Compared to baseline, the 2 hour post-prandial levels of plasma polyphenols, antioxidant capacity and superoxide dismutase (SOD) activity were significantly ($P<0.001$) higher following the red sorghum pasta (RSP) meal while the protein carbonyl level was significantly lower ($P=0.035$). Furthermore, net changes in polyphenols, antioxidant capacity and SOD activity were significantly ($P<0.001$) higher while protein carbonyls were significantly ($P=0.035$) lower following consumption of the RSP meal than the control meal. Pasta containing red whole grain sorghum flour, but not white sorghum flour, enhanced antioxidant status and improved markers of oxidative stress in healthy subjects. The increase in plasma polyphenols by the RSP meal may be attributed to its higher content of polyphenols. The potential limitations of this study include the short duration and use of only one postprandial blood collection. Furthermore, subjects in this study were healthy and their results may differ to people with oxidative-stress induced disease such as diabetes and obesity. Studies in subjects with mild to moderate oxidative-stress induced disease and who consume a sorghum-enriched diet daily over an extended period of time are required to further investigate potential effects on anti-oxidant processes from sorghum consumption.

Effects on cell-mediated immune responses

Cell mediated immune responses have been linked to cancer development. Epidemiological evidence dating back to the early 1980s has correlated consumption of sorghum with reduced incidence of oesophageal cancer, warranting closer attention to the potential chemopreventive properties of sorghum chemical components. Data from various sorghum-consuming countries in Africa and Asia has demonstrated lower esophageal cancer incidences compared to regions where wheat and maize were the major cereals consumed⁽¹⁸¹⁻¹⁸⁴⁾. However, contamination of maize in these communities by the *Fusarium* fungi, which convert nitrates to nitrites, known carcinogens, has been identified as a more likely cause of increased rates with maize consumption. Nevertheless, such epidemiological observations have driven research efforts towards understanding potential cell-mediated chemopreventive properties of sorghum grain components and their mechanisms of action not just against esophageal cancer, but other cancers of the gastrointestinal tract and beyond. Currently research is in its infancy, with growing numbers of cancer cell line studies exploring anti-inflammatory, anti-mutagenic and anti-proliferative effects that are important in prevention of carcinogenesis. Some animal studies have also shown that phenolic extracts derived from sorghum, on the basis of high antioxidant activity particularly from red, black and tannin sorghum varieties, have been able to effectively induce cell arrest and suppress tumor growth *in vivo*⁽¹⁸⁵⁾. Many more *in vitro* and animal studies are required before antioxidant effects of sorghum extract, aimed at cancer prevention and treatment can be justified in clinical trials. The role of sorghum consumption in cancer prevention is more likely to be examined in epidemiological studies with mechanistic studies contributing to the discussion on the plausibility of findings.

Table 2.4 Human studies incorporating sorghum-based test meals

Studies of vitamin metabolism

Lead Author/Year	Focus of investigations	Subjects/Experiment	Results
Gopalan et al. (1960) ⁽⁴⁸⁾	Role of amino acid imbalance (relative excess of leucine) in the pathogenesis of pellagra.	13 healthy & pellagrin subjects. 5g dietary leucine administered daily & changes in urinary excretion of N-methyl nicotinamide (NMN) measured.	Leucine increased urinary excretion of NMN in all subjects. Isocaloric replacement of rice by sorghum (jowar) resulted in increased urinary NMN excretion in all patients.
Deosthale et al. (1974) ⁽¹⁸⁶⁾	Sorghum molybdenum (Mo) consumption effects on copper (Cu) & uric acid excretion.	4 adult males (age not specified). Low (0.21 µg/g) & high (1.39 µg/g) Mo-containing grains were used in diets controlled for calories, protein, minerals, sulfur.	Uric acid increased only in high Mo intakes (10-15 mg Mo/day). Urinary Cu excretion was sig.ly increased with increasing levels of Mo. Faecal Cu excretion was unchanged.
Krishnaswamy et al. (1976) ⁽¹⁸⁷⁾	Vitamin B6, leucine absorption.	6 healthy males (25-35 yrs). Metabolic interrelations between excess dietary leucine & vitamin B6 studied.	Vit. B6 counteracted effects of leucine on urinary quinolinic acid excretion, in vitro nicotinamide nucleotide synthesis by erythrocytes; corrected abnormalities of 5-hydroxytryptamine metabolism induced by excess leucine.
Obizoba (1979) ⁽¹⁸⁸⁾	Mineral & vitamin metabolism.	5 healthy women (19-25 yrs). Fed 4 iso-nitrogenous mixed plant protein diets various blends based on whole wheat, navy bean & 3 sorghum flour varieties (Purdue normal, high lysine & Nigeria normal).	Various effects on measured Ca, Mg, Fe, Niacin, riboflavin, folic acid levels were reported & related to the contents in the test diet.
Wang et al. (1991) ⁽¹⁸⁹⁾	Fibre effects on niacin status/niacin utilisation.	10 healthy adult subjects. 28 g per day of ready-to-eat cereal (whole-ground sorghum flour) or cereal from decorticated sorghum flour (bran removed, polished). Urine, stool & fasting blood samples collected.	Whole grain sorghum cereal decreased fecal transit time, lowered urinary NMN excretions, but raised blood serum levels of NMN & nicotinamide when compared to polished grain sorghum cereal.
Schmid et al. (2007) ⁽¹⁴⁹⁾	Dietary intake analysis of mothers & their children in South India.	218 mothers (> 15 yrs) & their children (< 5 yrs) in South India. Comparison of dietary intake of subjects with & without intervention to manage malnutrition.	Mothers had sig. higher intakes of energy & protein in summer, & sig. higher intakes of energy, protein & Fe in rainy season. No differences in children. In mothers, sorghum contributed 29% energy, 33% protein, 53% iron.

Table 2.4 Human studies incorporating sorghum-based test meals (continued...)

Studies of iron (Fe) status/absorption

Lead Author/Year	Focus of investigations	Subjects/Experiment	Results
Derman et al. (1980) ⁽¹⁹⁰⁾	Fe absorption from maize & sorghum.	21 male & female South African subjects, healthy & Fe-deficient. Ages not specified. Study compared thin gruel, sorghum & wheat beers.	Ten times as much Fe was absorbed from the traditional maize & sorghum beer as from gruel made from the same ingredients.
Radhakrishnan et al. (1980) ⁽¹⁹¹⁾	Fe bioavailability.	12 healthy & 13 anemic subjects. Ages not specified. Diets based on high & low tannin sorghum.	In 12 healthy subjects, Fe absorption from the low & high tannin varieties was similar. In 6 anemic subjects, Fe absorption from low tannin sorghum was sig. higher. In the other 7 anemic subjects, there was no difference observed.
Gillooly et al. (1984) ⁽¹⁹²⁾	Fe absorption.	53 Fe-deficient Indian females (age not specified). 6 different experiments. Systematically examined effects on Fe absorption of polyphenol & phytate in sorghum.	When amounts of both compounds were reduced to low levels by pearling, there was a sig. increase in Fe absorption.
Haidar et al. (1999) ⁽¹⁹³⁾	Fe deficiency anaemia (IDA) status.	1449 pregnant & lactating subjects (15-49 yrs) in Ethiopia.	Overall status of IDA determined by hemoglobin level was 18.4 % with higher rates in maize, milk & sorghum staple areas.
Hurrell et al. (2003) ⁽⁵⁴⁾	Fe absorption.	34 males & 44 females (21-38 yrs). Measured the influence of phytic acid degradation on Fe absorption from cereal porridges.	Phytate degradation improves Fe absorption from cereal porridges prepared with water but not with milk, except from high-tannin sorghum.

Cholesterol control

Suhasini et al. (1991) ⁽¹⁷¹⁾	Effect of unrefined sorghum or maize on serum lipids.	6 males & 10 females (23-26 yrs). Grp 1 ate 100g unrefined sorghum. Grp 2 ate 50g of unrefined maize.	Both diets showed sig. reduction in serum total cholesterol & triglyceride levels with simultaneous increase in HDL cholesterol value over 3 weeks.
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Table 2.4 Human studies incorporating sorghum-based test meals (continued...)

Studies of diabetes/glycaemia/oxidative stress

Lead Author/Year	Focus of investigations	Subjects/Experiment	Results
Mani et al. (1993) ⁽¹⁶⁶⁾	Determination of glycaemic index of commonly consumed foods in India.	36 subjects (Type 2 DM). Glucose responses were measured 1 & 2 hrs after consumption of test foods (50g available carbohydrate) & compared to a 50g glucose load. 6 foods tested, 1 based on sorghum.	The mean glycaemic index of the sorghum meal was 77% +/- 8 (SE), indicating a relatively high glycaemic index. No sig. difference was observed in blood glucose level at the 1 h & 2 h time-points when compared with the corresponding reference.
Lakshmi et al. (1996) ⁽¹⁶⁷⁾	Glucose & insulin responses to sorghum-based meals.	3 males & 3 females with Type 2 DM (45-60 yrs). Consumption of whole grain sorghum meals compared with the same meals based on dehulled sorghum & other recipes prepared with wheat & rice.	The consumption of whole grain meals resulted in sig. lower glycaemic responses (P<0.05) in 6 subjects, in part due to differences in fibre content & cooking methods.
Abdelgadir et al. (2005) ⁽¹⁶⁸⁾	Glucose & insulin responses to 6 traditional Sudanese meals from wheat, sorghum, millet & maize flours.	Glucose & insulin responses in a randomised crossover design with 10 subjects with Type 2 diabetes mellitus (6 males & 4 females).	Millet porridge had the lowest post-prandial glucose & insulin responses followed by wheat pancakes, sorghum porridge & sorghum flat bread. Maize porridge induced higher glucose & insulin responses (as measured by mean iAUC).
Poquette et al. (2013) ⁽¹⁶⁹⁾	Glucose & insulin responses to sorghum-based meals.	Randomised-crossover design, 10 males consumed muffins containing 50g of total starch from either grain sorghum flour or whole wheat flour. Plasma glucose & insulin measured over 3 hrs.	Plasma glucose & insulin iAUC reduced by ~26% & 55% respectively. Glucose & insulin measures were sig.ly lower for the sorghum muffins (p<0.05) at different time points.
Khan et al. (2014) ⁽¹⁸⁰⁾	Plasma total polyphenols, antioxidant capacity & oxidative stress responses to sorghum pasta meal.	Randomised-crossover design, 22 males and females consumed pasta containing red or white whole grain sorghum flour (30% sorghum, 70% semolina) or a wheat control made from 100% semolina. One blood collection at 2 h time-point.	Plasma polyphenols, antioxidant capacity and superoxide dismutase (SOD) activity were significantly (P<0.001) higher following red sorghum pasta (RSP) meal & protein carbonyl level was significantly lower (P=0.035) following consumption of the RSP meal than the control meal (wheat).

Table 2.4 Human studies incorporating sorghum-based test meals (continued...)

Fibre studies

Lead Author/Year	Focus of investigations	Subjects/Experiment	Results
Cornu et al. (1981) ⁽⁷⁴⁾	Effect of fibre in sorghum on N digestibility.	12 healthy Cameroonian adult males whose habitual diet is based on a sorghum meal (2.4-4.2 g of crude fibre / 100 g DM). Subjects received successive diets of 3.3, 4.8, 5.4 g of crude fibre / 100 g of DM.	Increased fibre intake resulted in a sig. rise in quantity of fecal matter excreted (N & formic insoluble substances), but a reduction in urinary N losses.
MacLean et al. (1983) ⁽⁶⁷⁾	Effect of decortication & extrusion on the digestibility of sorghum.	9 children (7-24 mo). Sorghum provided 8% protein & 62% carbohydrate (kCal) in diet. Lysine was supplemented to 3% of protein. Casein provided 6.4% protein (kCal) in the control diet.	N absorption from sorghum & control not different but N retention lower than control. Fecal weights & energy losses showed minor differences. Decortication & extrusion improve protein quality & digestibility of sorghum.
Fedail et al. (1984) ⁽¹³⁴⁾	Effect of sorghum & wheat bran on the colonic functions.	10 males (22-24 yrs) healthy Sudanese subjects. Comparative study of normal diet, diet of 20 g/day sorghum bran, & 20 g/day wheat bran, for 3 wks. Wet stool wt, gut transit time, & freq. of bowel evac. noted.	The mean stool weight on normal diet was 136.6 ± 43.1 g/day, on sorghum bran 173.3 ± 48.4 g/day, & on wheat bran 219.1 ± 98.3 g/day (p<0.001). Both brans produced a similar number of bowel evacuations, stool weight & transit time.
Cornu et al. (1986) ⁽¹⁷⁴⁾	Effects of fibre on digestibility of sorghum lipids.	12 healthy Cameroonian adult males whose habitual diet is based on a sorghum meal (2.4-4.2 g of crude fibre / 100 g DM). Subjects received successive diets of 3.3 (A), 4.8 (B), 5.4 (C) g crude fibre / 100 g of DM.	Reduced lipid digestibility occurred in all diets. No difference was observed between A & B fibre diets but dropped with diet C. Lipid losses increased more rapidly than N losses with increasing fibre content. No sig. changes in concentrations of fecal fat.

Table 2.4 Human studies incorporating sorghum-based test meals (continued...)

Protein digestibility studies

Lead Author/Year	Focus of investigations	Subjects/Experiment	Results
Kurien et al. (1960) ⁽¹⁹⁴⁾	Metabolism of N, calcium & phosphorus.	7 boys (10-11 yrs). Effect on metabolism of N, Ca & P of replacing 25%, 50 th or 100% of rice in a poor Indian diet by Sorghum Vulgare was studied. Daily intake of N was constant in all diets.	Protein digestibility coefficients of protein & mean daily N retention diminished as sorghum increased. Sorghum led to: 1) higher Ca intake, but Ca retention decreased 2) higher P intake, which resulted in higher P retention.
Nicol et al. (1978) ⁽¹⁹⁵⁾	Utilisation of protein in cassava, rice & sorghum (Sativa) based diets	19 Nigerian men, 13 different feeding trials, each of 6 men. Net protein utilisation (NPU) of diets based on rice, sorghum or cassava, was compared to a minimal protein diet. Endogenous N excretion measured.	The NPU of a diet based on home-pounded, winnowed sorghum flour was higher than that of a diet based on milled whole-meal sorghum due to the low digestibility of the latter diet.
MacLean et al. (1981) ⁽¹⁵¹⁾	Protein quality & digestibility of sorghum	13 children (6-30 mo). Protein quality & digestibility of 2 high lysine (2.9-3.0 g/100 g protein) & 2 conventional varieties (lysine content 2.1-2.2 g/100 g protein) of whole grain sorghum milled were assessed.	Weight loss or poor weight gain was reported. No difference by variety in N absorption or retention. Stool weight & energy loss 2.5-3x control values. Total conc. essential amino acids was low as were conc. Lys & Thr. Lys was the limiting amino acid.

Table 2.4 Human studies incorporating sorghum-based test meals (continued...)

Therapeutic food studies

Lead Author/Year	Focus of investigations	Subjects/Experiment	Results
Weaning foods			
Dibari et al. (2013) ⁽¹⁹⁶⁾	Acceptability/safety of new ready-to-use therapeutic foods (RUTF) before use.	41 HIV/TB patients (>18 yrs) in Kenya. Cross-over RCT comparing soy/maize/sorghum RUTF (SMS-RUTFh) to control - 10 d measures of product intake.	SMS-RUTFh is acceptable & can be safely clinically trialed, if close monitoring of vomiting & nausea is included.
Bisimwa et al. (2012) ⁽¹⁹⁷⁾	Fortified soybean-maize-sorghum paste Vs fortified corn soy blend porridge in underweight infants.	6 mo Congolese infants randomly assigned to lipid-based ready-to-use complementary foods (RUCF, n=691) or fortified corn soy blend (UNIMIX, n=692) for 6 mo. Hemoglobin, triglyceride, & cholesterol noted.	No sig. differences in the concentrations of hemoglobin, serum triglyceride, & serum cholesterol were found between the 2 grps.
Human oral rehydration solutions based on sorghum			
Mustafa et al. (1995) ⁽¹⁹⁸⁾	Oral rehydration therapy for acute diarrhea using cereal-based solutions.	96 (children (6-40 mo) in Sudan. Comparative RCT (32 rice, 34 sorghum, 30 control). Safety & efficacy of rice or sorghum cereal-based oral rehydration solutions (ORS) relative to std. WHO ORS formulation.	Cereal-based ORS shortened the duration of diarrhea, reduced stool vol. & the freq. of diarrhoea & vomiting, & the mean total ORS intake. These effects were more marked with the sorghum-based ORS than with the rice-based ORS.
Molla et al. (1989) ⁽¹⁵²⁾	Food-based oral rehydration solution (maize, millet, wheat, sorghum, rice, potato).	266 children (1-5 yrs), history of acute diarrhea for ≤ 48 h. Digestibility of food-based ORS was assessed by stool pH, glucose content before & after acid hydrolysis & osmolality.	The mean stool output over the first 24 h in std ORT was sig. higher than food-based ORT. Food-based ORT showed substantial reduction in stool output.
Pelleboer et al. (1990) ⁽¹⁹⁹⁾	Oral rehydration therapy for acute diarrhea.	64 Nigerian children (2.5 mo-5 yrs). Comparative RCT - subjects consumed either the WHO recommended oral rehydration solution (WHO-ORS) or a solution, containing 60 g/l sorghum powder.	No sig. differences in amt. of fluid used, no. of stools & duration of diarrhea. No sig. difference in weight gain. 7 children died, 2 (6%) in the sorghum-ORS grp & 5 (17%) in the WHO-ORS grp. Sorghum-ORS was well accepted & tolerated.

Table 2.4 Human studies incorporating sorghum-based test meals (continued...)**Therapeutic food studies**

Lead Author/Year	Focus of investigations	Subjects/Experiment	Results
<i>Effects on immune status in people with HIV</i>			
Motswagole et al. (2013) ⁽²⁰⁰⁾	Effects of a sorghum meal on the immune status of adults with HIV.	132 HIV+ adults. Double-blind randomised placebo-controlled trial in Botswana. Micronutrient fortified sorghum meal including Vit. A (n=67) or control (n=65). Serum retinol, Fe, Zn, albumin, CD4 cell count & HIV viral load assessed over 12 mo.	Fortified sorghum meal did not influence serum retinol, CD4 cell count & HIV viral load.
Ayuba et al. (2014) ⁽²⁰¹⁾	Effects of supplementation with sorghum herbal preparation (Jobelyn®) on immune status.	61 HIV+ adults in Nigeria – 2 trials. 10 HIV+ patients not receiving antiretroviral therapy (ARVT). Patients consumed 500 mg Jobelyn® daily for 8 wks. Control with 51 HIV+ patients receiving ARVT.	Consumption of Jobelyn® contributed to improved hemoglobin levels & increased CD4 cell counts HIV+ patients.

Consumer/sensory studies

Kayitesi et al. (2010) ⁽⁵²⁾	Consumer opinions of marama /sorghum composite porridge.	30 males & 22 females. Descriptive sensory analysis, consumer testing, texture analysis, pasting & color.	The 100% sorghum porridge & the composite porridge with full-fat flour were the most acceptable to consumers.
Vazquez-Araujo (2012) ⁽²⁰²⁾	Consumer input for developing human food products made with sorghum.	Adults n=34 focus grps; n=1002 national survey; n=160 conjoint analysis	Heath aspects of grain products were the most appealing for consumers, whereas conjoint analysis showed that sensory attributes were the principal drivers for purchase intent.
Muhihi et al. (2013) ⁽²⁰³⁾	Sensory study: sorghum ugali (stiff porridge).	Overweight & obese Tanzanian adults. Pre- & post-tasting questionnaires were administered. A 10-point LIKERT scale used to rate attributes of 3 test foods. Sorghum ugali was consumed by 23% of participants.	All of the test foods were highly rated for smell, taste, color, appearance & texture. Taste was rated highest for unrefined maize ugali. Whole grain carbohydrates are highly acceptable.

2.4 Conclusions

There is an emerging body of scientific literature on sorghum (*Sorghum bicolor*), an underutilised cereal whole grain, that may provide health benefits and contribute to the prevention of chronic lifestyle related diseases, particularly in regions where associated morbidity and mortality rates are significant public health burdens⁽²²⁾. It appears that sorghum grain components may have an impact on metabolic disease processes through the delivery of slowly digestible starches, resistant starch, dietary fibre, polyphenols (including phenolic acids, flavonoids and condensed tannins), policosanols, unsaturated fatty acids and the food attribute of a high antioxidant capacity. However, the vast majority of studies available for consideration utilised extracts or purified compounds and were conducted in animal models. Few studies in humans have been reported, and there is a need to study sorghum as a whole grain in whole food formats, and in the context of a healthy diet. After all, food is complex, comprised of a myriad of compounds that act synergistically to elicit food effects⁽²⁰⁴⁾, which are further impacted and altered by processing⁽²⁰⁵⁾.

Substantiating potentially beneficial effects of sorghum foods on chronic lifestyle related disease risk factors requires human studies whereby consistent results from randomised controlled trials (RCTs) are reported, contributing to the highest level of evidence for practice⁽¹⁸⁵⁾. To date there appears to be fewer than five RCTs investigating sorghum metabolic disease-related effects in humans, and all interventions are short-term and have some experimental design shortcomings. Future RCTs should aim to directly examine a specific effect on chronic disease biomarkers or health outcomes between a control and sorghum-intervention diet, for a minimum of 3-6 months, enabling evidence for longer-term effects to emerge⁽¹⁸⁶⁾.

Specifically, studies should investigate lipid profiles, longer-term markers of glycaemic control, and body weight. While much of the research to date focuses on extracts and components, the impact of the whole food reflecting the synergy between the components needs to be considered (Chapter 3). It is also important to study the content of the background diet in these RCTs as background diet can confound results and may interfere with the ability to attribute effects to the dietary variable of interest⁽²⁰⁶⁾. In addition, for translation to practice, the impact of sorghum-based foods on chronic lifestyle related disease must be seen in the context of the whole diet, carefully monitored throughout trials (Chapter 3).

The evidence for a relationship between the antioxidant activity of sorghum and health benefits of its consumption is of particular importance due to the role oxidative stress plays in chronic disease development. To date, several *in vitro* and animal model studies have highlighted the potential of sorghum grain components, such as polyphenols, to scavenge free radicals⁽²⁰⁷⁻²¹¹⁾. Unfortunately, these studies are limited in their ability to attribute direct antioxidant effects of sorghum, as they do not account for metabolic transformations and interactions that influence bioavailability and biological activity of the polyphenols in the body after ingestion. For example, it is unclear what transformations polyphenols undergo, from the oral cavity, through the gastrointestinal tract and after absorption and metabolism⁽²¹²⁾. Thus, test regimens from such *in vitro* and animal studies need to be repeated in humans with mild to moderate oxidative-stress induced disease. Disease indicators, such as oxidative stress and inflammatory markers, can be measured in subjects who consume a sorghum-enriched diet daily over an extended period of time.

Finally, while the development of an evidence base for a link between sorghum consumption and chemoprevention is in its infancy, some evidence for strong antioxidant and anti-inflammatory effects that may mitigate cell proliferation, mutagenesis and carcinogenesis has been reported. Cell-line and animal studies have demonstrated the potential for such cell-mediated effects, but require substantiation in humans. Determining whether the concentration of sorghum grain extracts used in these studies may be feasibly consumed through dietary intake of sorghum-based foods is critical. Notwithstanding, the study of food effects on cancer is highly complex and clinical trials are problematic for ethical reasons. Even so, knowledge generated through various forms of experimental research adds to a general understanding of how the sorghum food matrix may be beneficial.

Overall, the scientific literature exposes a range of health areas in which nutritional effects of sorghum have been examined, such as energy balance, glycaemic control, lipids, gut microbiota, and cell-mediated immune responses, including antioxidant and anti-inflammatory effects. However, a paucity of human studies is evident. Therefore, researching effects of sorghum consumption on indicators of metabolic disease, such as weight, is warranted and elements of study designs from the reported literature were incorporated into the experimental components of this thesis.

CHAPTER 3:
Methodology

3.1 Overview of methodological framework

This thesis provides a scientific framework to investigate the potential effects of sorghum consumption on specific health outcomes in humans. Importantly, this is food-based research that must be considered within the context of a whole diet and hence “the assumption that in most cases the many substances in food have additive or more than additive effects on health”⁽²¹³⁾. To take this further, this concept suggests that food effects are the fundamental unit in nutrition, rather than their food constituents⁽¹⁴⁸⁾. Within the framework of this thesis, sorghum as a whole grain cereal food with inherent properties (nutrients, dietary fibre and other bioactive components) is considered with the knowledge that these individual components interact within the food matrix to demonstrate potential protective effects against chronic diseases in humans (Figure 3.1).

This holistic approach results in nutrition science that focuses on the interdependency of nutrients/components and their food matrix within a whole diet and the “additive” effects they have on health outcomes when consumed over longer periods of time. Therefore, while observed effects within this thesis are reviewed in the context of individual components to understand mechanisms of action, they must be evaluated more broadly with outcomes assigned to the whole sorghum food rather than isolated sorghum components. Specifically, research examining sorghum intake and chronic disease requires clinical studies to elucidate the potential protective roles of the sorghum grain as a constituent of foods within a whole diet. This thesis reviews the potential active ingredients within the sorghum food products based on existing literature identifying possible mechanisms associated with progression of metabolic disease, especially weight control (for example, mechanisms involving

slowly digestible starches and polyphenols). Body weight regulation and associated inflammatory and oxidative changes are particularly relevant in chronic disease prevention. Studies were therefore designed to examine potential mechanisms of food intake regulation, acute oxidative changes, effects of chronic consumption on markers of metabolic disease such as body weight, serum cholesterol and a range of inflammatory markers (Figure 3.1).

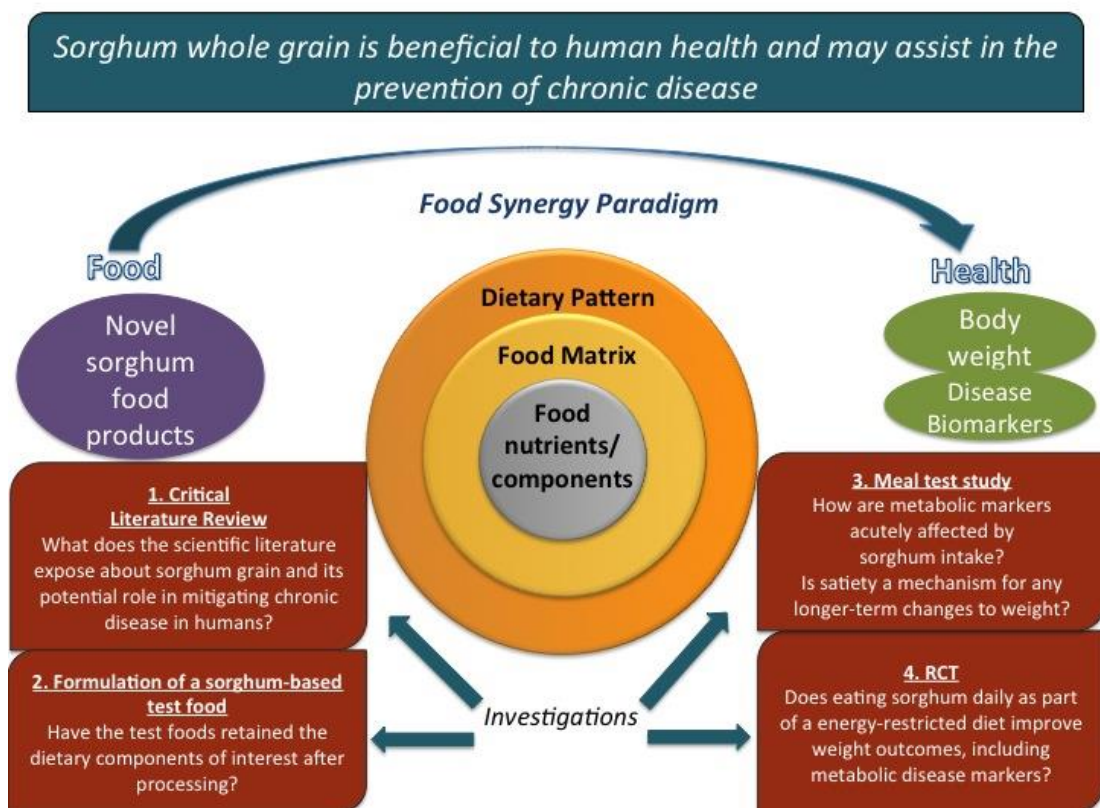


Figure 3.1 Conceptualisation of thesis framework

Sorghum whole grain has slow *in vitro* starch digestibility and some varieties are high in dietary fibre and rich in polyphenolic compounds that may contribute to effects on body weight outcomes and other weight-related clinical indices when it is consumed regularly as part of the human diet (Chapter 2). Food intake is a key

regulator of body weight and so examination of satiety-enhancing mechanisms, which may assist in reducing energy intake is an important component of investigations related to weight control. This may include changes to glucose, insulin and appetite-related gut hormone levels in response to altered intestinal changes that may occur post-prandially. Initially, evaluating subjective measures (subjective ratings of satiety sensations) together with food intake data, informed further studies aiming to identify any positive outcomes from longer-term weight loss interventions, such as body weight change. Collectively, the clinical investigations in this thesis explored the acute- and longer-term effects of sorghum consumption, adding to the *proof of concept* that eating foods made from whole grain sorghum may assist in the prevention of chronic disease, in part due to mechanisms associated with positive changes to body weight.

Well-designed clinical human trials, such as *meal test studies* (or mechanistic human studies) and *intervention trials* (or randomised controlled trials - RCTs), are important in scientific nutrition research. Mechanistic studies are highly controlled, laboratory-based experimental investigations that aim to expose mechanisms of action related to a specific component in food. On the other hand, RCTs involve randomisation of the study sample to a treatment or a control group to measure the effect of the treatment, thereby providing direct evidence of exposure to the dietary factor of interest⁽¹⁹⁴⁾. The existing knowledge on sorghum whole grain was found to be reasonably comprehensive and sorghum consumption appeared to have the potential to assist in the prevention of chronic disease. This knowledge formed the basis on which hypotheses for clinical investigations within the thesis could be developed. Food product formulation and careful characterisation of the test

foodstuff ensured that any effects associated with consumption of sorghum-based foods would be carefully considered. An acute mechanistic meal test study provided the basic understanding of the types of sorghum components that might be involved in altering health outcomes, as well as their mechanisms of action. Finally, a RCT provided direct evidence for the effects of *chronic* sorghum consumption on health outcomes.

3.2 Key hypothesis and study aims

The central concept underpinning this thesis is –

“Sorghum is a viable alternative to more commonly consumed whole grain cereals in the human diet and may have positive benefits on factors associated with metabolic health including weight management.”

To test this hypothesis, research (Figure 3.1) was undertaken with the aims of:

1. Elucidating the functional components of sorghum reported in the scientific literature with potential effects on human metabolic processes.

(Chapter 2: Nutritional composition of sorghum and evidence of its effects on health)

2. Formulating an optimised sorghum-based test product that contains the nutritional and chemical properties hypothesised to elicit clinical effects.

(Chapter 4: Formulation of a ready-to-eat sorghum flaked cereal food)

3. Investigating effects on proposed mechanisms associated with weight control and inflammation such as acute satiety, related hormonal responses and antioxidant potential in an acute meal test study.

(Chapter 5: Acute meal test study testing short-term effects of sorghum consumption, specifically targeting satiety mechanisms)

4. Testing for effects of functional components of sorghum foods within a controlled clinical trial over a longer time-frame with body weight and associated disease marker changes as outcomes.

(Chapter 6: Randomised controlled trial testing effects of chronic sorghum consumption, specifically targeting body weight outcomes)

3.3 Measurement Issues

Measurement of outcomes from food consumption is pivotal in this thesis. This considers how food “works” or impacts on both health and disease. Specifically, how whole grain sorghum consumption might benefit health and assist in chronic disease prevention is evaluated. To better understand the food/health/disease relationship it is important to consider the key factors of the “spectrum of life” – beginning with “healthy” and ending with “death”, intercepted by the appearance of disease risk factors and the diagnosis of disease along that continuum. This thesis focuses somewhere in the middle of the spectrum, to identify effects of sorghum food consumption on disease risk factors in the aim of evaluating its potential to assist in the prevention of chronic disease development in the longer term. To this end, the measurement of key indicators of disease risk was necessary, including measurement of body weight and body composition, as well as biochemical markers that expose

the status of different systems or processes in the body, such as the cardiovascular system (lipids), glycaemic regulation (glucose/insulin), inflammatory pathways and oxidative stress. These are linked to the progression of disease if they are not maintained within “healthy” reference ranges.

However, food consumption is intricately associated with human behaviour. There are both biological cues (for example, appetite-regulating gut peptides) and non-biological cues (environment, emotions, habits) that influence the food choices people make. Since satiety may be a mechanism of action driving body weight changes through altered energy intake, and since satiety encompasses both biological and behavioural drivers of food intake, it logically follows that measurement of specific satiety markers are also useful in studies measuring body weight outcomes. Beginning with behavioural aspects of food intake, the satiety markers will be discussed first in the next section, followed by a review of the major methodological issues related to the measurement of food intake in clinical trials. Finally an overview of the disease markers measured in this thesis will be presented.

3.3.1 Measuring Satiety, Satiation and Appetite

Satiety and satiation are processes that affect eating behaviour. Satiety is a feeling/sensation that influences the interval between meals or episodes of eating⁽²¹⁴⁾. Satiation occurs during the course of a meal and leads to a reduction or cessation of eating, and determines meal size⁽²¹⁵⁾. Collectively, satiety and satiation processes help to control the size of eating episodes and the frequency of eating. Investigating foods that have the ability to accelerate satiation, suppress hunger, extend satiety and reduce overall appetite are important for obesity/appetite researchers and for food

manufacturers designing innovative products that may assist with dietary control and weight management.

Studies assessing satiety, satiation and appetite commonly utilise a combination of measures. More objective assessments include gut hormone concentrations, gastric distention and emptying, brain activity and, potentially most related to outcomes, food intake. Although more objective in nature, these assessments can only be regarded as biomarkers or indicators, namely, because appetite cannot be directly measured using a specific test and we may consume food independent of our level of appetite. In particular, biomarkers for satiety measurement are of interest due to their potential to expose putative mechanisms of action⁽²¹⁶⁾. On the other hand, the subjective assessment of satiety (aiming to assess the desire-to-eat prior and in response to meals) is generally measured by “the magnitude or duration of changes in subjective ratings of appetite-related sensations”⁽²¹⁷⁾, often using Visual Analogue Scales (VAS) (Section 3.3.1.3).

Generally, biochemical analyses of hormone concentrations *plus* subjective measurements of appetite *and* food intake data are employed to evaluate satiety and appetite in clinical trials, particularly in laboratory-based meal test studies. This precise *suite* of assessments was selected to study sorghum’s potential satiety effects in the clinical trials conducted within this thesis.

3.3.1.1 Biochemical Measurements of Satiety: Gut Hormones

Gut hormones, including ghrelin, leptin, cholecystokinin (CCK), peptide-tyrosine-tyrosine (PYY), glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide

(GIP), are postulated to be part of the hypothalamic-gut-axis (or gut-brain-axis) that appears to be important in appetite and body weight regulation. The gut-brain-axis modulates short-term satiety and hunger responses to regulate the delivery and transit of nutrients through the gastrointestinal tract, enabling efficient digestion and storage of energy⁽²¹⁸⁾. Furthermore, the axis plays a role in glucose regulation, adipocyte function and energy expenditure, to ensure energy homeostasis after a meal⁽²¹⁹⁾. The precise mechanisms by which this axis controls food intake and eating behaviour are complex, and therefore beyond the scope of this review. However, evidence for the effects of gut hormones on appetite sensations and food intake that were evaluated in the meal test study conducted in this thesis, are reviewed here (namely, ghrelin, PYY, GLP-1, GIP). Glucose and insulin are also commonly investigated in meal test studies and their involvement in mechanisms that regulate appetite will also be discussed. It should be noted that leptin and CCK were not evaluated in this thesis as they are not the best fit for acute appetite investigations⁽²²⁰⁻²²³⁾.

Ghrelin

Ghrelin, an acylated 28-amino-acid peptide, plays an important role in the regulation of food intake. It is an endogenous ligand of the growth hormone secretagogue receptor type 1a (GHS-R1a). Ghrelin is primarily secreted by the X/A-like cells of the stomach⁽²²⁴⁾, and is also produced in low levels by the hypothalamus and by most peripheral tissues. As such, ghrelin may have both endocrine and paracrine effects. Ghrelin exists as both acylated and desacylated forms, with acylated ghrelin being more active. In this thesis, acylated ghrelin was assessed. The major biological functions of ghrelin include the secretion of growth hormone, the stimulation of

appetite and food intake, the modulation of gastric acid secretion and motility, and the modulation of the endocrine and exocrine pancreatic secretions.

Ghrelin is the only circulating orexigenic peptide that stimulates hunger and food intake in both animals and humans^(221; 225-227). Both central and peripheral administration of ghrelin in rats has been shown to induce food intake and reduce energy expenditure accounting for body weight increases^(221; 228; 229). In a study with healthy humans, intravenous infusion of ghrelin led to a 30% increase in food intake⁽²²¹⁾, however the dose was above physiological levels. Ghrelin is secreted in a pulsatile manner with highest concentrations measured during the fasted state and before the onset of meal consumption⁽²²⁷⁾. After eating, ghrelin levels drop relative to energy intake in people of normal weight⁽²³⁰⁾, but not as much in obese individuals⁽²³¹⁾. Concentrations remain low for approximately 1-1.5 hours, particularly following a mixed-macronutrient meal^(221; 232). This pulsatile secretion of ghrelin suggests that it may act as a signal for meal initiation and termination and has been directly correlated with hunger scores⁽²³³⁾, but again not in every case⁽²³⁴⁾.

The precise mechanisms underlying ghrelin's effects on hunger are not completely understood, however there is some evidence that ghrelin increases GI motility⁽²³⁵⁾, thereby increasing gastric emptying that consequently may reduce satiety. Further, elevated ghrelin levels have been inversely correlated with insulin levels, adding support to the association between high ghrelin levels and enhanced hunger⁽²³⁶⁾. However, ghrelin may be more important in long-term energy balance with reports showing that ghrelin levels are inversely correlated with body weight and they rise after weight loss^(237; 238). For example, in obese individuals, ghrelin is chronically

lowered compared to lean individuals⁽²³¹⁾, and rodents fed high fat diets have been shown to develop ghrelin resistance⁽²³⁹⁾. This suggests that ghrelin is down-regulated in a state of excess energy, acting as a longer-term moderator of body weight particularly through its interaction with leptin. In this light, ghrelin is less likely to have a role in meal initiation in obese subjects⁽²⁴⁰⁾.

The specific effects of ghrelin appear to be inconsistent and its underlying mechanisms of action are not fully elucidated. However, it is clear that ghrelin plays a role in meal initiation in lean individuals as well as longer-term weight management, justifying its use in satiety research. Still, when interpreting experimental study results, it is important to consider that the actual peaks of ghrelin concentrations may be related to non-physiological functions such as meal patterns and habitual diet, particularly in light of evidence showing that ghrelin levels may rise in anticipation of eating rather than actual feeding or inter-relationships with other hormone actions⁽²⁴¹⁾.

PYY

PYY is a 36 amino acid peptide that is produced and primarily released by the distal L cells of the gut, in particular from the ileum, colon and rectum⁽²⁴²⁾. It exists predominantly as two bioactive forms: PYY₃₋₃₆ and PYY₁₋₃₆. PYY₃₋₃₆ is the primary circulating active form and is produced when PYY₁₋₃₆ is cleaved by the enzyme dipeptidyl peptidase-4 (DPP-IV). PYY₃₋₃₆ levels are low in the fasting state and rise after food intake, for several hours, in proportion to the amount of energy ingested and especially after a protein-rich or high-fat meal⁽²⁴³⁾. For the purposes of this thesis, the generic term PYY will be used.

PYY has been shown to have strong anorectic properties when it is peripherally administered in both rodents and humans⁽²⁴⁴⁻²⁴⁷⁾. In a seminal *Nature* paper, Batterham and colleagues⁽²⁴⁸⁾ demonstrated a reduction in food intake and weight gain in rats when they received peripheral PYY injections. They also showed in humans that the infusion of normal postprandial concentrations of PYY significantly decreases appetite sensations (measured by VAS) and reduces food intake by 33% over 24 hours.

The postprandial elevation of PYY and thus the inhibition of feeding have been attributed to a gut–hypothalamic pathway. Specifically, altered neural activity mediated through the hypothalamus and the vagus nerve may be responsible for PYY’s anorectic effects⁽²⁴⁹⁾. PYY may also promote satiety through activation of the ‘ileal brake reflex’ that occurs when dietary macronutrients, particularly fats and carbohydrates, arrive in the ileum exposing it to an unusually high intraluminal nutrient content⁽²⁵⁰⁾. This reflex acts as a negative feedback mechanism inhibiting the emptying of nutrients from the stomach, essentially slowing gastric emptying and inhibiting transit of nutrients through the duodenum and jejunum, and as such placing a “brake” on hunger and food intake⁽²⁵¹⁾. Furthermore, PYY may regulate food intake through interactions with other hormones rather than acting alone, further highlighting the complexity of this regulatory system. For example, in a crossover study, participants ate 27% less at a buffet lunch after receiving an infusion of PYY₃₋₃₆ plus GLP-1, compared to infusions based on either PYY₃₋₃₆ or GLP-1 alone, or a saline control⁽²⁵²⁾.

There is a growing body of human studies that support PYY's role in reducing appetite and enhancing satiety. In both lean and obese subjects, blunted post-prandial rises in PYY have been reported, resulting in the reduction of food intake by approximately 25-30% at a subsequent buffet meal^(248; 249). In fact, there may be a dose-dependent decrease in food intake^(230; 246). When Degen et al.⁽²⁴⁶⁾ administered varying doses of PYY to subjects, the highest dose resulted in a 35% reduction in the amount of food eaten at a subsequent meal. However, the dose was not matched to physiological concentrations and was associated with adverse effects such as nausea, possibly confounding results. On the other hand, some research groups have not found a corresponding decrease in energy intake with exogenous PYY infusions⁽²⁴⁷⁾.

Obese individuals are more likely to have lower endogenous PYY levels, with fasting PYY levels shown to inversely correlate with body mass index (BMI)⁽²⁴⁹⁾; but not in every case⁽²⁵³⁾. Despite these inherently lower levels, obese subjects can still be susceptible to the anorectic effects of PYY when it is administered intravenously, in contrast to the reduction of leptin sensitivity that has been seen in obese individuals⁽²⁴⁹⁾. Furthermore, with sustained weight loss these levels may actually increase in the circulation⁽²⁵⁴⁾. Interestingly, exaggerated post-prandial PYY rises occur following Roux-en-Y gastric bypass surgery⁽²⁵⁵⁾, suggesting that this increased response may have an important role in the initial weight loss noted by this surgical treatment for obesity⁽²⁵⁶⁾. These variations in post-prandial circulating PYY levels and the associated anorectic effects, have stimulated strong research interest in the development of PYY-based therapies for the treatment of obesity. From a therapeutic food perspective, isolating ingredients/foods with the ability to continually stimulate PYY release is particularly interesting given that repeated PYY infusions and

injections have been shown to reduce food intake and body weight effectively in rodent models^(248; 257).

Although studies are not in complete agreement with regards to the precise effects of food intake on post-prandial PYY concentrations and there is some variation in reported levels, there is sufficient experimental evidence that demonstrates PYY's appetite-suppressing properties. This variation is more likely to again reflect the complexity of gut hormone mechanisms and actions as they relate to appetite regulation and food intake. Therefore, PYY remains an important hormone to study in appetite research.

GLP-1 and GIP

GLP-1 and GIP belong to the *incretin* gut hormones that potentiate insulin secretion after meal ingestion in a glucose-dependent manner. Overall, these peptides regulate islet hormone secretion, glucose concentrations, lipid metabolism, gut motility, appetite and body weight and immune function.

GLP-1 is formed from the cleavage of the preproglucagon precursor⁽²⁵⁸⁾ and exists in the circulation as two major bioactive forms GLP-1₇₋₂₆ and GLP-1₇₋₂₇. In this thesis, the generic term GLP-1 will be used. GLP-1, like PYY, is secreted from the L-cells of the gastrointestinal tract within minutes of food intake, in response to neural stimulation (via the vagus nerve)⁽²⁵⁹⁾ and in proportion to energy intake, especially glucose ingestion⁽²⁶⁰⁾. GLP-1 effects its actions through the GLP-1 receptor.

The key incretin effects attributed to GLP-1 include increased insulin release, in a glucose-dependent manner; decreased glucagon secretion and decreased gastric emptying⁽²⁶¹⁾. As such, GLP-1 has an important role in attenuating post-prandial glucose rises. GLP-1 is also involved in the “ileal break reflex” and has an anorectic effect on appetite⁽²⁶²⁾. Peripheral and central administration in animals has resulted in reduced food intake⁽²⁶³⁾. Similarly, in humans reduced food intake has been reported when GLP-1 is peripherally administered⁽²⁶⁴⁾, both at high and normal physiological levels⁽²⁶⁵⁾. Therefore, GLP-1 is likely to play an important role in the regulation of satiety.

In individuals with Type 2 diabetes, the incretin effect appears to be diminished, namely through decreased postprandial GLP-1 secretion⁽²⁶⁶⁾. Thus, the incretin properties of GLP-1 have been of particular interest to researchers in the treatment of Type 2 diabetes. Specifically, medications have been formulated as GLP-1 receptor agonists to improve glycaemic control and promote weight loss, especially because the glucoregulatory properties of GLP-1 remain functional in insulin resistant individuals⁽²⁶⁷⁾. A meta-analysis demonstrates that GLP-1 receptor agonists lead to weight loss in obese patients with and without Type 2 diabetes⁽²⁶⁸⁾. In this study, improvements to blood pressure and total cholesterol were also reported, however it is unclear whether these were related directly to weight loss effects rather than the GLP-1 receptor agonists themselves. Astrup et al.⁽²⁶⁹⁾ demonstrated sustained weight loss and improved metabolic disease markers over a two-year period when participants took a once-daily GLP-1 analogue called Liraglutide. On the other hand, and as may be expected, antagonists to GLP-1 have been shown to increase food intake and body weight⁽²⁷⁰⁾.

GIP is a 42 amino acid peptide that is produced predominantly in the duodenal K cells in the proximal small intestine. GIP effects are actioned via the GIP receptor (GIPR). GIP secretion, as for GLP-1, is stimulated by nutrient intake, with low circulating GIP levels in the fasted state that rapidly rise within minutes after food ingestion. The key physiological functions of GIP include: stimulation of intestinal glucose transport; release of insulin from pancreatic beta cells in the presence of glucose to facilitate nutrient storage⁽²⁷¹⁾; and uptake of glucose by adipocytes in an insulin-mimetic fashion⁽²⁷²⁾. Specifically, GIP promotes fat deposition through increased lipogenesis (via stimulation of lipoprotein lipase activity), enhanced fatty acid synthesis and incorporation into triglycerides, and down-regulation of glucagon-stimulated lipolysis⁽²⁷²⁾.

Although GLP-1 is decreased in individuals with Type 2 diabetes, GIP secretion from entero-endocrine cells appears to be normal or slightly elevated. However, impaired pancreatic β -cells appear to cause the insulinotropic effects of GIP and are responsible for the reduced incretin effects in these individuals⁽²⁷³⁾.

The dynamics of postprandial incretin secretion in diet-induced obesity are not clearly defined. GIP secretion is either increased⁽²⁷⁴⁾ or unaffected⁽²⁷⁵⁾ and GLP-1 secretion appears to be decreased⁽²⁷⁴⁾ or unchanged in obese, non-diabetic individuals. Interestingly, it has been shown those mice lacking the GIPR are resistant to diet-induced obesity⁽²⁷⁶⁾, although it is unclear if incretin secretion is affected by obesity *per se* or the high-energy diets that contribute to obesity. Nevertheless, the incretins are important biomarkers to monitor in studies investigating weight outcomes, including appetite research.

Glucose and Insulin

Glucose is the body's primary fuel source, exclusively for the brain, and insulin acts to control blood glucose levels in response to food ingested, especially carbohydrates. Both glucose and insulin appear to be involved in mechanisms of energy homeostasis, however the role of postprandial blood glucose and insulin levels in the regulation of short-term appetite in humans is less clear. Low blood glucose levels prior to eating a meal have been postulated as a cue to meal initiation but links to satiety remain weak⁽²⁷⁷⁾. On the other hand, higher levels of blood glucose have been associated with higher subjective satiety⁽²⁷⁸⁾ and thus meals that elicit a higher postprandial glycaemic response are expected to suppress short-term appetite to a greater extent than those with a slower glycaemic response. This is based on the *glucostatic theory* that links elevated blood glucose with *fullness and* low blood glucose levels with *hunger*⁽²⁷⁹⁾. Based on similar theories, higher insulin levels have also been associated with increased feelings of satiety.

Conversely, the *glycaemic index theory* proposes that attenuated post-prandial blood glucose and insulin levels are related to higher subjective satiety and feelings of fullness, such that foods with a low glycaemic index (GI) are more effective at alleviating hunger⁽²⁸⁰⁾. The rationale for this can be explained by the notion that low-GI foods are characterised by a slower rate of digestion and absorption, which in turn stimulate nutrient receptors in the gastrointestinal tract for a longer period of time, resulting in prolonged feedback (through satiety signals) to the hunger/satiety centre in the brain. However, neither the *glucostatic* nor the *glycaemic index theories* are able to describe the interrelationships between glucose, insulin and satiety with certainty, and there is even some suggestion that glucose and insulin may not be directly involved in appetite regulation.

A meta-analysis of meal test studies suggested that insulin, but not glucose, is associated with short-term appetite regulation in healthy, normal weight participants, but this relationship was not obvious in overweight and obese subjects⁽²⁸¹⁾. The authors concluded that the postprandial insulin response may be an important satiety signal, and that central nervous system insulin resistance in overweight might explain the blunted effect on appetite. However, Holt et al.⁽²⁸²⁾ found a significant negative association between individual insulin and satiety AUC responses to four test foods (containing 50 g of available carbohydrate as ordinary and quick-cooking rice and high- and low-amylose puffed rice), suggesting that increased rate of starch digestion and corresponding higher insulin responses are associated with lessened satiety, or greater feelings of hunger.

Interestingly, Raben et al.⁽²⁸³⁾ found that the consumption of a low GI raw potato starch meal that was high in resistant starch resulted in lower reported satiety and fullness compared with a highly digestible pre-gelatinised potato starch meal. Furthermore, the lower levels of satiety after the low GI/high resistance starch meal were concomitant with lower levels of GIP and GLP-1⁽²⁸³⁾. A more recent randomised crossover trial (involving 26 overweight or obese adults who received four diets: high GI/high carbohydrate; high GI/low carbohydrate; low GI/high carbohydrate; low GI/low carbohydrate at four different visits) showed that by reducing the GI or carbohydrate content of mixed meals, postprandial glycaemia and insulinaemia were also reduced over the course of a day⁽²⁸⁴⁾. However, there was no difference in subjective hunger and satiety ratings between the various diets, regardless of GI or carbohydrate content.

Insulin is therefore more likely to be involved in longer-term regulation of body weight, with fasting plasma concentrations and postprandial responses to a meal being correlated with body adiposity (acting via a negative feedback signal to recent energy intake and body fat)⁽²⁸⁵⁾. Verdich et al.⁽²⁷⁵⁾ and Flint et al.⁽²⁸¹⁾, propose that insulin is more likely to be a mediator of satiety sensations in the postprandial period, particularly decreasing *ad libitum* energy intake in normal weight individuals but not in the overweight and obese. Despite the lack of a direct association between insulin or glucose and satiety effects in humans, it is important to include measurements of both in appetite research in view of their physiological responses to the ingestion of food, their role in energy homeostasis and most importantly to help monitor long-term changes to glycaemia and insulinaemia in chronic studies of weight and disease risk.

3.3.1.2 Summary of gut hormone control in appetite regulation and some limitations

The regulation of appetite by gut hormones is complex and influences dietary intake through the “sum of many parts”. It is clear that ghrelin, PYY and the incretins (GLP-1 and GIP) have a role to play in this intricate system and justifiably should continue to be monitored in appetite studies. This review has highlighted the variation in study results for the postprandial effects of the various gut hormones in different types of studies - human, animal and cell-line. The reasons for these variations are complex and may be partly due to the wide array of immunoassays and test meals used in the studies. Furthermore, subjects’ background diet is often not defined; therefore, it is difficult to determine if usual eating habits have any potential effects on hormone secretion. Also, results from infusion studies need to be treated

with care when translating them to food intake and appetite sensations in humans, particularly where pharmacological and not physiological doses are used, as often these may lead to adverse reactions that can alter desire to eat and impair dietary intake. Furthermore, the effective translation of preclinical data into comparable human observations is hampered by the predominant use of young mice and rats in short-term preclinical studies; it is well established that there are clear differences in biological responses between younger and older animals. More clinical dietary studies that are designed to test hormonal responses after consumption of *actual* food in humans are needed to build on the promising animal and infusion studies conducted to date. Despite some controversy over their use as appropriate biomarkers of appetite⁽²⁸⁶⁾ in individuals and perhaps at a group level, the measurement of glucose and gut hormones can be justified to examine mechanisms and provide insight into potential functionality.

3.3.1.3 Subjective measurements of satiety: Visual Analogue Scales

Appetite sensations are used reliably to measure subjective desire-to-eat, prior to and in response to meals⁽²¹⁷⁾. Visual Analogue Scales (VAS) are commonly used as a methodological tool to quantify such feelings (Table 3.1), in a controlled environment, immediately before and following an eating *episode*, and then chronologically at regular time points⁽²⁸⁷⁾. In general, the VAS consists of a 100 mm horizontal line, anchored at each end with opposing extremes of a specific scale (for example, "not at all hungry" and "never been more hungry"). Participants place a vertical mark on the line to correspond with their appetite-related sensations. These feelings are quantified by measuring the distance from the left end of the line to the mark⁽²¹⁶⁾. Using the VAS tool in this manner results in an acceptable degree of

validity and reliability⁽²⁸⁷⁾. VAS have other advantages also; they are uncomplicated and straight-forward to use, they are reproducible in repeated protocols where the effect of different treatments are compared under similar conditions, and they exhibit a good degree of within-subject reliability^(217; 288). In most cases, subjects are untrained. Vinoy⁽²⁸⁹⁾ suggests that the use of untrained volunteers in studies using VAS poses a number of issues. For example, the inter-individual differences in gender, age, dietary restraint are likely to effect the experience of appetite and the VAS ratings, making it difficult to obtain significant discrimination of satiety effects using small samples⁽²⁸⁹⁾. In addition, some individuals may have limited introspection and poor awareness of their appetite sensations.

Table 3.1 Visual Analogue Scale Questions

Question	Extreme ends of the response	Factors of appetite tested
How hungry do you feel at this moment?	I am not hungry at all I have never been more hungry	Hunger
How satisfied do you feel at this moment?	I am completely empty I cannot eat another thing	Satisfaction
How full do you feel at this moment?	Not at all full Totally full	Fullness
How much do you think you can eat at this moment?	Nothing at all A lot	Prospective food intake

Other limitations of the VAS tool have been noted. For example, the satiety power of different foods may depend on very small differences in composition, such as the limited amounts of active ingredients found in cereal foods. Also, differences in both experimental designs and in the sensitivities of study participants may make between-study comparisons difficult.

3.3.2 Measurement of Dietary Intake

A key research benchmark in studies assessing appetite (satiation/satiety effects) is the measurement of dietary intake. Dietary intake, in particular energy intake, strongly influences body weight changes, and weight control is typically the long-term primary outcome measure of appetite research. Thus, it is imperative to quantify dietary intake in all appetite studies, both short-term and long-term, especially to enable the connection between short-term satiation/satiety effects (most likely observed in acute feeding trials) and longer-term weight control outcomes (usually assessed through dietary intervention trials). After all, the main goal with most appetite research is to reduce an individual's dietary intake to, for example control obesity. In the context of research investigating diet and chronic disease specifically, healthy weight is a primary preventative measure, thus long-term studies such as RCTs are the gold-standard to confirm that decreasing appetite will result in decreased dietary intake and total energy that will subsequently lead to weight loss. For this reason, an RCT was the obvious choice for testing longer-term effects of sorghum consumption in this thesis. However, there are several limitations associated with RCTs, some of which are relevant to this thesis and are briefly summarised in Table 3.2.

Table 3.2 Summary of some limitations associated with RCTs

Problems/Limitation	Steps taken in this thesis to overcome problem
Difficulty in blinding researcher to intervention/control treatments	Researcher not at subject interface at key times during testing
Background diets confounding results	Diet histories of usual intake and 3-day food records before testing starts
Control group also undergoes dietary intervention	Rigour in dietary recording, design and analysis in order to interpret results accurately

Dietary intake is measured by quantification of food consumed *ad libitum*. To assess satiation, the intake of the test food (preload) alone or as part of a meal is measured, whereas for satiety, energy intake at a test meal is quantified within a specified amount of time following the consumption of the test food⁽²¹⁶⁾. Typically, the test meal is offered in a buffet style or as a single meal, pre-selected by the participant from a menu offered by the researcher⁽²¹⁶⁾. There are various methods for measuring dietary intake in the research setting, depending on the study design, including: direct weighing of food consumed before and after intake; dietary recall usually for the 24-hour period preceding testing; diet history conducted by a trained professional or weighed food records completed by the subject. In the case of laboratory-based meal test studies, direct observation by the researcher is most commonly employed with diet histories/records or recalls assessing the pre-test intake and subject-recorded food records used to capture intake after testing. There are many obstacles and limitations that relate to obtaining an accurate record of food intake from subjects in dietary studies, mostly involving under-reporting and occasionally over-reporting⁽²⁹⁰⁾.

The food composition database is an important scientific tool in dietary intake measurement, however it presents with several limitations. Some obvious issues relate to: variability in the composition of foods, partial or limited coverage of food items and partial or limited coverage of nutrients. Currently, coverage of polyphenolic compounds is grossly limited in many databases. In this thesis, AUSNUT 2007 Database was utilised and like all food composition databases is not immune to some of the problems just described. Hence, to maximise the integrity of the research, it was an imperative that only qualified dietitians enter dietary information into the database enabling informed decisions when data was missing.

It is clear that human appetite and eating behaviour are complex functions, with many intervening variables (environmental, social and cognitive factors) that may influence them and ultimately an individual's intake. As described by Bornet et al.⁽²⁹¹⁾, "appetite is dependent upon interactions between biology and the environment. The environment contains extremely potent factors which for many people can readily overcome biological processes operating to maintain body weight". Laboratory-based meal test studies are designed to tightly control for and limit these intervening variables, and in doing so may actually make translatability to the free-living condition somewhat less realistic. The Stunkard and Messick questionnaire (also referred to as the three-factor eating questionnaire or TFEQ) has been designed to measure dietary restraint, disinhibition and hunger⁽²⁹²⁾. It is used in dietary studies to limit confounding factors relating to emotional or cognitive restraint or overeating. It is used in the screening phase to help recruit a similar study population whereby such emotional/cognitive drivers of food intake are reduced. Another point to consider in satiety studies is the fact that effects of a single eating

episode on satiety and energy intake may not be sustainable as repeated consumption could lead to either habituation and waning of the effect over time or to strengthening the effect due to learning⁽²¹⁶⁾. This further indicates the importance of longer-term intervention trials whereby subjects are eating the test food in their usual, natural environment, which was the aim of the RCT described in Chapter 6. Though meal test studies do not usually necessitate dietary standardisation prior to testing⁽²¹⁷⁾, in the acute meal test study described in this thesis, the evening meal prior to each visit was standardised. Importantly though, it was stratified to usual energy intake to ensure that subjects did not feel greater than “usual hunger”, or vice versa. The main reason for standardisation was to limit the variability in baseline plasma total antioxidant capacity (affected by polyphenolic content of foods) between testing days.

Clinical studies evaluating acute appetite effects post consumption of a test food often use buffet-style meals to assess energy intake at a subsequent mealtime. The amount of food offered in the buffet-style meal is deliberately in excess of participants’ usual intake and consists of a variety of food items, varying in macronutrient composition. However, this introduces a potential confounder in that more food may be eaten in this setting than in free-living conditions as the food is prepared for the subjects, is in abundance (higher quantity and greater choice) and is free. Thus it is difficult to distinguish whether energy intake is influenced more strongly by individuals’ food selections rather than the actual satiety-related effects of the test food.

In summary, understanding the limitations of different methodologies is essential to enable the design of rigorous clinical dietary trials and to maximise the opportunity to observe minimally confounded outcomes.

3.3.3 Measurement of Relevant Markers of Health/Disease Risk

Metabolic Syndrome (MetS) is defined by a cluster of associated metabolic abnormalities, including central obesity, dyslipidaemia, hypertension, hyperglycaemia, and insulin resistance. These metabolic disorders present significant risk factors for chronic disease development, including cardiovascular disease and diabetes. Dietary interventions and therapies specifically aim to prevent the development or advancement of such diseases, hence it is important to monitor the progression to MetS and its individual components when evaluating the diet-health relationship, or specifically in this thesis, the relationship between a sorghum-enhanced diet and health.

Traditional markers of metabolic disease relate to body measurements (such as weight, body mass index (BMI), and waist-to-hip ratio) and serve as indicators of obesity and risk of chronic metabolic disorders. In addition, biomarkers (also known as biological indicators) are used to monitor and predict the health of a population, to identify individuals with particular susceptibility or resistance to health problems, and to evaluate therapeutic or clinical interventions. Human dietary trials investigating effects on health outcomes require the measurement of such biomarkers, of which there are many, and each reflecting activity in at least one biological system such as the cardiovascular, metabolic or immune system. Selection of appropriate biomarkers to capture changes to clinical outcomes affected by dietary

intake is imperative. Blood pressure, heart rate, and pulse are commonly measured to indicate cardiovascular functioning. Serum cholesterol and triglyceride levels provide information on metabolic processes and have been used to evaluate the risk of coronary heart disease. Glucose and insulin are common indicators of glycaemic regulation and insulin resistance, providing useful information on the future risk of developing diabetes mellitus. Examining biomarkers of oxidative stress and inflammation is also essential, especially because oxidative stress is a key mediator of increased cytokine concentrations and low-grade systemic inflammation, precursors in the pathophysiology of chronic diseases. Therefore, measurement of cytokines (interleukins, tumor necrosis factor alpha, c-reactive protein) as well as the overall antioxidant potential of plasma itself (total antioxidant capacity) may be useful indicators of health/disease risk status.

A summary of the specific disease markers evaluated in the experimental studies in this thesis is provided in Table 3.3. Normal references ranges have been provided for all weight-related measures (BMI, waist circumference, percentage body fat) (Table 3.3a) and biochemical indices such as lipids, glucose and insulin levels (Table 3.3b). However, benchmarks for some of the inflammatory biomarkers are not yet available in the literature or accepted by pathology laboratories that may conduct such analyses. In these cases, the desired outcome would be to observe a decline in levels over time, especially after a beneficial weight-loss inducing dietary intervention (Chapter 6).

Table 3.3 (a) Summary of disease markers evaluated in the experimental studies in this thesis

Indication	Marker/ Measurement	Normal reference range	In which study was marker evaluated?	Primary (1^o) or Secondary (2^o) Outcome	
Obesity					
<i>Body Weight</i>	Weight		RCT	1 ^o	
	BMI	18.50-24.99 kg/m ²	RCT	1 ^o	
<i>Adiposity</i>	Body fat	Females	Males	RCT	2 ^o
	20-39 years	21-33%	8-20%		
	40-59 years	23-34%	11-22%		
	60-79 years	24-36%	13-25%		
	Waist circumference	< 80 cm (Females)		RCT	2 ^o
	< 94 cm (Males)		RCT	2 ^o	
Cardiovascular system					
<i>Blood Pressure</i>	Diastolic blood pressure	<80 mmHg	Acute Meal Test & RCT	2 ^o & 2 ^o	
	Systolic blood pressure	<120 mmHg	Acute Meal Test & RCT	2 ^o & 2 ^o	

Table 3.3 (b) Summary of disease markers evaluated in the experimental studies in this thesis

Indication	Marker/ Measurement	Normal reference range	In which study was marker evaluated?	Primary (1^o) or Secondary (2^o) Outcome
Metabolism				
<i>Lipid metabolism</i>	Total cholesterol	3.6 - 5.2 mmol/L	RCT	2 ^o
	LDL cholesterol	< 2.59 mmol/L	RCT	2 ^o
	HDL cholesterol	0.9 - 2.0 mmol/L	RCT	2 ^o
	Triacylglycerides	< 1.70 mmol/L	RCT	2 ^o
<i>Glucose metabolism</i>	Glucose	3.6 - 5.5 mmol/L	Acute Meal Test & RCT	2 ^o & 2 ^o
	Glycosylated haemoglobin	4 - 6 %	RCT	2 ^o
<i>Insulin regulation</i>	Insulin	2.6 - 10 mIU/L	Acute Meal Test & RCT	2 ^o & 2 ^o

Table 3.3 (c) Summary of disease markers evaluated in the experimental studies in this thesis

Indication	Marker/ Measurement	Normal reference range	In which study was marker evaluated?	Primary (1^o) or Secondary (2^o) Outcome
Inflammation/ Oxidative Stress Status				
<i>Cytokines</i>	Interleukin 6 IL-6	Not defined	RCT	2 ^o
	Interleukin 8 IL-8	Not defined	RCT	2 ^o
	Interleukin-1B	Not defined	RCT	2 ^o
	Tumour Necrosis Factor- α	Not defined	RCT	2 ^o
	c-Reactive Protein	0-5 mg/L	RCT	2 ^o
	Hydroperoxide	0-2.3 mmol/L H ₂ O ₂	RCT	2 ^o
<i>Antioxidant potential</i>	Total antioxidant capacity	1.07-1.53 mmol/L Trolox	Acute Meal Test & RCT	2 ^o & 2 ^o

3.3.3.1 Biomarkers of Inflammation and Oxidative Stress: A brief overview

Indications of the extent of inflammation and oxidative stress caused by ROS and RNS are very important in the assessment of health and/or disease status. Clinical trials aiming to measure the outcomes of food consumption on different human biological systems therefore require measurement of specific oxidative stress and inflammation biomarkers. A “panel” of disease markers, including cytokines, c-reactive protein and hydroperoxide, was selected to monitor changes to inflammation and oxidative stress status in the RCT described in this thesis, with the aim of enhancing the overall evaluation of the effectiveness of the intervention (Table 3.3 (c)).

Cytokines

Cytokines are important regulators of adipose tissue metabolism. Adipocytes (fat cells) within the adipose tissue synthesise a range of cytokines including tumour necrosis factor alpha (TNF- α) and several interleukins (IL), notably IL-1beta (IL-1 β), IL-6 and IL-8⁽²⁹³⁾. In MetS, adipocyte dysfunction is frequently present and is associated with an increase in M1 macrophage population within the adipose tissue. The M1 macrophages secrete these pro-inflammatory cytokines, which in turn act through a number of cell signaling pathways to induce insulin resistance and abnormal glucose metabolism⁽²⁹⁴⁾. Through their inflammatory properties, these cytokines have been implicated in endothelial cell damage within blood vessels that leads to vascular dysfunction and atherosclerosis. Studies have shown that elevated levels of TNF α , IL-6, IL-8 and IL-1 β are associated with MetS and increasing levels are associated with more severe MetS (also assessed by hypertriglyceridemia, hypertension, and fasting glucose levels)^(295; 296). Cytokines can therefore serve as

potential biomarkers of inflammation and progression to MetS, and in the longer-term, progression to more serious chronic disease. In the context of this thesis, TNF α , IL-6, IL-8 and IL-1 β were selected as biomarkers of disease-risk status after a 3 month dietary intervention (Chapter 6), specifically monitoring the impact of dietary modifications and weight loss on these markers.

C-reactive protein

C-reactive protein (CRP) is an acute-phase protein produced by the liver in response to inflammation. The cytokines TNF α , IL-6 and IL-1 β are widely reported to induce synthesis of CRP by hepatocytes (liver cells)⁽²⁹⁷⁾. In relation to metabolic disease, CRP is secreted in the presence of atherosclerotic inflammation and is perhaps the most well-known cardiovascular biomarker. In fact, CRP is commonly measured in health screening assessments in clinical practice⁽²⁹⁸⁾. Moreover from a nutrition perspective, a recent meta-analysis of 17 studies found evidence that consumption of a healthy dietary pattern was associated with significant reductions in CRP (weighted mean differences:-0.75 (-1.16, -0.35), p=0.0003)⁽²⁹⁹⁾. CRP, specifically high sensitivity CRP (hs-CRP), was measured in this thesis based on its widespread use and effectiveness as a predictor of disease progression or health status in previous clinical studies and in practice.

Hydroperoxide

Lipid peroxidation, mediated by free radical activity, has been implicated in several disease states including cancer and rheumatoid arthritis, as well as in the degenerative processes associated with ageing⁽²⁹⁹⁾. There is also evidence that circulating lipid hydroperoxides (HPX) play a pivotal role in atherogenesis⁽³⁰⁰⁾ and

thus coronary heart disease, the leading cause of death in Western societies such as Australia⁽³⁰¹⁾. Lipid peroxidation initiates a cascade of events that lead to enhanced uptake of low-density lipoproteins by macrophages and formation of lipid-laden foam cells, one of the earliest indicators of atherosclerosis. Given the study cohort in the RCT (Chapter 6) was overweight and mildly obese, and presumably under greater oxidative stress, plasma HPX was also measured as a biomarker. It was anticipated that HPX would provide insights into the effectiveness of the dietary intervention (and more specifically its constituent antioxidant food components) to reduce the extent of lipid peroxidation and more broadly alleviate overall oxidative stress.

Total Antioxidant Capacity

The concept of “total antioxidant capacity” (TAC) originated in chemistry but in recent times it has been applied to medicine, biology and nutrition as an indicator of antioxidant status both *in vitro* and *in vivo*. Researchers in the food and nutritional sciences have been interested in evaluating TAC of foods (such as fruits, vegetables, grains and wine)⁽³⁰²⁾, as well as measuring the TAC of plasma in human studies as an indicator of antioxidant status⁽¹⁸⁰⁾. Plasma TAC considers the cumulative action of all the antioxidants present in plasma, thus providing an integrated parameter rather than the simple sum of measurable antioxidants⁽³⁰³⁾. Hence, the capacity of known and unknown antioxidants and their synergistic interaction is assessed. Studies have explored acute effects of eating foods with high TAC on plasma TAC, such as high tomato consumption⁽³⁰⁴⁾, while other studies have evaluated nutritional interventions containing foods with a high TAC on disease risk and prevention⁽³⁰⁵⁾. For example, Pitsavos et al.⁽³⁰⁵⁾ demonstrated that adherence to Mediterranean dietary practices

was positively associated with plasma TAC levels and negatively associated with oxidation of the atherogenic LDL-cholesterol. In the present thesis, TAC of the test foods was measured, as was plasma TAC in both the acute meal test study and the longer-term RCT (measured at baseline and after 3 months). However, there is some criticism that the TAC assay excludes enzymatic activity from the total measure of antioxidant activity, precluding meaningful application to *in vivo* conditions⁽³⁰²⁾.

3.4 Overview of thesis research design

In view of the opportunities to develop new sorghum products for human consumption, this thesis begins by asking the questions: what are the nutritional properties of sorghum and what do we know about the effects of consumption on humans? The first part of the thesis involved review of the literature to describe sorghum grain structure and the specific chemical, nutritional and functional attributes that relate to mechanisms by which sorghum grain components may influence weight management and chronic disease risk. In addition, the evidence reported in the scientific literature on effects of sorghum consumption on health outcomes related to chronic disease prevention. Next, a sorghum based food product was developed with a food manufacturer and two experimental studies were conducted to examine effects of consumption on relevant chronic disease indicators. Overall, these thesis components integrate to arrive at a position on sorghum's potential as an underutilised whole grain cereal to assist in chronic disease prevention in humans.

CHAPTER 4:

Formulation of Ready-To-Eat Sorghum Flaked Breakfast Biscuits

A component of this chapter is published in the article:

Stefoska-Needham, A., Beck, E.J., Johnson, S.K., Chu, J., Tapsell, L.C. (2015) Flaked sorghum biscuits increase post-prandial GLP-1 and GIP levels and extend subjective satiety in healthy subjects. *Molecular Nutrition and Food Research*. 60:1118-1128.

A component of this chapter is published in the article:

Stefoska-Needham, A., Beck, E.J., Tapsell, L.C. (2015) Perspective: The path to confirming and exploiting potential satiety-enhancing effects of sorghum-based foods for human diets. *Quality Assurance and Safety of Crops & Foods*. (DOI: 10.3920/QAS2015.0695).

The experimental work in this chapter was undertaken in collaboration with Sanitarium (the Industry Partner of the ARC Linkage Grant LP100200125) and the use of their pilot processing facility to formulate the biscuits. All biscuit analyses were completed at the Department of Food Science and Technology, Curtin University, Western Australia, and were led by Associate Professor Stuart Johnson. The candidate attended Curtin University in 2012 and was trained in analysis techniques detailed in this Chapter and engaged in all decisions around methods and foods/ingredients requiring testing. Scanning Electron Microscopy and interpretation of micrographs was performed by the candidate at the University of Wollongong.

4.1 Introduction

Healthy dietary patterns are positively associated with indicators of health and wellbeing⁽¹⁷⁾ and contain specific foods and food components to which potent positive effects on disease markers may be attributed. Though food has been used historically to improve the health of people, modern food and nutrition research has focused on improving and innovating food *itself*, through a greater understanding of the links between food components, the whole diet and health. This approach has facilitated improvements in the quality (or *functionality*) of food products, enhancing their potential to benefit health and reduce the risk of disease when they are consumed regularly. Today such foods are commonly referred to as “functional foods”.

Global food mega-trends, particularly in the developed countries, indicate a strong and growing consumer interest in “naturally functional” food products, including those that promote greater feelings of fullness and satiety^(11; 306). Food companies have responded by investing in innovative product development and reformulations that have resulted in novel satiety-enhancing food products. Diets containing foods with optimised satiety attributes could be beneficial for appetite regulation and longer-term weight management⁽³⁰⁶⁾ and may have the potential to assist in the prevention of obesity-related chronic diseases. It has been shown that plant-based diets rich in whole grains, with naturally higher fibre content and lower energy value, promote satiety⁽³⁰⁷⁾. This thesis focuses on sorghum as a specific example of foods that may assist in driving satiety or decreasing energy intake, and may be particularly beneficial in populations where excess weight is a key driver of metabolic disease.

4.2 The opportunity for sorghum-based foods with satiety attributes

Food choice at breakfast represents an important opportunity to increase satiety in the morning and reduce energy intake at lunch and during the rest of the day⁽³⁰⁸⁾. Consequently, developing breakfast products from whole grain cereals with inherent satiety-enhancing functional properties is a growing area of interest for researchers and food companies alike. Sorghum, with its reputation for being satiating⁽²³⁾, may be a viable high-value ingredient for such food applications. Food manufacturers are also interested in sorghum for its gluten-free attribute. Traditionally, sorghum has been used in a variety of foods particularly in Africa, India and Central America, including breads, porridges, steamed and boiled products, beverages and snack foods (such as popped sorghum)^(60; 309).

In more recent years in Western cultures, sorghum's light color (characteristic of some cultivars), neutral flavour and pleasing texture has made it suitable for use in non-traditional food products including breakfast cereals, baking mixes for bread, gluten-free bread, cakes/brownies, pancakes, bars and gluten-free beer⁽³¹⁰⁾. A US consumer survey⁽²⁰²⁾ identified several drivers for purchase intent of sorghum grain products including: local origin (domestically-grown grain), inherent health benefits (for example, antioxidants), and appealing sensory characteristics (such as crunchy texture in breakfast cereals). To date, most of the sorghum-based products that have been launched are marketed for their gluten-free attribute rather than the more broadly applicable health effects of sorghum grain.

4.3 Developing an optimised sorghum-based breakfast cereal

In this thesis, a ready-to-eat (RTE) sorghum-based breakfast cereal was formulated as whole grain flaked cereal biscuits, mainly because this food format is well accepted by consumers as a breakfast cereal option based on sales data of an equivalent wheat-based product already on the market. A gluten-free alternative to traditional wheat flaked biscuits has been long sought after, particularly by individuals with a wheat or gluten intolerance such as coeliac disease. Of additional value to sorghum's inherent gluten-free attribute would be any potential appetite and weight regulating functionality. Before testing for these potential effects in humans, it is important to confirm that the sorghum flaked cereal biscuits actually contain the implicated chemical and nutritional components and that processing has not negatively altered their physicochemical properties.

For the meal test study (Chapter 5), the flaked cereal biscuits were derived from three different sorghum cultivars (red-, white- and brown-grained sorghums) and a single wheat control biscuit (Figure 4.1). Wheat was chosen as the suitable food control because it has a different polyphenolic profile to red sorghum but has similar nutritional attributes and, from a behavioural perspective, wheat cereal products are readily interchangeable with sorghum breakfast cereal products. For the RCT (Chapter 6), the same red sorghum and wheat control biscuits were tested. Great effort was made to formulate a similar control biscuit (not disparate in appearance and taste) in order to minimise confounding effects that might emerge if the control was markedly different to the other treatment biscuits.



Figure 4.1 (a) Red sorghum flaked breakfast biscuits



Figure 4.1 (b) Brown sorghum flaked breakfast biscuits



Figure 4.1 (c) White sorghum flaked breakfast biscuits



Figure 4.1 (d) Wheat (control) flaked breakfast biscuits

4.3.1 Processing Methods

White sorghum grain (variety Liberty - a commercial hybrid) and red sorghum grain (variety Alpha - an in-bred line), were grown and supplied by Lochabar Enterprises Pty Ltd (Tara, QLD, Australia) using organic conditions specifically for human food use. Brown sorghum grain, variety IS8237C (an in-bred line), was supplied by the Queensland Government Department of Agriculture, Fisheries and Forestry and grown at the Hermitage Research Station (Warwick, QLD, Australia). Non cultivar-specific Australian Prime White (APW) hard wheat, grown in central New South Wales, Australia and siloed at ambient temperature was supplied by Sanitarium Health and Wellbeing (Cooranbong, NSW, Australia). The bleach test of Waniska, Hugo and Rooney⁽³¹¹⁾ was used to determine the presence or absence of tannins in the grains of different sorghum varieties used for the manufacture of the breakfast cereals. The white and red sorghum gave no blackened grains, indicating the absence of tannins. However blackened grains were observed for the brown sorghum indicating the presence of tannins in this variety.

The test biscuits were prepared according to the following recipe: 2.5 L of water was added to 25 kg of whole sorghum grains and the mixture steamed for 30 minutes in a rotating pressure vessel. A small amount of malt extract was added (precise quantities not available due to a confidential formula). The cooked grains were then air dried to approximately 24% moisture before being passed through a flaking mill, reducing their thickness to approximately 0.1 mm. This differs from the “shredded wheat” process, in that the flakes were not cut into strips at any stage. These moist flakes were then pressed into a biscuit shape by hand and baked for 25 minutes at 130 °C, drying the biscuits to approximately 7% moisture (weight basis). Prepared

biscuits were labeled in non-descript packaging with coding such that both the researcher at the participant interface and the subjects were blinded to the test foods.

4.3.2 Analyses of biscuit contents

To enable proximate and dietary fibre analysis, the test biscuits were ground to a fine particle size using a Grindomix GM200 mill (Retsch, Haan, Germany). All analyses were done in duplicate using standard methods (AOAC, 2008) and expressed as g/100g *as is*. Moisture content was determined by loss of mass on drying by AC 945.15. Protein content was determined by Kjeldahl nitrogen determination (nitrogen conversion = 6.25) by AOAC 991.20. Fat content was determined by Soxhlet extraction according to AOAC 2003.05. Ash content was determined after combustions at 550 °C as described in AOAC 923.03. Total dietary fibre was determined by enzymatic-gravimetric analysis according to AOAC 991.43. The available carbohydrate of the samples was calculated by difference and the energy content calculated using Atwater factors according to the Australian Food Standards Code⁽³¹²⁾.

To determine polyphenolic content and antioxidant capacity, the flaked breakfast biscuits were ground in a bench-top blender to pass 100% through a 500 µm sieve. Free and bound phenolic extraction was performed in duplicate, as described by Adom and Liu⁽¹¹⁷⁾. Extraction of free phenolic compounds was performed by shaking 1g of ground sample with 2 mL of 75% acetone for 10 minutes and centrifuging at 20 °C for 10 minutes at 4000 x g to recover the supernatant. The pellet was re-extracted twice more in the same manner and the supernatants pooled and made up to a final volume of 10 mL with 75% acetone. The extracts were stored at -20 °C in the

dark until analysis. Bound phenolic compounds were extracted from the residue after the extraction of free phenolics. Twenty-five millilitres of 2M sodium hydroxide was added to each residue and digestion was performed for 1h at room temperature under nitrogen gas whilst shaking. Concentrated HCl was used to lower the pH to 2 and then the mixture was extracted twice with 10 mL hexane for 10 minutes with shaking, followed by centrifugation for 10 minutes at 4000 x g at 20 °C. After each extraction the hexane upper layer was discarded. The lower aqueous phase was extracted five times with 15mL ethyl acetate by shaking for 10 minutes, followed by centrifugation for 10 minutes at 4000 x g and 20 °C. The pooled ethyl acetate fractions were evaporated to dryness at 30 °C using vacuum rotary evaporation. The phenolic residue was re-dissolved in 10 mL of 75% acetone and stored at -20 °C in the dark until analysis.

The phenolic content of extracts was measured using a method by Singleton and Rossi⁽³¹³⁾ (adapted from Adom and Liu⁽¹¹⁷⁾). Duplicate 50µL samples of the extracts of free or bound phenolics were diluted with 650 µL ultrapure water. Fifty microliters of Folin-Ciocalteu reagent was then added followed by 500µL of 7% solution sodium carbonate in water. The sample was mixed by vortex and incubated at room temperature for 90 min. The sample absorbance was measured at 750 nm against a 75% acetone blank. Known amounts of gallic acid (0 – 250 µg/mL) were used to prepare a calibration curve. The phenolic content was expressed as gallic acid equivalents (mg of GAE/g as is).

In associated investigations (to be reported elsewhere)¹, liquid chromatography-mass spectrometry (LC-MS) was used in the identification and characterisation of the different phenolic compounds of both the unprocessed whole grains and the final flaked breakfast cereals.

Determination of antioxidant capacity was based on the method reported by Awika et al.⁽³¹⁴⁾, whereby duplicate 150 µL samples of the extracts of free and bound phenolics and standards of 0 – 150 µg/L Trolox in absolute ethanol were reacted with 2850µL of 60 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in methanol for 8 h at room temperature in the dark whilst shaking. The absorbance at 515nm was measured against a blank (150µL absolute ethanol +2850 µL of DPPH solution). A Trolox standard curve was generated to calculate the antioxidant capacity of the samples expressed as Trolox equivalents (µmol TE/g as is).

An enzymatic colorimetric method (K-TSTA 04/2009 kit; Megazyme International Ireland Ltd, Wicklow, Ireland) was used to determine total starch content (in duplicate) of the flaked breakfast cereals as g/100g *as is*. *In vitro* starch digestibility was determined by a method using rapid glucometry⁽³¹⁵⁾ after manual grinding through a 1mm sieve. At time intervals of 0, 10, 20, 45, 60, 90 and 120 minutes from addition of pancreatin mixture in the *in vitro* simulation of digestion, duplicate glucose readings were made using an AccuCheck® Performa® glucometer (Roche Diagnostics Aust. Pty. Ltd, Castle Hill, Australia) and corrected against a reagent blank.

¹ Price, W., Kang, J., Fang, Z., **Stefoska-Needham, A.**, Tapsell, L.C., Johnson, S.K. Nutritional and phytochemical properties of flaked sorghum breakfast cereals. *Food Research International* (In draft).

Equation (1) was used to calculate digested starch D (g per 100g dry starch) at each time point.

$$D = \frac{c G_G M_G V}{W S [100 - M]} \quad (1)$$

Where G_G is glucometer reading (mM/L), V is volume of digesta (mL), M_G is molecular weight of glucose ($M_G=180$), W is weight of sample (g), S is starch content of sample (g per 100g dry starch), M is moisture content of sample (g per 100 g sample), and c is stoichiometric constant for starch from glucose contents ($c=0.9$). Rapidly digestible starch RDS (g/100g dry starch) was calculated as $G_G = G_{20} - G_0$. Slowly digested starch SDS (g/100g dry starch) was calculated as $G_G = G_{120} - G_{20}$ and resistant starch RS (g/100g dry starch) was calculated as $G_G = 100 - RDS$ (g/100g dry starch) – SDS (g/100g dry starch).

Additional physicochemical profiling was enabled with scanning electron microscopy (SEM), particularly showing characteristics of the starch granules in the different biscuits.

4.4 Results and Discussion

Optimised flaked breakfast biscuits were successfully formulated by Sanitarium Health and Wellbeing on a pilot line in their manufacturing plant at Cooranbong, NSW, Australia. Analyses of proximate and dietary fibre composition and energy content (on *as is* basis per 100g) identified modest differences between the control and different sorghum varieties (Table 4.1). The wheat control had the lowest levels of total energy, fat and available carbohydrate compared to the sorghum biscuits.

Total dietary fibre content of the wheat biscuit was similar to the brown sorghum biscuit but significantly higher than the red and white sorghum biscuits ($p < 0.05$).

Table 4.1 Proximate and dietary fibre composition and energy content of flaked breakfast cereals^{1,2,3}

Flaked cereal biscuit type	Moisture (g/100g as is)	Protein (g/100g as is)	Fat (g/100g as is)	Ash (g/100g as is) ³	Total dietary fibre (g/100g as is)	Total available carbohydrates (g/100g as is)	Energy content (kJ/100g as is)
Wheat control	6.6±0.0	13.7±0.2 ^d	1.7±0.0 ^a	1.6±0.0	9.6±0.1 ^b	66.9±0.1 ^a	1508±1 ^a
White sorghum	6.6±0.0	10.7±0.0 ^b	2.9±0.0 ^b	1.6±0.0	7.3±0.4 ^a	71.0±0.4 ^b	1553±4 ^c
Red sorghum	6.6±0.0	9.4±0.1 ^a	2.9±0.1 ^b	1.6±0.0	7.7±0.0 ^a	71.9±0.1 ^b	1550±0 ^c
Brown sorghum	6.1±0.0	11.7±0.1 ^c	2.9±0.1 ^b	2.1±0.0	9.9±0.2 ^b	67.4±0.3 ^a	1530±1 ^b

¹Means (n=2) ± standard deviation

²Values within a column with the same superscript letter denotes no significant difference ($p > 0.05$)

³Statistical comparison not possible as some replicates identical

The wheat biscuit also contained the highest level of protein compared to all three sorghum biscuits ($P < 0.05$). Free and bound polyphenolic (PP) content (mg GAE/g *as is*) and antioxidant capacities ($\mu\text{mol TE/g as is}$) of the test foods were also determined (Table 4.2). As expected, red and brown sorghum biscuits had the highest PP levels, on account of the high anthocyanin and tannin contents previously reported for these varieties⁽²³⁾, and lower levels of total PP were reported for white sorghum and wheat biscuits. Preliminary results from the LC-MS analyses (unpublished data) identified different types and amounts of free, bound and total phenolic compounds in both the unprocessed whole grains and the flaked sorghum biscuits. The types of phenolics acids identified were: protocatechuic acid, caffeic acid and ferulic acid. The flavonoids eridictyol, quercetin, luteolin, naringenin and apigenin, were also identified in the samples, as were luteolinidin and apigeninidin, two types of 3-deoxyanthocyanidins unique to sorghum.

Table 4.2 Free, bound and total phenolic content and antioxidant capacity of flaked breakfast cereals^{1,2}

Flaked cereal biscuit type	Free PP (mg GAE/g as is)	Bound PP (mg GAE/g as is)	Total PP (mg GAE/g as is)	Free AC (μ molTE/g as is)	Bound AC (μ molTE/g as is)	Total AC (μ molTE/g as is)
Wheat control	0.71 \pm 0.01 ^a	0.67 \pm 0.22 ^a	1.37 \pm 0.21 ^a	2.64 \pm 0.01 ^a	2.67 \pm 0.01 ^a	5.31 \pm 0.01 ^a
White sorghum	0.65 \pm 0.04 ^a	0.82 \pm 0.04 ^a	1.46 \pm 0.07 ^a	3.39 \pm 0.18 ^a	2.08 \pm 0.01 ^a	5.46 \pm 0.20 ^a
Red sorghum	1.27 \pm 0.01 ^b	1.00 \pm 0.11 ^{ab}	2.27 \pm 0.12 ^b	11.25 \pm 0.57 ^b	9.16 \pm 0.62 ^b	20.40 \pm 1.18 ^b
Brown sorghum	1.46 \pm 0.04 ^c	1.51 \pm 0.06 ^b	2.99 \pm 0.01 ^c	16.36 \pm 0.23 ^c	14.69 \pm 0.55 ^c	31.05 \pm 0.79 ^c

¹Means (n=2) \pm standard deviation²Values within a column with the same superscript letter denotes no significant difference (p>0.05)
PP, polyphenolic; AC, antioxidant capacity; GAE, gallic acid equivalents; TE, Trolox equivalents

Data for the *in vitro* starch characterisation of the biscuits only showed significant differences in RS, with higher levels occurring in brown sorghum biscuits compared to the wheat control (p=0.044) (Table 4.3). Levels of SDS and RDS did not differ between biscuits.

Table 4.3 Starch properties of flaked breakfast cereals^{1,2}

Flaked cereal biscuit type	Rapidly digested starch (g/100g dry starch)	Slowly digested starch (g/100g dry starch)	Resistant starch (g/100g dry starch)
Wheat	31.9 \pm 1.6 ^a	47.9 \pm 4.2 ^a	20.2 \pm 3.9 ^a
White sorghum	30.2 \pm 1.6 ^a	46.5 \pm 3.2 ^a	23.3 \pm 3.5 ^{ab}
Red sorghum	31.9 \pm 4.2 ^a	46.9 \pm 7.7 ^a	21.2 \pm 3.6 ^{ab}
Brown sorghum	29.3 \pm 3.2 ^a	43.2 \pm 1.6 ^a	27.6 \pm 2.6 ^b

¹Means (n=2) \pm standard deviation²Values within a column with the same superscript letter denotes no significant difference (p>0.05)

SEM show a consistent degree of starch gelatinisation within all breakfast biscuits (Figure 4.2), further supporting the results of the starch profiling studies which identified minimal differences in starch properties between biscuits (Table 4.3). Minimal differences in the degree of gelatinised starch between biscuits are a positive result since this has implications for the post-prandial clinical effects and may have confounded results if significant differences existed.

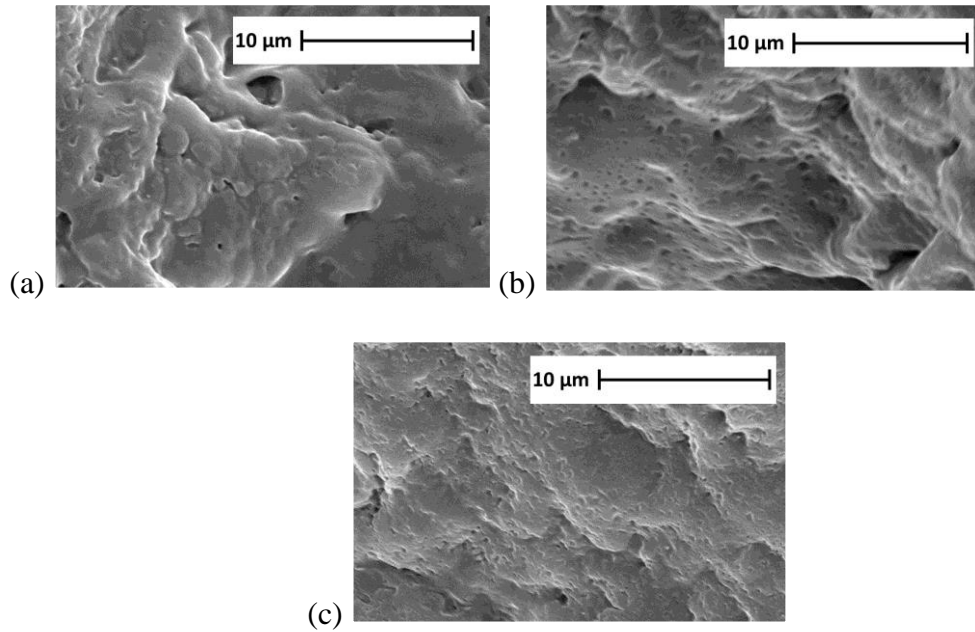


Figure 4.2 Scanning electron micrographs (15kV) of (a) white sorghum flaked biscuit (b) red sorghum flaked biscuit (c) brown sorghum flaked biscuit depicting gelatinised starch granules.

4.5 Conclusions

This study confirmed that the flaked cereal biscuits retained the chemical and nutritional properties inherent to native sorghum whole grains, particularly with respect to polyphenolic content (key differences emerging between cultivars, as expected) and similar levels of SDS. These results validate the utilisation of the formulated sorghum breakfast biscuits as suitable test foods in the clinical dietary trials described in this thesis. They also support the use of this breakfast cereal format for the formulation of novel sorghum-based cereal foods. Consideration of the effects of processing on the biscuits' functional, nutritional and chemical attributes was imperative to enable more rigorous interpretation of clinical results from the dietary trials conducted in this thesis and represents a key strength of the overall research strategy.

CHAPTER 5:

Acute meal test study investigating satiety-related effects of flaked sorghum biscuit intake

The majority of this chapter is the substantive content of the published article:

Stefoska-Needham, A., Beck, E.J., Johnson, S.K., Chu, J., Tapsell, L.C. (2015) Flaked sorghum biscuits increase post-prandial GLP-1 and GIP levels and extend subjective satiety in healthy subjects. *Molecular Nutrition and Food Research*, 60:1118-1128.

Discussion regarding the use of a standardised of dinner meal in this study design:

Needham, A., Beck, E., Tapsell, L. (2014) Does a standardised dinner meal consumed the evening prior to testing add methodological integrity to an acute meal test study design? *Nutrition and Dietetics*, 71 (Suppl. S1), 26. (Oral presentation at DAA Conference, 2014, Brisbane, Australia).

Discussion relating to meal test study design exploring sorghum's potential satiety-enhancing effects:

Stefoska-Needham, A., Beck, E., Tapsell, L. (2014) Acute effects of consuming sorghum-based breakfast meals on objective and subjective measures of satiety. *Cereal Foods World*. 59, No.6 (Suppl):A29, 2014. (Oral presentation at AACCI Annual Meeting 2014, Rhode Island, U.S.).

5.1 Introduction

Whole grain consumption is postulated to decrease chronic disease risk, largely due to beneficial effects on body weight regulation⁽³¹⁶⁾. Studies of whole grain constituents, including slowly digestible starches (SDS), dietary fibre and phytochemicals, implicate their effects on energy balance, glycaemic control, lipids and inflammation. Sorghum grain constituents have also been shown to produce such effects (Chapter 2).

Recent studies demonstrate that sorghum foods can be formulated and processed to deliver SDS⁽⁷²⁾. Sorghum foods with both higher levels of SDS and dietary fibre content have the greatest potential to assist in improving blood glucose control and possibly to influence appetite-related gut hormones such as ghrelin, GLP-1, GIP and PYY. Influences on gut hormone profiles may be a key mechanism of satiety-enhancing action. GLP-1 and GIP are *incretin* gut hormones that potentiate insulin secretion after meal ingestion in a glucose-dependent manner. They are secreted within minutes of food intake and are associated with increased satiety⁽³¹⁷⁾. PYY has been shown to have strong anorectic properties in both lean and obese subjects⁽²⁴⁵⁾, reducing appetite and food intake by approximately 25-30% at a subsequent meal⁽²⁴⁸⁾. Ghrelin, an orexigenic hormone, decreases immediately after ingestion of food and stimulates hunger and food intake⁽²²¹⁾. Understanding how sorghum grain intake acts acutely, for example affecting these key satiety hormones, may help to justify and formulate hypotheses for the effects of chronic sorghum consumption in longer-term weight reduction intervention trials. In addition, consideration can be given to any observed effects in relation to known effects of individual components within sorghum, such as polyphenols, starches and fibre.

5.2 Study Aims and Hypothesis

The aim of this double-blinded, randomised, crossover feeding study was to test the effects of eating three different RTE whole grain sorghum flaked breakfast biscuits on appetite responses in healthy subjects. The biscuits were derived from white, red or brown whole grain sorghum, or wheat (control), all high in SDS levels but differing in polyphenolic content and dietary fibre levels (Chapter 4). It was hypothesised that the consumption of sorghum-based breakfast cereal foods would reduce appetite compared to a wheat control. Specifically, sorghum-based products would: (1) increase acute subjective satiety; (2) alter responses of appetite-regulating hormones (plasma ghrelin, GIP, GLP-1, PYY and insulin) and plasma glucose; and (3) reduce food intake at a subsequent meal and during the remainder of the test day. It was postulated that mechanisms involving polyphenolic, starch and dietary fibre components will synergistically drive satiety changes. A secondary aim was to review any differences in satiety outcomes between the sorghum varieties to better inform which components may drive any benefits of sorghum consumption when consumed as whole cereal foods.

5.3 Materials and Methods

5.3.1 Subjects

Healthy subjects, 20 males and 20 females, aged 18-50 years (BMI range from 20–31 kg/m²) were sought within the local community through paid media advertisements and institutional emails. Subjects with serious illness including diabetes, with known food allergies, restrictive eaters (identified using the TFEQ described in Chapter 3) and those who were smokers, were excluded. Pregnant,

breastfeeding and post-menopausal participants were also excluded. Height, weight, waist circumference and percentage body fat (Tanita Scales Model no.TBF-662) were recorded. A diet history (DH) and three-day weighed food records were collected from subjects prior to the start of the testing phase in order to design an ad-libitum meal within the subjects' taste preferences and based on familiar foods. Twenty-four hour dietary recalls were collected for the day prior to each study visit (Figure 5.1).

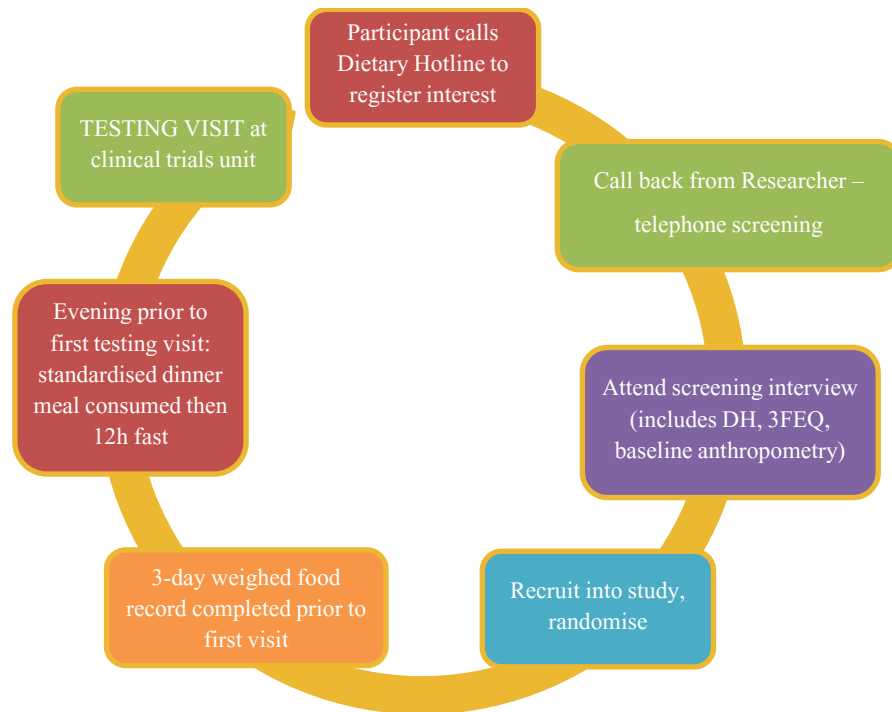


Figure 5.1 Participant engagement before randomisation into study and prior to first testing visit

5.3.2 Test Protocols

Subjects consumed four different breakfast cereal meals on four separate occasions after a 12-hour overnight fast with a minimum of three days between visits. Subjects consumed a standardised frozen meal for dinner the evening before testing, stratified

to subjects' usual energy intake. Subjects were advised to refrain from vigorous physical activity and alcohol consumption in the 24-hour period prior to testing. Female subjects were tested within the same phase of their menstrual cycle⁽³¹⁸⁾. The study was approved by the University of Wollongong Human Research Ethics Committee (application number HE12/410) and was prospectively registered with the Australian New Zealand Clinical Trials Registry (ACTRN12614000640606). See Figure 5.2.

5.3.3 Test Foods

The flaked whole grain biscuits were made from three different sorghum cultivars (red, white and brown grained) and a single wheat control, as per the protocol discussed in Chapter 4. Each test breakfast meal consisted of 3 biscuits (50 g total) served with 200 mL water. An additional 400 ml water was provided to participants during the course of the four-hour testing period. Analyses of proximate, dietary fibre, energy and polyphenolic contents of the test biscuits were completed in accordance with methods detailed in Chapter 4. Results of these analyses are also reported in Chapter 4, together with scanning electron microscopy studies.

At each study visit, participants were also instructed to complete a product evaluation form shortly after eating the test biscuits (Appendix 2). This comprised both open-ended questions and also 100mm VAS lines. Specifically, questions related to organoleptic properties of the biscuits and asked about subjects' perceptions of taste, texture, and crunch.

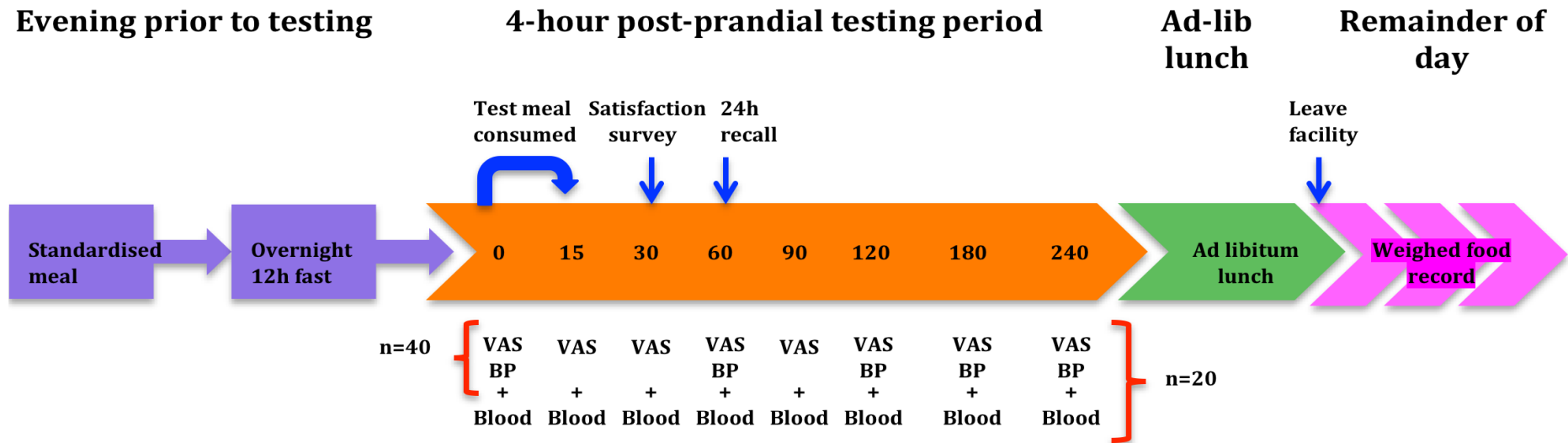


Figure 5.2 Overview of protocol for acute meal test study

5.3.4 Appetite Markers and Measures

On arrival to the clinical trials unit, subjects had anthropometric measurements and baseline blood pressure measured. A cannula was inserted and initial fasting blood samples were collected. The subject then ate one of the four test breakfast meals within a 10-min period. Fifteen minutes from commencement of the test breakfast meal, the next blood sample was taken. Further samples were then collected at 30, 60, 90, 120, 180 and 240 min. All biochemical analyses were performed by an accredited Pathology Laboratory, according to standard protocols for the respective assays. Glucose analysis was performed by the glucose hexokinase method (Roche Diagnostics, Australia). Analyses of insulin, active ghrelin, PYY and GIP were performed using a multiplexed Merck Millipore Human Metabolic Hormone Panel. Active GLP-1 was assayed using an Epitope Diagnostic Elisa kit. Plasma total antioxidant capacity (TAC) was measured using a method adapted for a Cobas Mira (Roche Diagnostics, Australia). Plasma was incubated with met-myoglobin and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid). After incubation, hydrogen peroxide was added and the sample incubated again. Absorbance was measured spectrophotometrically to determine TAC.

At each of the eight time-points for blood collection, subjects completed a four-question visual analogue scale (VAS) related to appetite⁽²¹⁷⁾. The questions were: How hungry do you feel at this moment? How satisfied do you feel at this moment? How full do you feel at this moment? How much do you think you can eat at this moment? The VAS consisted of a 100 mm horizontal line, anchored at each end with opposing extremes of the scale (for example, "not at all hungry" and "never been more hungry"). The study participants were instructed to place a vertical mark on the

line to correspond with their feelings. The subjective sensation was quantified by measuring the distance in millimetres from the left end of the line to the mark. Four hours after completion of the breakfast, a buffet lunch was served, consisting of a variety of sandwiches (cut into bite-size pieces), a cold pasta salad, dried fruit, yoghurt and water (totaling approximately 7500 kJ and containing 50% carbohydrate, 20% protein and 30% fat) (Figure 5.3). All the foods were made to standardised recipes and were weighed before the meal was consumed and at completion to enable calculation of the amount of food eaten.



Figure 5.3 Buffet (ad-libitum) lunch

5.3.5 Statistical Analysis

Sample size was calculated based on previous appetite studies measuring satiety-related biochemical markers and VAS scores. Flint et al.⁽²¹⁷⁾ suggested that 18 subjects should identify statistically significant changes using a paired design and a

study power of 0.8 if VAS ratings vary by at least 5 mm. However, Beck et al.⁽³⁰⁸⁾ concluded that a sample size of 37 would be required for results to reach statistical significance in a paired design with a power of 0.8 (alpha 0.05). Specifically for detection of biochemical differences, a sample size of 14, with seven subjects of each gender, was suggested as being sufficient to see significant results⁽²²⁰⁾. In consideration of these studies and the fact that sorghum has not been previously tested, a target sample size of 36-40 subjects of equal genders was set to undergo subjective satiety assessment through VAS, and a subset of 18-20 subjects to undergo biochemical analysis. Results for VAS scores, biochemical analyses and second-meal dietary intake were entered into SPSS for windows, Version 21.0 (SPSS Inc., Chicago, IL, USA). Differences in VAS responses between the breakfasts were identified using repeated measures analysis of variance (RMANOVA) with post-hoc Bonferroni adjustments. The area under the curve was assessed using trapezoidal methods for all biochemical data with values corrected for baseline and inclusive of all incremental area below the curve, as well as the area below the fasting concentration (net incremental AUC or net iAUC as previously described⁽³¹⁹⁾). Differences in net iAUC for glucose, insulin, ghrelin, GLP-1, GIP and PYY between the breakfasts were identified using RMANOVA with post-hoc Bonferroni adjustments. For meal intake values, RMANOVA of kilojoules consumed was performed. Data was analysed for individual and combined sexes as past studies have identified some gender-related differences in satiety markers⁽²²⁰⁾. Regression analysis relationships were explored between biochemical, subjective and meal intake data and 24 h dietary recalls. A $p < 0.05$ was considered statistically significant.

5.4 Results

5.4.1 Subjects

A total of 81 potential subjects expressed interest in the study, of which 65 were contacted to undergo further preliminary screening via telephone. From this group, 43 people were eligible for further assessment and attended an interview to determine final eligibility. A total of 40 subjects were then randomised into the study. All subjects attended the required four study visits, resulting in a 100% participant retention rate. Twenty male and twenty female subjects were aged 21-50 years (average ages were 27.7 and 31 years, respectively). Further subject characteristics are summarised in Table 5.1.

Table 5.1 Subject characteristics at baseline (mean \pm standard deviation)

	Male (n=20)	Female (n=20)
Age (years)	27.7 (\pm 6.921)	31.0 (\pm 8.891)
Age range (years)	21-43	21-50
Weight (kg)	80.50 (\pm 8.094)	63.84 (\pm 7.814)
Height (m)	1.84 (\pm 0.073)	1.67 (\pm 0.053)
BMI (kg/m²)	23.78 (\pm 2.025)	22.94 (\pm 3.00)
Waist circumference (cm)	83.05 (\pm 5.196)	75.85 (\pm 8.53)
Body fat (%)	16.81 (\pm 3.15)	28.35 (\pm 5.287)
Blood glucose (mmol/L)	4.71 (\pm 0.303)	4.80 (\pm 0.371)
Blood insulin (mmol/L)	254.60 (\pm 47.350)	289.50 (\pm 89.022)

5.4.2 Test Food Analysis

Test foods in the form of flaked cereal biscuits were consumed by subjects with no adverse reactions. Responses to the product evaluation questionnaire did not identify differences in subjects' perceptions about taste, texture and crunch between the different sorghum biscuits and the wheat control (data not presented), suggesting that

they were equally acceptable to the study sample. Analyses of proximate and dietary fibre composition and energy content, as well as the free and bound polyphenolic (PP) content (mg GAE/g *as is*) and antioxidant capacities ($\mu\text{molTE/g as is}$) of the test foods, were reported in Chapter 4, and confirm that the formulated RTE biscuits were suitable test foods for this trial. Specifically, analyses of proximate and dietary fibre composition and energy content (on *as is* basis per 100g) identified modest differences between the control and different sorghum varieties (Table 4.1), with the wheat control containing the highest soluble fibre levels (4%), followed by brown sorghum (2.3%), red sorghum (1.5%) and finally white sorghum (1.2%). Free and bound polyphenolic (PP) content (mg GAE/g *as is*) and antioxidant capacities ($\mu\text{molTE/g as is}$) of the test foods were also determined (Table 4.2). As expected, red and brown sorghum biscuits had the highest PP levels, due to the high anthocyanin and tannin contents previously reported for these varieties⁽²³⁾, and lower levels of total PP were reported for white sorghum and wheat biscuits. Assessment of starch properties showed no difference in the amount of total starch, RDS and SDS between biscuits (Table 4.3). RS was highest in the brown sorghum biscuit compared to all other biscuits ($p < 0.05$).

5.4.3 Baseline Measurements

Dietary recalls for the 24-hour period prior to each visit averaged 8867 kJ (4871 kJ – 15 780 kJ). Average intake for females was 7587 kJ and for males 9949 kJ. As expected, regression analysis indicated some contribution of total energy intake consumed in the previous 24 h to the prediction of total lunch time ad-libitum intake ($R^2 = 0.281$, $p < 0.001$), as well as intake during the remainder of the day ($R^2 = 0.153$, $p < 0.001$). No relationship was identified between the 24 h recalls and baseline

measurements of individual VAS questions, fasting glucose, insulin, ghrelin, GIP, GLP-1, PYY and TAC levels. There was some correlation between polyphenolic content of the standardised dinner meal consumed the evening prior to testing and baseline plasma TAC levels, adding support to the decision to standardise this meal in this study design (Table 5.2).

Table 5.2 Correlation between polyphenol content of standardised dinner meal and baseline plasma TAC

Correlations	Male (n=8)	Female (n=8)
Pearson Correlation (r)	0.863	0.451
Significance (2-tailed)	0.006	0.088
Effect size (Cohen's*)	Large	Medium

*Due to the small sample size, Cohen's values were applied to determine effect size.

5.4.4 Subjective Satiety

The measurement of appetite sensations using VAS showed the treatment effect was significant in all four questions ($p < 0.001$, RMANOVA of means for all questions) (Table 5.3). Pairwise comparisons using Bonferroni adjustments indicated differences primarily between the control (wheat biscuit) and all three sorghum biscuits (red, brown and white) for all four questions ($p < 0.05$ for all three comparisons in each question). No differences between sorghum biscuits were identified using Bonferroni adjustments for multiple comparisons. Overall, means of VAS responses showed significantly greater hunger and lower satiety ratings after consumption of wheat biscuits compared to all three sorghum treatments over the 4 h testing period (also shown in Figure 5.4).

Table 5.3 Mean VAS ratings (mm) for all 4 questions after intake of different breakfast cereal biscuits¹

Flaked cereal biscuit type	Q1 Hunger	Q2 Satisfaction	Q3 Fullness	Q4 Desire to eat
Wheat control	58.9 ± 24.9 ^a	36.6 ± 24.3 ^a	35.8 ± 24.5 ^a	65.4 ± 22.9 ^a
White sorghum	54.4 ± 26.7 ^b	41.9 ± 26.6 ^b	39.8 ± 27.2 ^b	61.7 ± 25.9 ^b
Red sorghum	52.8 ± 26.4 ^b	43.9 ± 26.6 ^b	42.9 ± 27.7 ^b	60.4 ± 26.1 ^b
Brown sorghum	53.9 ± 27.1 ^b	41.4 ± 27.6 ^b	41.1 ± 27.6 ^b	60.2 ± 26.9 ^b

¹Values within a column with different superscript letters denotes significant differences (p<0.001). Values with the same superscript letter denote no significant difference (p>0.05).

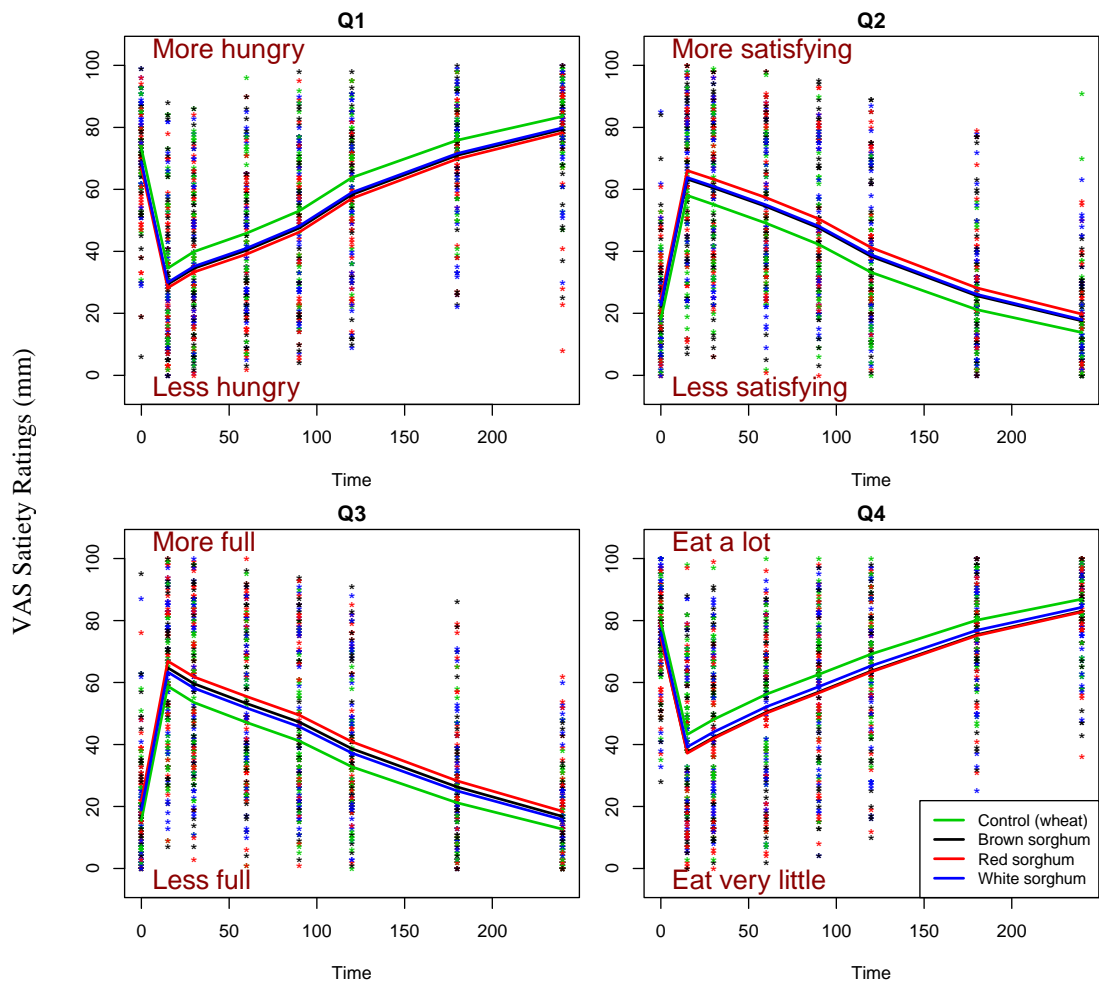


Figure 5.4 RMANOVA of mean satiety ratings(mm) for all 4 questions (Q1, Q2, Q3, Q4) after intake of different breakfast cereal biscuits at different time points over 4h^{1,2}

¹Q1, Q4: Increasing values on y-axis associated with greater hunger/lower satiety sensations

²Q2, Q3: Increasing values on y-axis associated with greater satiety/lower hunger sensations

5.4.5 Biochemical Indices

Between meal differences in iAUC postprandial responses of glucose, insulin and various gut hormones are shown in Table 5.4. RMANOVA of insulin iAUC data (corrected for baseline) indicated a significant difference between responses over the 4 h testing period ($p=0.016$). Post-hoc comparisons using Bonferroni adjustments showed differences of statistical significance between the red sorghum biscuit and the wheat control ($p=0.032$), with a greater insulin response after the red biscuit treatment.

Similar analysis of iAUC responses for the incretin gut hormones, GIP and GLP-1, also showed significant differences over 4 h ($p=0.031$ and $p=0.018$ respectively), identified using RMANOVA. Post-hoc comparisons using Bonferroni adjustments showed significant differences in GIP responses for the overall 4 h period only between red sorghum and the wheat control ($p=0.028$) (Figure 5.5d).

Although overall, between-treatment variations in GLP-1 responses were statistically different ($p=0.018$), Bonferroni post hoc comparisons only showed a trend towards a higher response after red sorghum biscuit intake compared to control ($p=0.127$), with no differences between the control and the other two sorghum varieties ($p>0.05$) (Figure 5.5c).

Overall, differences in PYY iAUC responses between treatments during the 4 h testing period did not reach statistical significance ($p=0.197$), although the greatest release of PYY was after the consumption of red sorghum biscuit (Figure 5.5e). When gender-specific data was reviewed using Bonferroni adjustments, the PYY

concentrations were significantly higher in males after red sorghum intake compared to wheat ($p=0.036$). No significant between-treatment differences were found in glucose and ghrelin responses during the testing period (Figure 5.5a and 5.5f). RMANOVA of TAC iAUC did not identify differences between treatments (data not presented). No significant correlations between biochemical indices and flaked biscuit properties were identified (data not presented).

5.4.6 Energy intake at ad-libitum lunch meal and for the rest of the day

No significant differences in lunch energy intakes were detected ($p=0.955$) for the group or individual genders between test diets (data not presented). Similarly, when analysing total energy content from the rest of the day, or that intake combined with the ad-libitum buffet lunch, no significant differences were detected (data not presented).

Table 5.4 Postprandial responses of glucose, insulin and various gut hormones over 4h (expressed as mean net iAUC \pm standard deviation)

		Wheat control	White sorghum	Red sorghum	Brown sorghum	p-value (RMANOVA)
Glucose	Δ mmol/L.min	67.03 \pm 84.03	47.17 \pm 85.94	55.30 \pm 90.28	47.68 \pm 85.94	0.841
Insulin	Δ pg/ml.min	53524.93 \pm 31550.26	52787.32 \pm 29370.34	69282.49 \pm 40375.97	64218.02 \pm 35412.16	0.016*
Ghrelin	Δ pg/ml.min	2229.28 \pm 4861.99	4690.35 \pm 5561.33	4224.62 \pm 4504.79	846.86 \pm 7357.46	0.361
GIP	Δ pg/ml.min	7200.08 \pm 3891.26	7892.01 \pm 3796.86	10155.30 \pm 3860.78	8853.11 \pm 3722.51	0.031*
GLP-1	Δ pM.min	-30.69 \pm 211.62	82.57 \pm 124.70	104.19 \pm 107.98	72.71 \pm 113.74	0.018*
PYY	Δ pg/ml.min	1025.66 \pm 4035.30	2489.46 \pm 5093.02	3610.03 \pm 2739.10	2387.58 \pm 3043.18	0.197

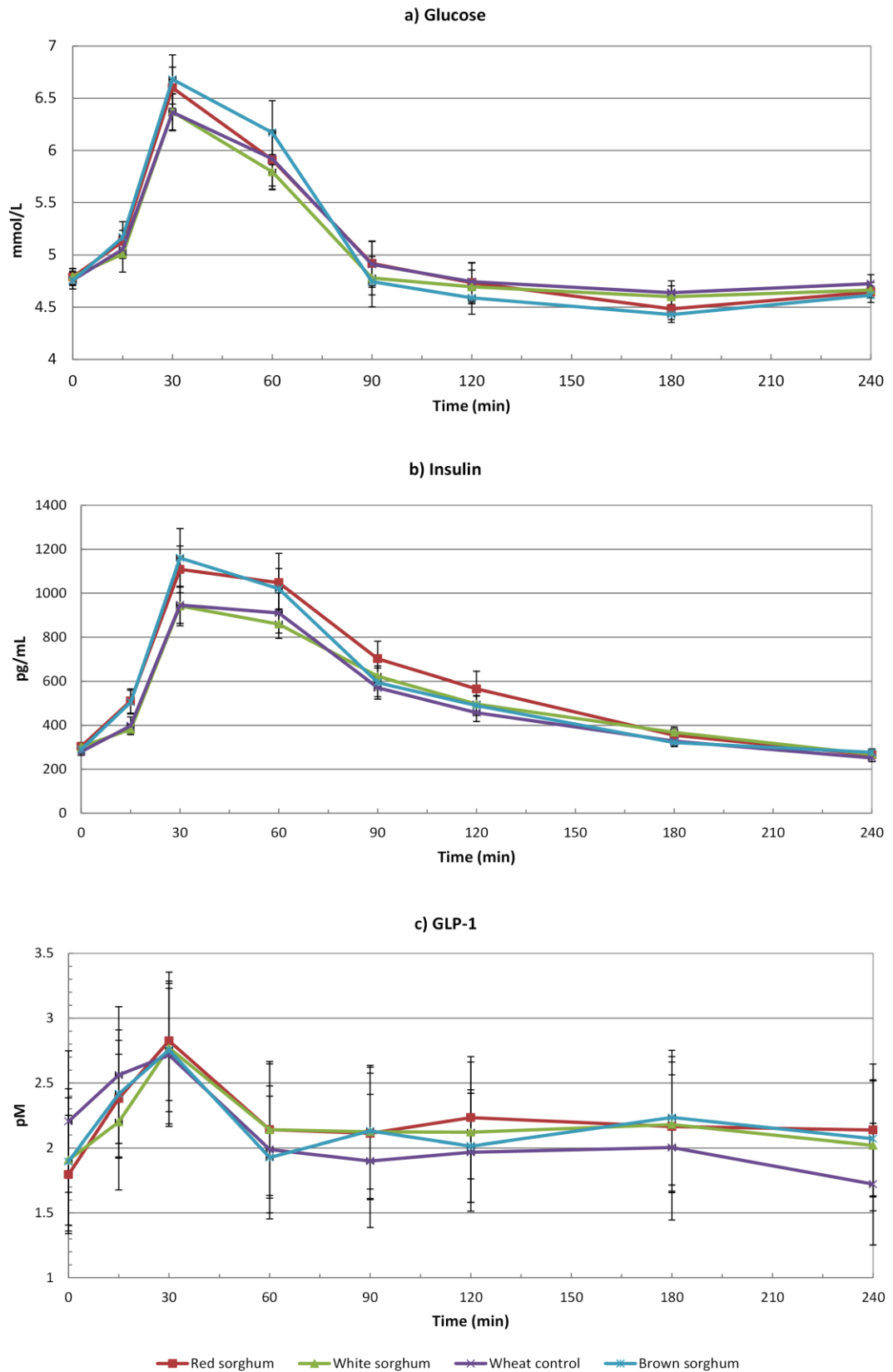


Figure 5.5 Changes in mean plasma concentrations of (a) glucose, (b) insulin, (c) GLP-1 over time

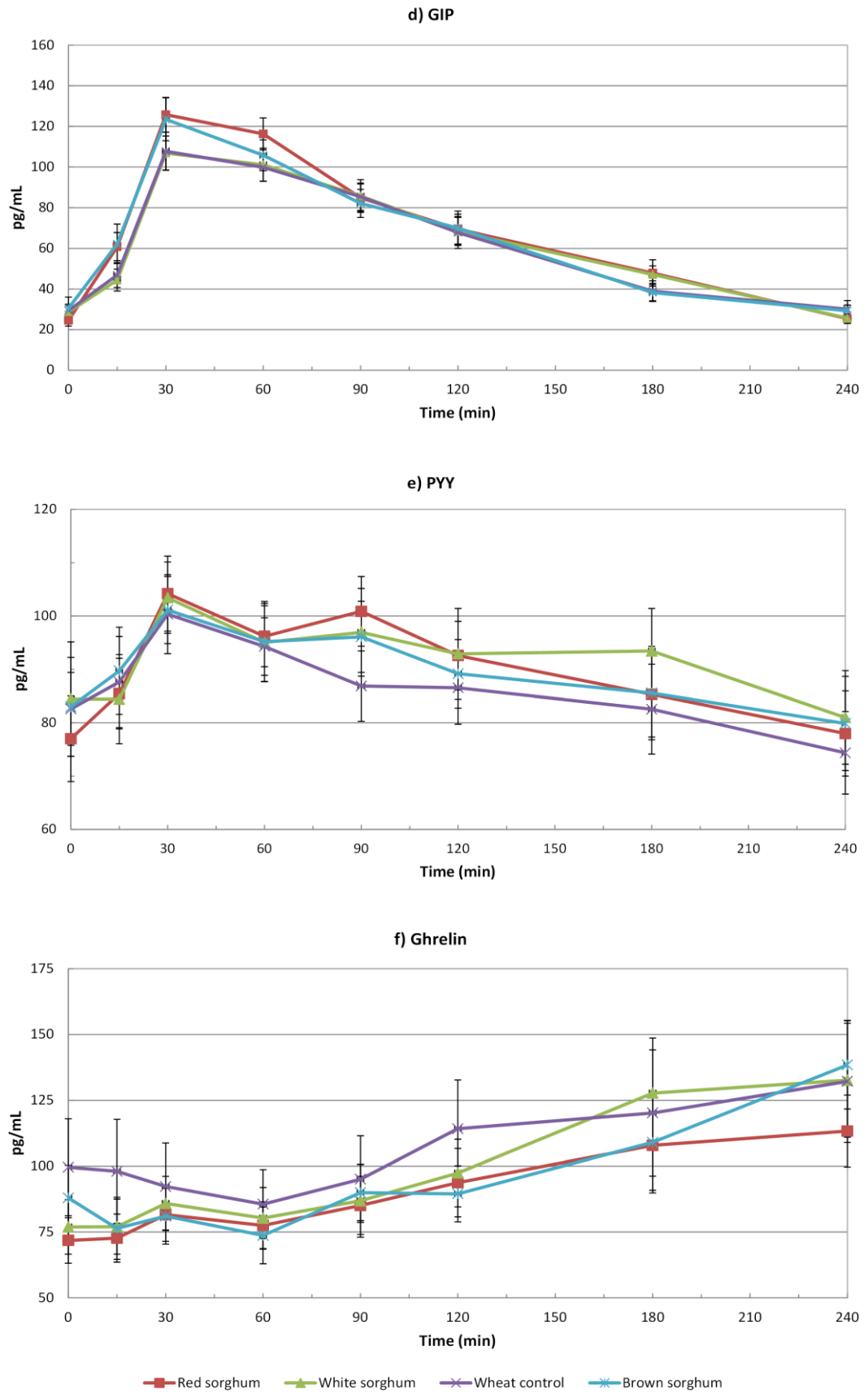


Figure 5.5 Changes in mean plasma concentrations of (d) GIP, (e) PYY and (f) ghrelin over time

5.5 Discussion

The VAS ratings for subjective appetite/hunger showed that sorghum flaked breakfast biscuits significantly decreased hunger and increased satiety sensations compared to wheat, and in particular the red sorghum showed variations in appetite hormones associated with satiety. Although this did not translate to differences in intake at the subsequent ad-libitum meal, the presence of wide standard deviations in the food intake data indicated there may have been a lack of power to detect differences at this level. Nevertheless, the subjective satiety results align with traditional experiences of some African communities who report feelings of satiety after eating sorghum foods⁽²³⁾.

Enhanced satiety has been attributed to SDS in sorghum grain and their role in lowering glycaemic index (GI) of sorghum foods⁽¹⁶⁹⁾, particularly those made from polyphenol-rich sorghum varieties⁽⁶²⁾. In our study, it seems SDS in isolation did not play a role in satiety-enhancing mechanisms between treatments, because SDS levels were high in all the flaked biscuits (Chapter 4)⁽³²⁰⁾. RS has been shown to increase satiety⁽⁷³⁾, however in the present study the higher RS levels in the brown sorghum biscuits compared to wheat did not coincide with greater satiety responses post-prandially. Hence, we must turn to other non-starch components present within the food matrix of the sorghum biscuits, such as dietary fibre and polyphenolic compounds concentrated in the bran component of the grain, to explain the observed differences in satiety effects in this study.

In general, foods rich in fibre may promote satiety due to bulking⁽²⁸³⁾, relatively low energy density⁽³²¹⁾ and increased viscosity of intestinal contents, delaying gastric

emptying and slowing absorption of macronutrients⁽³²²⁾. Fibre may also affect secretion of gut peptides, independent of glycaemic responses, which may act as satiety-enhancing factors⁽³²¹⁾. The total dietary fibre in the sorghum biscuits was relatively high, ranging from 7.3-9.9%, and similar to the control food, wheat (9.2%). The soluble fibre, often associated with acute satiety, was highest in the wheat so it is also difficult to specifically ascribe effects on satiety from sorghum to the fibre in these food products. The absolute differences in total and soluble fibre between the wheat biscuit and the red biscuit, for example, was modest at 0.95 g and 0.14 g per serve, respectively. These doses of fibre are possibly too small to drive experimentally discernable differences in satiety outcomes. Future dose response studies, with specific manipulation of the fibre types (soluble and insoluble) found in sorghum, would be useful to more precisely investigate the role of fibre in satiety-enhancing mechanisms of sorghum-based foods.

Dietary polyphenols have also been implicated in appetite regulation mechanisms, mainly through the inhibition of α -amylase and α -glucosidase, the key enzymes responsible for digestion of dietary carbohydrates to glucose⁽⁶⁶⁾. The sorghum biscuits were confirmed to have higher levels of total and free polyphenolic components compared to the wheat biscuit, however no correlations between polyphenolic contents and clinical measures were identified. It is interesting that the red sorghum showed the greatest alteration in appetite hormones, but this did not translate to differences in the subjective appetite responses in comparison to the other sorghums, only to the wheat control. This indicates that the specific combination of components in the red sorghum biscuit requires further investigation. Mechanisms invoked by polyphenolic components and dietary fibre, for example, may not be

discernable in appetitive and digestive responses over four hours⁽³²³⁾. Hence, review of hormones over greater periods of time in a study with chronic ingestion, and with different sorghums varieties, would be useful. This would also help to identify potential differences in hormone release for acute versus chronic sorghum consumption.

Of note, plasma glucose responses were not altered. Despite similar glucose responses, satiety ratings differed significantly between sorghum and wheat biscuits, supporting the case for insulin (and not glucose) being a more likely mediator of satiety sensations in the postprandial period in normal weight individuals^(275; 281). Flint et al.⁽²⁸¹⁾ showed in a meta-analysis that insulin response was significantly and inversely related to post-prandial hunger in normal-weight subjects, particularly decreasing *ad libitum* energy intake at a second meal. In the present study, consumption of red sorghum biscuits produced the highest insulin response, as well as the lowest ratings of hunger. Interestingly though, insulin was lower after the white sorghum biscuit compared to the red sorghum biscuit but resulted in the same subjective satiety ratings, so this again does not explain the entire mechanism.

Postprandial GLP-1 and GIP responses were also higher for all sorghum biscuits compared to the wheat. GLP-1 in particular has an important role in attenuating post-prandial glucose rises and is involved in the *ileal break reflex*, having an anorectic effect on appetite⁽²⁶²⁾, with some studies showing reduced food intake when it is peripherally administrated⁽²⁶⁴⁾. However, positive changes in GLP-1 and GIP in both sexes, and PYY levels in men, did not translate to decreased food intake at a second meal in the present study.

The key limitation in interpreting results from the present study was the inability to detect significant differences in food intake data at the ad libitum meal and for the rest of day. A larger sample size may have enabled these differences to emerge and to corroborate the significant subjective satiety ratings (VAS scores) and also the biochemical indices (insulin, GIP, GLP-1, PYY). Importantly, such a small breakfast as the test meal, followed by 4 hours of fasting, may have increased hunger to an extent that blunted any subtle differences in food intake. Factors that relate to non-biological drivers of appetite and food intake such as environment, social and cognitive influences are also relevant to consider in the present study. For example, the amount of food offered in the ad-libitum lunch was deliberately in excess of participants' usual intake and consisted of a variety of appealing food items that may have led to more food being eaten simply because the food was in abundance and pre-prepared. Differences in fibre content between biscuits, although modest, limited the ability of researchers to confidently ascribe effects to specific grain components such as polyphenolics. While controlling for fibre content may more clearly elucidate mechanisms in future work, the present study defined results within a standard serve size, relevant to consumers.

5.6 Conclusions

In conclusion, exploration of the use of sorghum whole grain as an ingredient in the formulation of foods targeted for weight control through appetite regulation is justified in future research. In the present study, whole grain sorghum-based flaked cereal biscuits (red-, white- and brown- grained) were formulated and were shown to increase short-term subjective satiety ratings compared to a wheat biscuit control. Differences in polyphenolic contents between the red, white and brown sorghum

flaked biscuits appear to play a role in satiety-enhancing mechanisms, although their precise actions are not clear. Actions of SDS and dietary fibre may contribute to these effects, reflecting the benefits of the whole cereal food delivering synergistic effects of individual grain components into the body. Additional mechanisms involving interactions between insulin and the incretin hormones, GLP-1 and GIP, as well as PYY, may be contributory factors to enhanced satiety effects of sorghum flaked biscuits. Choice of a test food based on red sorghum is justifiable in randomised controlled trials that aim to directly examine effects of sorghum intake on chronic disease biomarkers or health outcomes. Chapter 6 describes a 3-month intervention trial examining these outcomes.

CHAPTER 6:

Examining the longer-term effects of chronic sorghum consumption: a randomised controlled trial

The majority of this chapter is the substantive content of the article:

Stefoska-Needham, A., Beck, E.J., Johnson, S.K., Batterham, M., Grant, R., Ashton, R., Tapsell, L.C. (2016) *A diet enriched with red sorghum flaked biscuits, compared to a diet containing white wheat flaked biscuits, does not enhance the effectiveness of an energy-restricted meal plan in overweight and mildly obese adults.* Journal of the American College of Nutrition. (Accepted for publication)

6.1 Introduction

The acute satiety study described in Chapter 5 showed that flaked biscuits made from red sorghum grain, a variety rich in anthocyanin flavonoids, elicited the greatest overall acute satiety-enhancing responses. Neither the dietary fibre nor the starch contents of the biscuits could explain the responses, but overall differences in polyphenol contents, or a synergy between polyphenols, dietary fibre and SDS, may have played a role. While these initial acute satiety results were promising, longer-term effects on chronic disease biomarkers or health outcomes from a sorghum-enriched diet required investigation using a “gold-standard” RCT design. To maintain the integrity of the methodological framework used to explore the key hypothesis, the equivalent flaked breakfast biscuits were used in this RCT, with the obvious choice of red sorghum as the treatment on account of its superior acute satiety effects. The RCT results are important to provide the level of evidence necessary to guide clinical practice, inform science-based food messages (such as those found in Dietary Guidelines and health claims) and to support commercial investments by food industry and hence product development.

6.2 Study Aims and Hypothesis

This study was a 3 month randomised controlled dietary intervention trial, aiming to test effects of whole grain red sorghum included in an energy-restricted meal plan. The primary outcome was difference in weight loss between the control and intervention groups. Secondary outcomes included a variety of biochemical measures linked with change in body weight and disease markers, such as glucose, insulin, cholesterol and various anti-inflammatory/oxidative stress markers, as well as subjective satiety measures. Overall, overweight individuals following a

nutritionally-balanced, energy-restricted diet including sorghum whole grain foods would be expected to lose more weight than if they followed the same diet without sorghum-based foods, driven by reduced energy intake as a result of satiety actions of sorghum grain. Specifically, it was hypothesised that: (1) subjects receiving foods made from whole grain sorghum would lose more weight than the subjects receiving control foods (wheat-based); (2) weight loss would be accompanied by changes in metabolic disease risk factors, which may be anticipated given the higher polyphenolic content of sorghum.

6.3 Materials and Methods

6.3.1 Subjects and Recruitment

This study was a 3 month, double-blinded, parallel, randomised controlled trial with a total of 60 subjects, 46 females and 14 males. There were two arms to the study: *sorghum intervention* and *wheat control*, with both groups receiving advice on an energy-restricted diet from an Accredited Practising Dietitian. All subjects were provided with cereal products, in the form of flaked breakfast biscuits, to include in their diets (Figure 6.1). The target sample size of 30 subjects per group was based on previous human intervention studies incorporating cereal/high fibre foods into hypocaloric diets⁽³²⁴⁻³²⁶⁾. Difference in weight loss between groups was used as the primary outcome measure to establish power for the study. To observe a two-kilogram weight difference between groups, at a power of 80% and to be significantly different at level of α 0.05, 26 subjects would be required per group. Therefore, setting the recruitment target at 30 subjects per group would account for an approximate dropout rate of 15%.

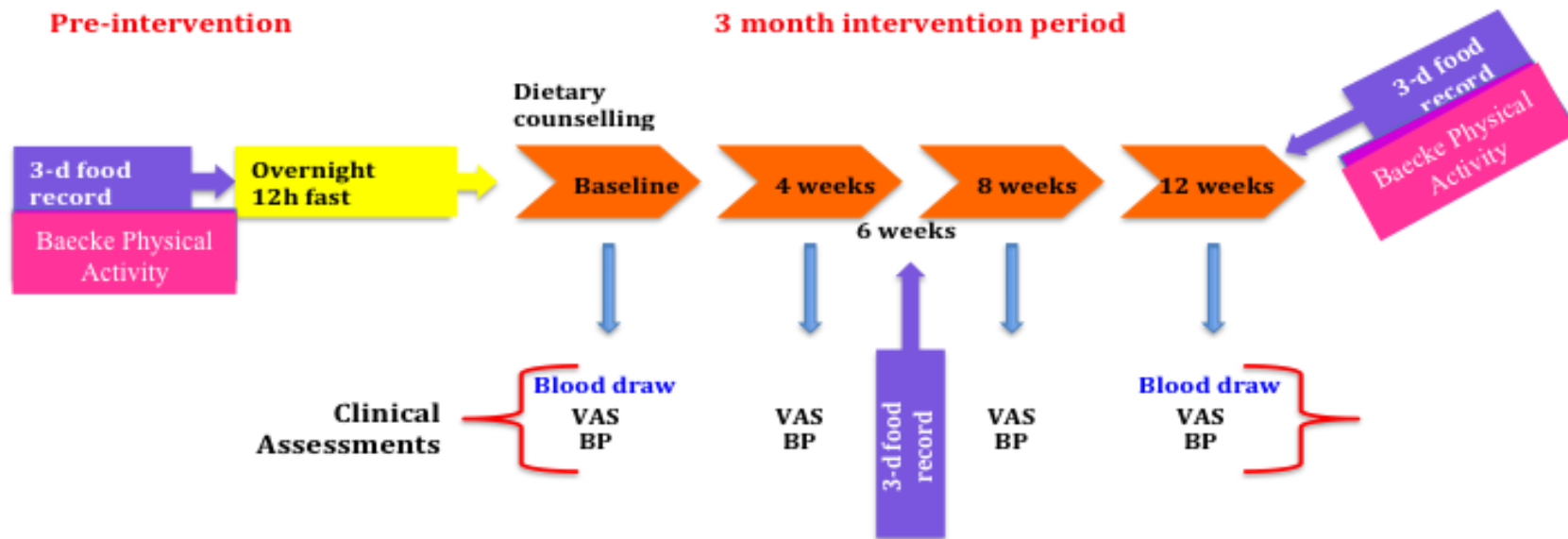


Figure 6.1 Overview of protocol for RCT

Eligible subjects (aged 18–65 years, BMI range from 25–35 kg/m²) were sought through paid media advertisements and institutional emails. Subjects with serious illness including diabetes, with known food allergies, and those taking appetite-altering medications were excluded from the study. Restrictive eaters (identified using the TFEQ described in Chapter 3) and subjects who were smokers, were pregnant, breastfeeding and post-menopausal were also excluded. All procedures were approved by the University of Wollongong, Human Research Ethics Committee (approval number HE14/100). The trial was also prospectively registered with the Australian New Zealand Clinical Trials Registry (#12614000640606). Figure 6.1 provides an overview of the study protocol.

A total of 115 potential subjects expressed interest in the study of which 103 were contacted to undergo further preliminary screening via telephone (Figure 6.2). From this group, 80 people were eligible for further assessment in the form of a detailed screening questionnaire. This led to sixty-one people attending an interview to determine final eligibility with sixty subjects randomised into the two study groups by a researcher independent of the subject interface, stratified by sex, block randomised using STATA Version 12 and using the RALLOC command (StataCorp, 2011, College Station, Texas, USA). The researcher/dietitian (myself) and all subjects at the subject interface were blinded to the randomisation. The subjects attended the clinical research trials unit (CRTU) at the University of Wollongong, Australia, on five occasions. The first visit included collection of background dietary data using a validated diet history interview⁽³²⁷⁾ and instruction on the completion of a 3-day weighed food record. The subsequent visit included collection of fasting blood samples (baseline) and dietary education. Two follow-up dietary visits

occurred at the 1-month and 2-month time-points, with collection of fasting blood samples at 3 months (final visit). All the subjects had their height, weight, percentage body fat (Tanita Scales Model no.TBF-662) and waist circumference recorded at each visit to the CRTU. Visual analogue scales (VAS) related to appetite⁽²¹⁵⁾ and food records were all completed at baseline, half-way through the study (at the 6-week point) and within the final week of the study. The Baecke Physical Activity Questionnaire⁽³²⁸⁾ was completed at baseline and at the completion of the study.

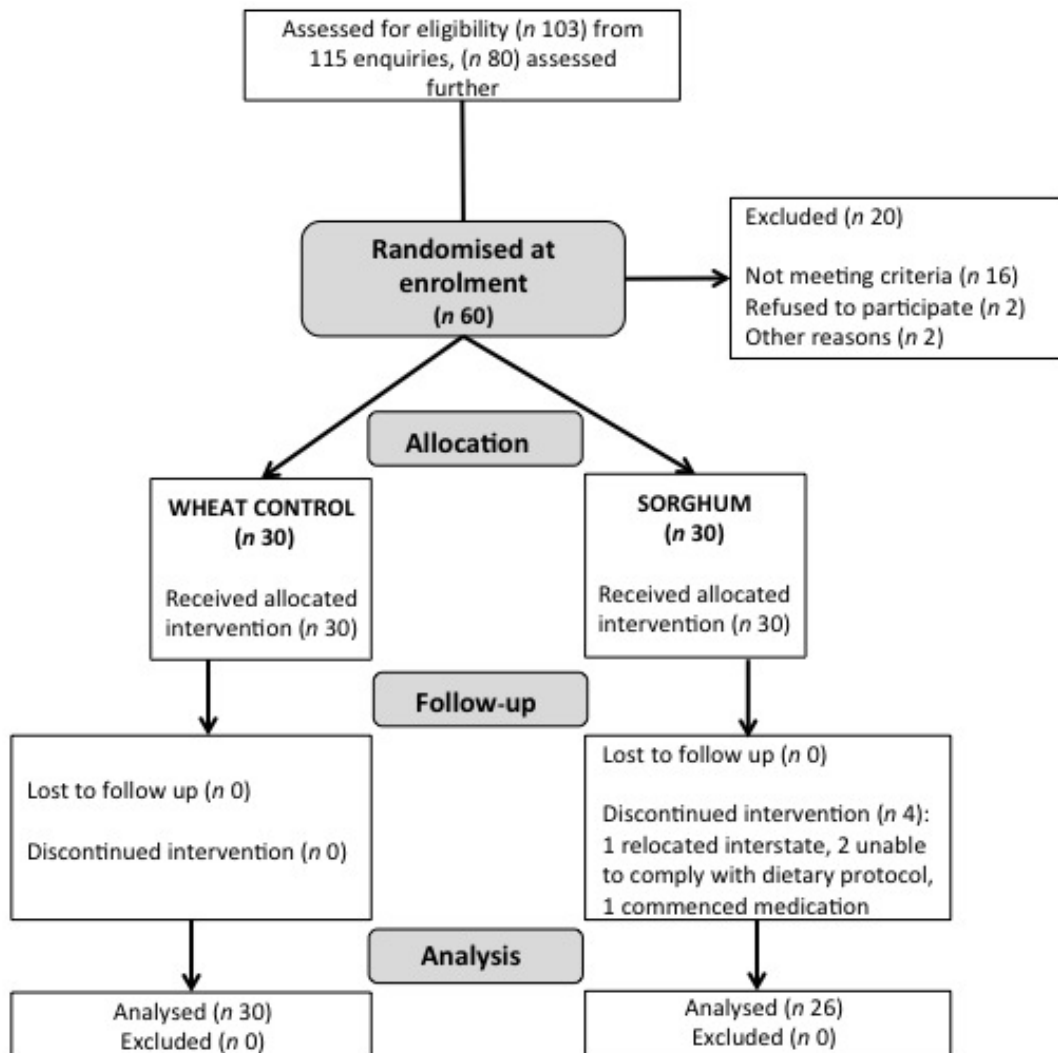


Figure 6.2 Flow of participants through the trial

For the appetite VAS, the subjects recorded their appetite responses on individual forms at six different time- points throughout the day: immediately before each of the three main meals and 2 h after each of these meals but before their subsequent snack. The VAS consisted of a 100 mm horizontal line, anchored at each end with opposing responses (for example, "not at all hungry" and "never been more hungry"). The study participants were instructed to place a vertical mark on the line to correspond with their feelings and the subjective sensation was quantified by measuring the distance in millimeters from the left end of the line to the mark. All nutritional analysis was performed using FoodWorks 2007, version 5 (Xyris Software, Brisbane, QLD, Australia) with nutrient contents of study foods added as required.

6.3.2 Dietary Intervention

For each subject, a basal metabolic energy requirement was calculated using the Mifflin-St Jeor equation where a BMI equal to 25 kg/m² was included⁽³²⁹⁾. A low activity factor (1.3) was chosen to estimate energy requirements (given the overweight sample with limited usual physical activity) and then this level was reduced by 20% to drive weight loss. Intervention diets were designed to control for all macronutrients with the only variation being the trial cereal products given, as described in Table 6.1. The sorghum intervention group received sorghum-based cereal biscuits and the wheat control group received wheat-based cereal biscuits, matched by serve size (3 biscuits) and therefore overall mass (45 g per day).

Table 6.1 Dietary prescription (totaling 5, 500kJ) for a typical participant

Food Group	Portion or serve size	Number of serves / day
Control or intervention products	1 flaked cereal biscuit equivalent to 15g	3 (2 biscuits at breakfast and 1 at afternoon tea)
Breads/cereal/starchy vegetables	1 slice bread or 1/2 cup cooked pasta or 1/3 cup cooked rice or 1 small potato	4
Other vegetables	1/2 cup cooked or 1 cup raw	2.5 cooked or 5 raw
Fruit	1 piece fresh or 3/4 cup canned and drained	2
Milk / alternatives	150ml light or 200ml skim, 100g low-fat yoghurt	2-3
Meat / alternatives	30g meat or 50g fish or 20g low fat cheese	3-4
Fats	1 tsp oil or margarine or 1 tbsp avocado	3-4

6.3.3 Test food products

Test foods were manufactured in the form of RTE flaked cereal biscuits, as for the acute test study (method detailed in Chapter 4). The sorghum flaked cereal biscuits were processed from the same red sorghum grain, variety Alpha, grown and supplied by Lochabar Enterprises Pty Ltd (Tara, QLD, Australia). The wheat flaked cereal biscuits were made from non-cultivar-specific Australian Prime White (APW) wheat (grown in central New South Wales, Australia) and was again supplied by Sanitarium Health and Wellbeing (Cooranbong, NSW, Australia). The subjects were instructed to eat two cereal biscuits (30g) at breakfast and one biscuit (15g) at afternoon tea daily. The proximate, dietary fibre and energy content (on *as is* basis per 100g) (Table 6.2), polyphenolic (PP) content (mg GAE/g *as is*) (Table 6.3) and starch properties (per 100g *as is*) (Table 6.4) between the sorghum and wheat biscuits were reported and were analysed according to the methods described in Chapter 4.

Analyses identified modest differences between the control and red sorghum biscuits with respect to energy, macronutrients and dietary fibre (Table 6.2). Notably, both total and soluble fibre levels were higher in the wheat control compared to the sorghum biscuits, however the actual difference between biscuits was modest at 0.86 g and 0.13 g per 45 g test serve, respectively. Individual macronutrient levels were not controlled for because this study was designed to test whole foods as consumed in real-life settings, hence the serve size rather than specific components were matched. As expected, red sorghum biscuits had higher PP levels compared to the control (Table 6.3), most likely due to high anthocyanin contents characteristic of this sorghum variety⁽³³⁰⁾. Assessment of starch properties showed no difference in the

amount of total starch, rapidly digestible starch (RDS) and slowly SDS between biscuits, and in particular the levels of SDS in each serve classified as high (Table 6.4).

Table 6.2 Proximate and dietary fibre composition and energy content of flaked breakfast cereals^{1,2,3,4,5}

Flaked breakfast cereal type	Moisture (g/100g as is)	Protein (g/100g as is)	Fat (g/100g as is)	Ash ³ (g/100g as is)	Total dietary fibre (g/100g as is)	Soluble Fibre ⁴ (g/100g as is)	Total available carbohydrates ⁵ (g/100g as is)	Energy content (kJ/100g as is)
Wheat (control)	6.6±0.0	13.7±0.2 ^b	1.7±0.0 ^a	1.6±0.0	9.6±0.1 ^b	4.0	66.9±0.1 ^a	1508±1 ^a
Sorghum (red)	6.6±0.0	9.4±0.1 ^a	2.9±0.1 ^b	1.6±0.0	7.7±0.0 ^a	1.5	71.9±0.1 ^b	1550±0 ^b

¹Means (n=2) ± standard deviation

²Values within a column with the same superscript letter denotes no significant difference (p>0.05)

³Statistical comparison not possible as some replicates identical

⁴Analysis provided by manufacturer – standard deviations not available and statistical comparison not possible

⁵Available carbohydrate calculated by difference

Table 6.3 Free, bound and total phenolic content and antioxidant capacity of flaked breakfast cereals^{1,2}

Flaked breakfast cereal type	Free PP (mg GAE/g as is)	Bound PP (mg GAE/g as is)	Total PP (mg GAE/g as is)	Free AC (µmolTE/g as is)	Bound AC (µmolTE/g as is)	Total antioxidant capacity (µmolTE/g as is)
Wheat (control)	0.71±0.01 ^a	0.67±0.22 ^a	1.37±0.21 ^a	2.64±0.01 ^a	2.67±0.01 ^a	5.31±0.01 ^a
Sorghum (red)	1.27±0.01 ^b	1.00±0.11 ^a	2.27±0.12 ^b	11.25±0.57 ^b	9.16±0.62 ^b	20.40±1.18 ^b

¹Means (n=2) ± standard deviation

²Values within a column with the same superscript letter denotes no significant difference (p>0.05)

PP, polyphenolic; AC, antioxidant capacity; GAE, gallic acid equivalents; TE, Trolox equivalents

Table 6.4 Starch properties of flaked breakfast cereals^{1,2,3}

Flaked breakfast cereal type	Total starch ³ (g/100g as is)	Rapidly digested starch (g/100g dry starch)	Slowly digested starch (g/100g dry starch)	Resistant starch (g/100g dry starch)
Wheat control	54.2 ± 0.2 ^a	31.9±1.6 ^a	47.9±4.2 ^a	20.2±3.9 ^a
Sorghum (red)	57.7 ± 2.3 ^a	31.9±4.2 ^a	46.9±7.7 ^a	21.2±3.6 ^a

¹Means (n=2) ± standard deviation²Values within a column with the same superscript letter denotes no significant difference (p>0.05)³Total starch was determined using a Megazyme K-TSTA 09/14 Total Starch Kit (Bray, Ireland) which excludes low molecular weight starch hydrolysis/breakdown products that may have resulted during the manufacturing process

6.3.4 Clinical Indices

Fasting blood samples were collected at 0 and 3 months into clot gel tubes, centrifuged and stored at -80° Celsius until analysis could be completed according to standard protocols for glucose, insulin, total cholesterol, high-density lipoprotein (HDL-c), low-density lipoprotein (LDL-c), triacylglycerides (TAG) and high-sensitivity c-reactive peptide (hsCRP) at Sydney Adventist Hospital Pathology (Wahroonga, NSW, Australia). Glycosylated haemoglobin (HbA1c) was performed by Southern Pathology (Wollongong, NSW, Australia) after samples were collected into tubes containing potassium EDTA and stored at 4° Celsius until analysis was conducted. Additional markers for inflammation and oxidative stress, Interleukin 1 β (IL1 β), Interleukin 6 (IL-6), Interleukin 8 (IL-8), hydroperoxide (HPX), tumour necrosis factor alpha (TNF α) and total antioxidant capacity (TAC) were performed by the Australasian Research Institute, (Sydney Adventist Hospital, Wahroonga, NSW, Australia) according to established protocols.

6.3.5 Statistical Analysis

Data for all anthropometry, blood analysis, VAS measurements and dietary intake were entered into SPSS for windows, version 21.0 (IBM SPSS 21.0, IBM

Corporation, Armonk, NY, USA). Repeated measures ANOVA (RMANOVA) using the general linear model with group (control, sorghum) as the between subjects factor, was used to identify primary changes in each parameter over time and also differences between the groups. Post hoc analysis using Bonferroni adjustments was reviewed to detect specific differences between the control and intervention groups. Regression analysis was used to identify correlations between group anthropometric and biochemical indices.

6.6 Results

6.6.1 Baseline Data

There were twenty-six subjects in the sorghum intervention group (after four subjects withdrew) and thirty subjects in the control group. Reasons provided for their withdrawal were: relocation, commencement of a contraindicated medication and the lack of motivation to adhere to an energy-restricted meal plan. For the final analysis, fifty-six subjects were included, although numbers in each calculation varied because some subjects did not complete all forms (food records or VAS). There were no significant differences between group anthropometric, dietary and metabolic measures at baseline (Table 6.5), except for a slightly lower protein intake in the sorghum group. Baseline energy intakes seemed low, suggesting under-reporting by some subjects. Consequently at subsequent study visits, dietitians specifically addressed potential under-reporting with subjects when reviewing their individual food records. The subjects were mildly obese but overall they did not exhibit impaired glucose tolerance or insulin resistance⁽¹⁵⁾, nor were they hyperlipidaemic⁽³³¹⁾ or hypertensive⁽³³²⁾.

Table 6.5 Baseline characteristics of study subjects (means and standard deviations)

VARIABLE	CONTROL		SORGHUM		DIFFERENCE
	Mean	SD	Mean	SD	<i>p</i> -value
Age (years)	48.6	11.4	48.1	10.3	0.859
Weight (kg)	86.1	9.8	87.1	12.9	0.732
BMI (kg/m ²)	31.6	2.8	31.2	3.5	0.632
Waist (cm)	105.3	9.5	102.5	12.0	0.311
Fat (%)	40.3	5.8	38.7	6.3	0.309
Fasting Blood glucose (mmol/L)	5.2	0.4	5.3	0.6	0.297
Fasting Insulin (mIU/L)	12.5	5.7	12.3	6.9	0.893
HbA1c (%)	5.3	0.3	5.2	0.4	0.554
Total Cholesterol (mmol/L)	5.6	1.0	5.3	1.0	0.173
LDL-c (mmol/L)	3.5	0.9	3.2	0.8	0.193
HDL-c (mmol/L)	1.4	0.4	1.5	0.3	0.533
TAG (mmol/L)	1.6	0.8	1.3	0.7	0.155
IL1 β (pg/ml)	0.9	1.0	0.9	0.9	0.841
Il-6 (pg/ml)	1.5	1.6	0.8	0.7	0.052
Il-8 (pg/ml)	6.2	2.8	5.2	1.8	0.096
TNF α (pg/ml)	2.9	1.3	3.2	1.2	0.483
hsCRP (mg/L)	2.7	2.6	2.4	2.1	0.662
TAC (mmol/L Trolox)	1.2	0.2	1.3	0.2	0.476
HPX (mmol/L H ₂ O ₂)	2.5	0.5	2.4	0.6	0.495
Diastolic BP (mm Hg)	82.3	9.6	80.4	7.4	0.407
Systolic BP (mm Hg)	126.2	16.9	122.3	14.4	0.349

6.6.2 Dietary Intervention

The flaked cereal biscuits were consumed by subjects with no adverse reactions and dietary compliance was relatively high at approximately 85%, as determined by a manual count of uneaten biscuits upon study completion. Food record analysis indicated no significant difference between the groups for protein, carbohydrate and fat intakes at any of the time-points once the study commenced. Review of the overall dietary composition of baseline diets compared with the 3 month end-point showed a significant decrease in percentage energy from fat ($p < 0.001$), as well as saturated fat ($p < 0.001$). Percentage energy from protein and carbohydrate was not altered over time (Table 6.6).

Compliance with a weight reduction meal plan was identified by overall weight loss and energy reduction reported in the food records. Total energy intake was significantly lower at the mid-point (mean 7136kJ (SD 2164 kJ)) and the end-point (mean 6880 (SD 1815) kJ) ($p < 0.001$) of the trial compared with the baseline (mean 8742 (SD 2656) kJ), using RMANOVA with treatment as the between-group effect and time as the within-subject variant. The lack of an interaction effect over time ($p = 0.671$) suggests that there were no differences between groups in energy intake over time. There were no significant differences in activity scores between the groups at any time-point, indicating that changes in physical activity were not a factor in influencing overall results.

6.6.3 Clinical Indices

The majority of the anthropometric and biochemical indices changed over 3 months; however, there were no significant differences between the groups for any of the measured clinical parameters over this time (Table 6.7). The average weight loss for the study sample was significant at 5.3 kg ($p < 0.001$) (or 6.1% of baseline body weight) over the 3 month intervention. The mean weight changes ranged from a 2.1 kg gain to a 14.7 kg loss, however weight loss was not significantly different between the groups ($p = 0.369$). Waist measurements and percentage body fat decreased significantly compared to baseline ($p < 0.001$). Regression analysis indicated that weight loss significantly predicted waist change ($p < 0.001$) and percentage body fat ($p < 0.001$). As expected, these predictions were strong (R^2 0.389 and R^2 0.615). Fasting blood glucose ($p < 0.001$) and fasting insulin levels decreased over 3 months ($p < 0.001$) but HbA1c did not change. No between group differences

Table 6.6 Energy and macronutrient intakes reported by subjects at baseline, mid-point and 3 months, with p-values for repeated measures ANOVA between control and sorghum groups (mean values and standard deviations)

VARIABLE	BASELINE				MID-POINT				3 MONTHS				P-VALUES		
	CONTROL		SORGHUM		CONTROL		SORGHUM		CONTROL		SORGHUM		Group	Time	Interaction
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
Energy (kJ)	9274.0	2696.2	7897.3	2429.8	6993.0	2244.8	7363.9	2076.2	6752.8	2090.6	7082.0	1297.1	0.110	<0.001*	0.671
Protein (g)	106.6	33.2	89.5	30.8	88.9	35.0	95.4	32.6	81.7	26.2	89.6	24.7	0.049*	0.008*	0.909
% E Protein	20.0	5.2	19.5	4.1	22.4	6.5	21.9	4.0	20.7	3.4	21.5	4.1	0.56	0.059	
CHO (g)	234.6	84.5	190.6	60.7	187.8	74.4	181.4	43.5	177.9	56.8	183.2	40.9	0.144	0.040*	0.306
% E CHO	39.9	5.1	39.1	7.09	42.6	8.3	40.0	5.2	42.6	6.3	41.5	6.0	0.811	0.161	
Total Fat (g)	85.1	25.7	77.2	31.5	54.9	23.5	61.9	26.0	57.1	23.8	57.6	15.3	<0.001*	0.381	0.979
% E Total Fat	34.0	5.2	35.5	5.3	28.5	5.4	30.4	4.9	30.8	7.0	30.1	5.2	0.435	<0.001*	
SFA (g)	34.3	12.4	29.8	14.3	18.4	7.4	22.9	10.9	20.3	8.6	20.4	5.8	0.161	<0.001*	0.976
% E SFA	13.7	3.2	13.6	3.3	9.7	2.0	11.3	2.8	11.0	3.0	10.7	2.2	0.090	<0.001*	
PUFA (g)	12.7	5.3	12.9	6.6	9.7	5.8	9.3	5.0	9.1	4.7	9.5	4.2	0.829	0.003*	0.946
% E PUFA	5.1	1.7	6.1	2.8	4.9	2.0	4.5	1.5	4.9	1.9	4.9	1.6	0.234	0.081	
MUFA (g)	31.6	10.8	28.8	14.2	20.3	10.5	23.0	11.0	21.3	6.5	21.5	6.5	0.486	<0.001*	0.970
% E MUFA	12.6	2.8	13.1	3.2	10.4	3.2	11.2	2.6	11.3	4.0	11.3	2.8	0.789	0.005*	
Total Fibre (g)	26.3	9.0	25.6	9.5	24.4	9.1	24.9	7.2	24.1	9.2	27.2	10.2	0.432	0.626	0.660

E – energy; CHO – carbohydrate.

* Significant values (p<0.05) were measured using repeated measures ANOVA.

Table 6.7 Changes in anthropometric and biochemical indices over time between control and sorghum groups (means and standard deviations)

CLINICAL INDICES	ALL GROUPS				CONTROL				SORGHUM				P-VALUES		
	Baseline data		Mean change		Baseline data		Mean change		Baseline data		Mean change		Group	Time	Interaction
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
Weight (kg)	86.6	11.6	-5.3	3.7	86.1	9.8	-5.4	3.6	87.1	13.6	-5.2	4.0	0.369	<0.001*	0.772
BMI (kg/m ²)	31.3	3.1	-1.9	1.4	31.6	2.6	-1.9	1.3	31.1	3.4	-1.9	1.4	0.754	<0.001*	0.601
Waist (cm)	104.2	10.9	-10.9	5.4	105.3	9.5	-11.8	5.6	102.9	12.5	-9.9	5.2	0.209	<0.001*	0.594
Body fat (%)	39.4	6.0	-2.1	2.3	40.3	5.8	-2.1	2.3	38.3	6.9	-2.3	2.5	0.876	<0.001*	0.194
Glucose (mmol/L)	5.26	0.50	-0.13	0.4	5.18	0.36	-0.11	0.3	5.35	0.62	-0.15	0.4	0.705	0.009*	0.162
Insulin (mIU/L)	12.32	5.78	-2.86	3.3	12.55	5.8	-3.17	3.0	12.06	5.86	-2.53	3.7	0.481	0.000*	0.905
HbA1c (mmol/L)	33.95	4.2	0.10	3.1	33.97	3.89	0.20	3.2	33.92	4.60	0.00	2.9	0.843	0.843	0.896
HbA1c (%)	5.26	0.37	0.00	0.3	5.26	0.34	0.00	0.3	5.25	0.41	0.00	0.2	0.926	0.926	0.912
Total cholesterol (mmol/L)	5.47	0.99	-0.40	0.7	5.64	0.98	-0.50	0.7	5.28	0.99	-0.30	0.6	0.139	<0.001*	0.384
HDL-c (mmol/L)	1.42	0.38	-0.07	0.2	1.40	0.40	-0.10	0.2	1.45	0.37	-0.10	0.2	0.674	0.008*	0.454
LDL-c (mmol/L)	3.39	0.85	-0.15	0.5	3.52	0.90	-0.20	0.6	3.24	0.79	-0.10	0.4	0.225	0.05	0.379
TAG (mmol/L)	1.46	0.76	-0.40	0.6	1.59	0.81	-0.50	0.7	1.30	0.67	-0.30	0.5	0.393	<0.001*	0.174
IL1β(pg/ml)	0.93	0.97	-0.15	0.4	0.92	1.03	-0.10	0.4	0.94	0.92	-0.20	0.3	0.565	0.004*	0.943
Il-6 (pg/ml)	1.19	1.29	-0.22	0.3	1.48	1.59	-0.20	0.4	0.86	0.73	-0.20	0.3	0.752	<0.001*	0.071
IL-8 (pg/ml)	5.73	2.46	-0.49	1.1	6.20	2.84	-0.40	1.2	5.19	1.86	-0.60	0.9	0.621	0.001*	0.075
hsCRP (mg/L)	2.52	2.41	-0.28	1.8	2.65	2.64	-0.30	2.1	2.37	2.15	-0.20	1.3	0.059	0.207	0.728
TNFα (pg/ml)	3.06	1.23	-0.76	0.8	2.95	1.27	-0.60	0.7	3.19	1.18	-0.90	0.8	0.110	<0.001*	0.748
HPX (mmol/L H ₂ O ₂)	86.6	11.6	-5.3	3.7	86.1	9.8	-5.4	3.6	87.1	13.6	-5.2	4.0	0.296	0.174	0.150
TAC (mmol/L Trolox)	1.26	0.18	-0.06	0.1	1.24	0.15	-0.10	0.1	1.29	0.20	-0.10	0.1	0.759	<0.001*	0.247

c – cholesterol; TAG – triacylglycerides; HbA1c – glycosylated haemoglobin; IL1β – Interleukin 1β; Il-6 – Interleukin 6; IL-8 – Interleukin 8; hsCRP – high sensitivity c-reactive protein; TNFα – tumour necrosis factor alpha; TAC – total antioxidant capacity.
 * Significant values were measured using repeated measures ANOVA

were identified for any of these clinical indices (Table 6.7). The inflammatory cytokines IL1 β , Il-6 and Il-8, decreased significantly compared to baseline ($p<0.001$), as did TNF α ($p<0.001$). TAC levels also decreased significantly compared to baseline ($p<0.001$). There were no significant changes over time in hsCRP and HPX levels. The reduction in total cholesterol was not significantly different between the groups, with mean decreases of 0.3 mmol/L (SD 0.6 mmol/L) for the sorghum group and 0.5 mmol/L (SD 0.7 mmol/L) for the control. There was a significant decrease in total cholesterol over 3 months for all subjects ($p<0.001$), as well as significant decreases in HDL-c ($p<0.001$), LDL-c ($p<0.05$) and TAG ($p<0.001$) levels over this time.

6.6.4 Subjective Satiety

VAS results were reviewed for the six daily time-points at 0, 6 and 12 weeks to compare any differences that may have existed between the control and test groups (RMANOVA). No significant differences were identified, demonstrating that all individuals reported similar feelings of hunger/fullness at the same test points (data not shown).

6.7 Discussion

As a consequence of this dietary intervention, a reduction in energy intake was achieved in the overweight study sample. The reduced energy intake in both groups was an effective driver of weight loss over the 3 month period. Regardless of the diet subjects followed, they lost weight and experienced beneficial changes in waist circumference, fasting glucose, fasting insulin, lipids and inflammatory markers. If maintained over time, the mean weight loss of 6.1% and reduced waist

circumference (10.9 cm (SD 5.4 cm)) for the study sample, could decrease risk of insulin resistance, impaired glucose tolerance and dyslipidaemias⁽³³³⁾, as well as lower the incidence of developing type 2 diabetes⁽³³⁴⁾.

The present study failed to confirm differentiating effects of sorghum grain consumption on weight loss in an energy-restricted diet over 12 weeks where only the test foods were different. This design is necessary, however, for rigorous testing of individual foods where other dietary confounders are cancelled out. Despite the previous acute satiety study showing promising results (Chapter 5)⁽³³⁵⁾, the present study showed that differences in meal satiety do not necessarily translate to weight reduction differences in the long term. Bearing in mind that the energy restriction of the total diet was the same for both groups, subjects receiving foods made from sorghum included in the dietary advice, did not lose more weight than the subjects receiving control foods and similar overall dietary advice. Thus, the meal-based satiety mechanisms postulated to involve sorghum polyphenols did not have an enhancing effect on body weight changes over 3 months, nor on overall reduction in metabolic disease risk factors.

Previous research conducted by our group has also shown that where both the control and intervention diets focus on healthy eating patterns and control for energy intakes, a difference in weight loss is not seen⁽³¹⁵⁾. Ostensibly this is because weight loss is dependent on control of energy intake and hence the percentage energy reduction in both arms was matched. Typically also, no differences are seen when structured dietary advice to both the control and intervention groups is provided^{(336;}
³³⁷⁾, as was the case in the present study. However, differences in other risk factor

variables (affected by dietary attributes other than total energy, such as dietary fat^(338; 339)) have been observed. Given our assumption that a higher polyphenol intake from sorghum may have had similar effects, the study design was appropriate, but we may now need to look to other potential synergistic components of the diet, or a higher dose to pursue our hypothesis further.

The lack of differences between groups in changes in lipid levels was not consistent with another study investigating the effects of consuming sorghum foods on serum lipid levels⁽¹⁷¹⁾. In this study, a significant reduction ($p < 0.05$) in total cholesterol, TAG and HDL-c was observed in 10 males and 6 females after daily consumption of 100 g of unrefined sorghum in the form of pancakes over 3 weeks. However, this study used a small sample size (with low statistical power), reducing the likelihood that a statistically significant result reflects a true effect. Further, the content of subjects' background diets was not adequately reported, making assessment of dietary confounders and final conclusions difficult. Overall, these results are ambiguous hence more human evidence for lipid effects in relation to sorghum consumption is warranted, and may be more relevant where weight loss inducing diets are not prescribed so as to discern lipid lowering effects independent of weight loss.

A further strength of the present study was that the polyphenolic composition of the test foods was profiled. From a physiological perspective, consideration was given to the results of a recent randomised, controlled, crossover human study, involving 22 healthy adults, assessing the acute effects of consuming pasta containing red or white whole grain sorghum flour (30% sorghum, 70% semolina) on plasma total

polyphenols, TAC and oxidative stress markers compared to a wheat control made from 100% semolina⁽¹⁸⁰⁾. Pasta containing red whole grain sorghum flour (RSP), but not white sorghum flour, enhanced antioxidant status and improved markers of oxidative stress in healthy subjects ($p < 0.001$). The increase in plasma polyphenols by the RSP meal was attributed to its higher content of polyphenols. In the present study, despite significant differences in the levels of polyphenols and TAC between the test food products, differences in the levels of inflammatory biomarkers and plasma TAC between the sorghum intervention and wheat control groups were not detectable (Table 6.3). The reasons are not clear as to why the TAC levels unexpectedly decreased over time even though the inflammatory markers showed significant improvement. While standard protocols were followed and precautions taken, possible oxidation of blood samples during preparation or storage, cannot be ruled out⁽³⁰²⁾. Furthermore it may be that the effects of ingesting sorghum grain components are short-lived and subtle, hence the positive outcomes observed in acute studies may not translate to whole of diet studies conducted over a number of months.

The lack of differences in subjective satiety scores was perhaps expected. VAS are validated for use in highly controlled situations⁽²¹⁷⁾, such as acute meal test studies. The inclusion of satiety-related VAS was justified to enable a possible link between reported ratings in this study and those of the previous acute satiety study⁽³³⁵⁾ (Chapter 5), though realistically, the small differences from month to month in the present study were unlikely to be captured accurately by the subjects using the VAS tool. An alternative appetite questionnaire, validated for use in free-living conditions⁽³⁴⁰⁾, may have been more informative.

Separating out the effects of individual components within a food, in this case the flaked cereal biscuit matrix, is difficult within a human intervention trial. To this end, a key strength of the present study is the comprehensive characterisation of the physicochemical properties of the test foods used in the intervention diets, enabling the link between clinical effects and components within the food. In the previous acute study⁽³³⁵⁾, determination of polyphenolic compounds, dietary fibre, profiling of starch contents and proximate analyses of the RTE sorghum flaked biscuits, guided the hypothesis that the polyphenolic compounds in particular played a role in observed acute satiety effects that would drive weight loss in the present RCT. Overall, the lack of positive results should not infer that sorghum is not a beneficial ingredient in cereal foods, as subjects consumed the foods successfully, lost weight, decreased their serum cholesterol and showed other positive outcomes related to metabolic health. The whole grain sorghum product was as effective as the whole grain wheat product in the present study. Sorghum, like other whole grain cereals, delivers beneficial, health-promoting components and properties (such as antioxidants, dietary fibre, SDS and valuable energy) when it is consumed as part of a healthy food within a healthy diet.

As with most human dietary intervention trials, there are a number of confounding variables in the present study that require some consideration. Firstly, the trial was only a 3 month intervention and this period of time may not be long enough to detect differences between the subjects' compliance with an energy restricted diet and the actual effects of the dietary intervention. It has been shown that subjects who reported greater compliance with a weight loss protocol lost more weight regardless of the dietary intervention⁽³⁴¹⁾, suggesting that strategies to increase adherence may

be more important than the actual composition of the diet. Validated “measures of adherence” to enhance compliance to dietary advice in the context of clinical interventions would therefore be useful in future studies⁽³⁴²⁾.

Secondly, the present study included similar test food products for both the intervention group and the control group, which only varied in the source of grain (sorghum versus wheat). The effect of individual grain components within the cereal biscuits, even if positive, are likely to be relatively small and hence showing a statistical difference between the groups would be difficult, especially over 3 months. Significant differences between the treatment and control groups may have been detected if the whole wheat control biscuits were lower in dietary fibre and higher in RDS compared to the sorghum biscuits. Further, instructing subjects to eat food products at both breakfast and at afternoon tea as part of the compliance criteria for the dietary intervention protocols, may have actually increased overall energy consumption at times when the subjects might otherwise have chosen not to eat or to eat less in either arm of the study.

Thirdly, it is difficult to control the dietary intake of human subjects. Although food records and discussions with subjects during study interviews indicated high compliance with consumption of test products, the subjects were less compliant with the energy restriction as evidenced by the lower-than-predicted weight loss. All four subjects that withdrew from the study were from the intervention group, which could infer the greatest difficulty with compliance, but this is less likely given the reasons provided for their withdrawal. Lastly, large standard deviations existed within all the analysed datasets, limiting the ability to detect significant results. The large standard

deviations indicated that individual fluctuations are more varied than any overall effect of sorghum intake.

6.8 Conclusions

In summary, although the groups experienced significant weight loss and general improvement in all clinical measures such as fasting glucose, insulin, cholesterol and key inflammatory biomarkers, no effects appeared specifically related to sorghum consumption. Overall, there were no discernable differences between an energy-restricted diet containing a wheat-based cereal food and that containing a sorghum-based cereal food. With a longer intervention and greater exposure to the test foods, some differences might have been detected, as subtle effects of components in the cereal foods would become more prominent. Nevertheless, the consumption of sorghum flaked breakfast cereal biscuits in the context of an energy-restricted diet is not deleterious in overweight and mildly obese individuals, and in fact represents a viable gluten-free alternative to wheat in food products.

CHAPTER 7:

Conclusions and Recommendations

A component of this chapter is contained within the article:

Stefoska-Needham, A. (2016) Progressing the position of sorghum, a potentially sustainable cereal crop, through the food product innovation pipeline: an Australian perspective. *Agroecology and Sustainable Food Systems*. (Under Review)

7.1 Summary of Findings

“Food is not just food. Food is a many splendoured thing”

Anita Stefoska-Needham... Inspired by Kaisa Poutanen (Dietary Fiber Conference, Paris, 2015)

Food represents a complex set of compounds. In nature these compounds act synergistically and serve a biological purpose in the original organism (plant or animal)⁽⁶³⁾. Even more complex, is the understanding of a food’s capacity to contribute to beneficial effects in the human body. The complexity of dealing with food was demonstrated in this thesis, which presented a case study of the effects of a novel food on health. This case study investigated the potential of whole grain sorghum as an ingredient in whole foods to assist in the prevention of chronic disease, particularly by examining hypotheses relating to body weight regulation as a key strategy for management of metabolic disease. Specifically, it was hypothesised that sorghum was a viable alternative to existing whole grain cereals and may have positive benefits on markers of metabolic health including weight management. In a broad sense, by focusing on a single food ingredient – the sorghum grain – this thesis asks whether sorghum might have a more significant place in the diets of Australians.

The research undertaken for this thesis could be seen as a particular *form* of case study on how research on new foods for human consumption might take place. After positioning sorghum as a nutritious food, the research focused on the effects of a novel food product developed in partnership with a food manufacturer (Sanitarium Health and Wellbeing) and facilitated through the Australian Research Council Industry Linkages program. The examination of sorghum’s potential to assist in the

prevention of chronic disease exposed a number of the complexities which scientific research needs to address in dealing with food effects on health. Findings from this thesis add to the knowledge base for sorghum as a viable human food, particularly within the Australian context where interest from food companies, researchers, farmers and consumers is just beginning to grow. The thesis considers an important, albeit gradual, transition for sorghum as a low-value livestock feed to a value-adding human food ingredient. With the backdrop of a changing Australian economy (where the agricultural sector is becoming a more significant economic stimulator), and a growing priority for sustainable agriculture in the face of climate change⁽⁵⁾, sorghum appears as an appealing and appropriate choice of crop. After all, sorghum is relatively cheap to produce, with remarkable adaptability, enabling it to survive in harsh climates common to many regions of Australia.

An examination of the existing scientific research on sorghum provided a picture of the state of the evidence base for sorghum consumption assisting in the prevention of chronic disease. It also exposed a myriad of nutrients and bioactive compounds in sorghum grain with potential actions in metabolic processes. The sorghum grain components included slowly digestible starches, non-starch polysaccharides, proteins, fats and a vast array of phytochemical compounds, including phenolic acids and flavonoids. Studies of these components implicated effects on energy balance, glycaemic control, lipids, gut microbiota, and cell-mediated immune responses, including antioxidant and anti-inflammatory processes. Put together, it also appeared likely that, as a result of food synergy, the whole food is likely to be more potent than its individual components⁽¹⁴⁸⁾. For this reason, whole sorghum-based foods were used to test the effects of sorghum intake. The critique of effects of bioactive

components within sorghum gained from the literature review guided the direction and formulation of valid hypotheses for subsequent human studies reported in the thesis, and informed the development of an optimised sorghum-based food product for testing. This was a necessary and innovative step in the thesis, and reflected the absence of existing sorghum-based foods in the Australian diet.

The food product innovation transformed sorghum from its raw grains into a whole food product (flaked cereal breakfast biscuits), appealing to the Australian palate. To link the potential actions of food components within the sorghum flaked biscuits with effects, it was necessary to comprehensively characterise the physicochemical properties of the test foods and this was a major strength of this thesis. Aside from typical analyses such as proximate determinations, evaluation of polyphenolic/antioxidant compounds and starch properties was also made. Full details on the processing of the sorghum grain were considered including unique information from scanning electron microscopy, which intimated details on the status of starch granules in the final test foods (mainly with respect to gelatinisation). Collectively, this data assisted with ascribing clinical effects to particular components within the sorghum food matrix. This information also guided specific recommendations made to the food manufacturer around future formulations of flaked sorghum biscuits. In particular, consideration was given to lowering the input and final moisture levels in order to minimise starch gelatinisation, to further increase the content of slowly digestible starches and to potentially lower the glycaemic index of the final product. Importantly, this information also provided evidence for potential mechanisms of action that may contribute to outcomes in the subsequent trial testing the longer-term effects of chronic sorghum consumption.

This thesis contributed to the evidence that whole grain sorghum enhances acute satiety and is likely to be equivalent to wheat when consumed as an ingredient in whole foods. Meal test results showed an increase in anorexigenic hormones (GLP-1 and GIP in both sexes and PYY in males) over four hours after sorghum whole grain was ingested in the form of flaked breakfast biscuits, especially after red sorghum intake. Likewise, subjective satiety was increased acutely after sorghum biscuits were consumed compared to wheat biscuits. Interactions between polyphenols, dietary fibre and SDS within the food matrix may have contributed to the observed elevated satiety effects of sorghum flaked biscuit consumption (compared to wheat), although the involvement of other nutrients and bioactive components may also be relevant. These may include additional effects from gut hormone stimulation. While differences in polyphenol content may be relevant, no individual component was definitively implicated in the mechanistic pathways, supporting the food synergy concept whereby “many substances in food have additive or more than additive effects” on health outcomes⁽³⁴³⁾.

As part of an energy-reduced diet, sorghum produced significant weight loss and the majority of clinical measures (fasting glucose, insulin, serum cholesterol and key inflammatory biomarkers) improved over 3 months during the dietary intervention trial. However, these effects were not significantly different to an energy-reduced wheat control diet. Overall, it was not possible to conclude that whole grain sorghum assisted specifically with weight management, or was superior to wheat (another whole grain product). However, the lack of differentiating results between chronic consumption of sorghum and wheat should not infer that sorghum is an inferior ingredient in cereal food products, especially since subjects consumed the sorghum-

based foods successfully and experienced beneficial effects. What is evident from this research is that a whole grain sorghum product appears as effective as a whole grain wheat product in the context of an energy-restricted diet.

Sorghum, like other whole grain cereals, delivers beneficial, health-promoting components and properties when it is consumed as part of a healthy food within a healthy diet, and it represents an excellent gluten-free whole grain alternative to wheat. This knowledge assists with future marketing of sorghum as a food with potential health benefits, to consumers. Today more people are motivated by the idea that healthy eating encompasses wholesome foods and drinks, which are minimally processed, to fuel the body and provide long-lasting energy⁽¹¹⁾. Weight management is often an underlying driver for consumer food choice, particularly in Western societies where rates of overweight and obesity are prevalent. Foods that are able to assist in appetite control are of particular interest to both consumers and food companies alike. Equally appealing is the use of alternative and/or novel ingredients that are naturally functional, the so-called “ancient grains” being a good example⁽¹¹⁾. Market researchers predict that the “healthy living” movement will continue⁽¹¹⁾, resulting in more consumers prioritising their personal health and demanding naturally healthy, minimally processed whole food product innovations, which could include products from sorghum.

Following this demand will be interest from the food industry to make health claims about these novel “healthy” food innovations, and thereby an increased demand for science to substantiate these claims is expected. For now, specific health claims about weight regulation, appetite and satiation/satiety enhancement are not permitted

in Australia under the FSANZ regulations⁽¹²⁾, though this may change with more scientific evidence demonstrating appetite-regulating effects of different foods. This would firstly require the development of guidance documents on satiety-related health claims, similar to those produced by Health Canada⁽²¹⁶⁾. Should this become available, the results from the research in this thesis would make a contribution to the required evidence base.

7.2 Limitations and considerations for further research

As with all research, the major limitations of this thesis relates to the breadth and depth of analysis that is made available through the study designs. Nevertheless, the choices made were consistent with convention. Food-based research has historically used mechanistic studies to explain health effects of food consumption, though this has often proven futile for supporting claims on health outcomes mainly because the human body is complex and the pathways that lead to improved health or disease development are multifaceted. Adding to this intricacy is the complexity of food itself and the myriad of interactions between food components and body processes. A good example of this complexity comes from the acute study in this thesis, whereby insulin response was lower after the white sorghum biscuit compared to the red sorghum biscuit but resulted in the same subjective satiety ratings. If greater insulin responses are indeed associated with enhanced acute satiety effects⁽²⁸¹⁾, then why did the subjects not perceive greater feelings of satiety? Similarly, although the satiety related hormones GLP-1 and GIP were elevated after red sorghum consumption, the release of the hunger hormone ghrelin was not different between treatments despite differences in subjective satiety sensations. There is no clear answer to fully explain these results, although human behaviour may be the most

powerful determinant of people's food choices. We all experience eating food in response to non-biological cues such as emotions, hedonic hunger (eating to obtain pleasure in the absence of an energy deficit), environmental influences, and habits. For example, if satiating/satiety effects could override other factors on eating, then the sorghum group may have eaten less, but this was not the case. More consideration should therefore be given to assessing human behaviour in these types of studies.

Given the complex nature of food effects, translating scientific research into practical food and nutrition advice on disease prevention is difficult. Nevertheless, mechanistic studies of food components add to our knowledge about nutrition and contribute to inferences about important causal pathways. In this vein, the acute meal test study in this thesis identified important satiety-enhancing actions occurring within the sorghum grain (ascribed potentially to SDS, polyphenols, dietary fibre), as well as highlighted potential physiological processes involving gut hormones. The subsequent trial then served as a platform to test whether these effects could be seen over a longer time frame and within the context of a whole diet, which after all, more closely resembles the natural context of human eating behaviour. However these studies were conducted with a single food – and while well-characterised to define the ingredient, it is difficult to ascertain how an ingredient will translate into another food.

The RCT design (Chapter 6) was the necessary choice for conducting this “translational” nutrition research because its internal validity for comparing an intervention to a control is considered to be the “gold standard” and results from

RCTs are usually reliable for causal inferences. However, where both the control and intervention diets focus on healthy eating patterns and control for energy intakes, as was the case in the RCT in this thesis, it seems that a difference in weight loss is not likely to be seen⁽³³⁶⁾. This is because weight loss is dependent on control of energy intake and the percentage energy reduction was matched in both arms in the present RCT. Usually though, differences in other risk factor variables (not affected by total energy) can be observed, as seen in other studies of similar design⁽³³⁹⁾. It was postulated that a difference in serum cholesterol, glucose, insulin and inflammatory markers may be detected between groups (partly due to differences in bioactive components such as polyphenolic compounds), but this was not the case. Possibly, the amount of sorghum grain in the serve size of the test cereal food products was inadequate to elicit differences in satiety and metabolic markers over 3 months. Although it is not novel to consider that serve size/dose is important in food studies, it is an important factor especially in cereal food studies because clinical effects may be dependent on small components within the food matrix.

The RCT results raise the important question about whether the strategy to match the percentage energy restriction in both the control and intervention arms is actually a limitation. An alternative option is to design studies with additional study arms, whereby subjects consume their usual diet without energy restriction and eat the test foods of interest daily. To demonstrate this using the RCT in this thesis as an example, there would be four study groups: 1) usual diet + sorghum; 2) usual diet + wheat; 3) energy-restricted diet + sorghum; 4) energy-restricted diet + wheat. Effectively, this suggests two controls and two intervention groups; a control for overall dietary composition (including total energy) and eating behaviour and a

control for the test food being studied (cereal). It should be acknowledged that this might be prohibitive from a practical point of view as costs are increased with more subjects and subject recruitment becomes a challenge when volunteers are not promised a weight loss intervention. Nevertheless, an alternative strategy such as this may be worthwhile to increase opportunities of detecting differences in weight loss or biomarkers between groups. Greater study power achieved through a larger sample size and/or conducted for a longer time-frame may have allowed differences between groups to emerge, though this presents with other challenges typical of RCTs including confounding and compliance issues (Chapter 6).

Although energy restriction is pivotal to weight loss, and weight loss is a key preventative strategy for disease prevention, it does not reflect the overall quality of an individual's dietary intake. In fact, energy restriction could compromise nutrient intake and lead to an unbalanced diet. Aside from the energy-restricted dietary prescription provided to subjects in the RCT (based on the Dietary Guidelines of Australia), diet quality was not formally monitored. Grafenaer et al.⁽³⁴⁴⁾ state that the emphasis on diet quality in a weight-loss context recognises the interrelationships between foods and food components, and considers the relationship between the dietary pattern and overall health. Upon reflection, this is a key component of the research framework of this thesis and to this end, a formal assessment of diet quality using a validated tool, for example the Food Choices Score (FCS)⁽³⁴⁴⁾, would have provided useful information about the types of food choices individuals made and how those selections influenced their eating behaviour, rather than using adherence to total energy reduction as the sole gauge of dietary intake and in the end, body weight outcomes.

7.3 Key recommendations for future studies

Specifically in relation to sorghum, ongoing investigation is warranted on the use of sorghum whole grain as an ingredient in the formulation of foods targeted for weight control through appetite regulation, in both a research and commercial context. The outcomes of this thesis identify the following key points and raise important recommendations and questions for prospective studies using sorghum foods.

1. Part of the effects of sorghum consumption on acute satiety appears to relate to stimulation of gut hormones such as GLP-1, GIP and PYY. These effects were demonstrated over 4 hours, hence future research should use this time frame as the minimum testing period. However, review of hormones over greater periods of time in a study with chronic ingestion, and with different sorghums varieties, would be useful, especially since mechanisms involving polyphenolic components and dietary fibre appear to be quite subtle. These hormones would be best reviewed during meal test studies pre- and post-long-term interventions, given that a single fasting level taken at a particular point in time over 3 months is not likely to be representative. Although, plasma glucose responses were not altered in the acute study (probably due to similar starch properties between treatment foods), specific testing of the flaked biscuits' GI and insulin index would have provided additional mechanistic information. This may have also helped to better understand the increased insulin response observed after red sorghum biscuit intake. Numerous studies suggest that insulin plays a greater role in acute satiety than glucose⁽²⁸¹⁾, however ideally reduced insulin responses may be more valuable in diabetes prevention. Controlling for fibre content may more

clearly elucidate mechanisms and future dose response studies with manipulation of the sorghum fibre types would be useful to more precisely investigate the role of fibre in satiety-enhancing mechanisms of sorghum-based foods.

2. Studies should aim to determine whether differences in satiety would persist with chronic sorghum intake – and in turn whether these differences would influence long-term food intake and thence weight management. Specifically, evidence from future RCTs (aiming to directly examine effects on health outcomes between a control and sorghum-intervention diet) are needed to better understand the health-promoting potential of sorghum consumption – as this remains ambiguous in this thesis. However, food-based RCTs present with substantial design challenges, particularly when examining weight loss outcomes. Developing research strategies to test weight loss outcomes in free-living individuals where energy intake is not controlled but scientific rigour is preserved, should be a key focus in this endeavor. Additionally, a focus on diet quality in a weight-loss intervention trial and more broadly in research aiming to link food and health (such as sorghum consumption and health), would be advantageous as improved diet quality exposes individuals to health promoting food components such as dietary fibre and antioxidant compounds and these factors can be better evaluated and considered in the overall study outcomes.
3. More-commonly consumed food formats should be tested in future studies of sorghum grain consumption, including bread, pasta, porridge and other

breakfast cereal options. Invariably, foods are processed from cereals using different methods that impact on both sensory and nutritional aspects of the food. The acute satiety study in this thesis may have been improved with at least an additional test food included in the crossover design, one based on an alternative food format such as porridge. This would have assisted with evaluating the influence of processing on the sorghum grain and subsequently the impact on biomarkers. Various chemical and physical reactions are initiated during food processing that may alter the properties of the sorghum grain and food matrix, impacting on the fate of the food once ingested. It is imperative in future sorghum-based studies to broadly define the post-processing physicochemical properties of the test foods in order to make plausible links between observed clinical effects and potential mechanisms of action at the food level.

7.4 Concluding Remarks

Sorghum is an excellent crop suitable for sustainable agriculture, particularly in Australia which is experiencing negative impacts of climate change and global warming⁽⁵⁾. This research suggests that commercial investment in sorghum food product development is warranted. Further, with consumer trends indicating a growing demand for naturally functional, minimally processed and satiety-enhancing foods⁽¹¹⁾, whole grain sorghum represents a novel ingredient in food formulations, especially those targeted for appetite control. ‘Value-adders’ would benefit not only from sorghum’s gluten-free attribute, but its source of nutrients, slowly digestible starches, dietary fibre, antioxidant compounds, and its application (especially red sorghum) as a natural food colour. Not only do the findings from this thesis provide

new knowledge that may improve health outcomes for consumers of sorghum-based products in the longer term, but they also contribute to developing and growing the sorghum industry globally. Multinational corporations and smaller entrepreneurial food companies now need to invest in launching sorghum-based product formats that have the potential to become popular within these markets.

7.5 Post-Script

By the completion of this PhD thesis, a sorghum-based food product was launched successfully in the market by the food manufacturer associated with this research; a business decision that was supported by scientific developments from this body of work. This joint innovation project reflects the value of collaboration between food industry and academic research to explore a food's potential through rigorous science.



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APPENDICES

APPENDIX 1

Appetite-Related Visual Analogue Scales (VAS)

QUESTIONS ON APPETITE AND DESIRE FOR FOOD

INSTRUCTIONS:

Make a vertical mark | on each line that best matches how you are feeling **at this moment**.

For Example:

I am not hungry at all	_____ _____	I have never been more hungry
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How hungry do you feel at this moment?

I am not hungry at all	_____	I have never been more hungry
---------------------------	-------	----------------------------------

How satisfied do you feel at this moment?

I am completely empty	_____	I cannot eat another bite
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How full do you feel at this moment?

Not at all full	_____	Totally full
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How much do you think you can eat at this moment?

Nothing at all	_____	A lot
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APPENDIX 2

Product Evaluation Questionnaire: Flaked Cereal Biscuits

ABOUT YOUR BREAKFAST CEREAL

Instructions

For questions that appear like this, draw a vertical mark (|) on each line that best matches how you feel about the breakfast cereal you have eaten

Dislike very much _____ Like very much

1. How much did you **like** or **dislike** the **taste** of the breakfast cereal?

I disliked the _____ I liked the
taste a lot taste a lot

2. How would you describe the taste of the breakfast cereal you have eaten?
(please tick the boxes which correspond to your selection)

Sour

Bitter

Sweet

Salty

Other (please specify):

3. How much did you **like** or **dislike** the **texture** of the breakfast cereal?

I disliked the _____ I liked the
texture a lot texture a lot

4. How much did you **like** or **dislike** the **crunchiness** of the breakfast cereal?

I disliked the _____ I liked the
crunchiness a lot crunchiness a lot

5. How **satisfying/filling** was the breakfast cereal after you had eaten it?

Not very filling _____ Very filling

6. Do you usually add any additional food items such as fruit, sugar, yoghurt or honey (apart from milk) to your breakfast cereal?

7. If you were allowed to add additional food items such as fruit, sugar, yoghurt or honey to the breakfast cereal you have eaten, how likely would you be to eat this cereal (please circle one):

Be more likely to eat the cereal

Be equally likely to eat the cereal

Be less likely to eat the cereal

8. If you were able to buy this breakfast cereal from a grocery shop and it was priced similarly to other breakfast cereals, how **likely** would you be to **buy** it? (Please tick one)

Definitely Would Buy

Probably Would Buy

Unsure

Probably Wouldn't Buy

Definitely Wouldn't Buy